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PROF. P. K. BANDYOPADHYAY

DEPARTMENT OF ZOOLOGY
University of Kalyani, Kalyani-741235
West Bengal, India

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Advances in Parasitology :
A Novel Approach Towards a Disease Free World

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PREFACE

It is my proud privilege to extend my cordial and warm greetings to the distinguished and celebrated scientists, erudite researchers, professors, clinicians of national and international repute, faculties, scholars and students who have assembled in the 22nd National Congress of Parasitology organized by the Department of Zoology, University of Kalyani, Kalyani, West Bengal in collaboration with the Indian Society for Parasitology and Zoological Survey of India, Kolkata held during the period 30th October, 2010 to 1st November, 2010.

During the three days of the congress, it became an important podium for the dissemination and argument on the new findings of chemotherapy, immune response, molecular biology and biochemistry, host parasite interaction, parasite infections in aquatic ecosystem, taxonomy, systematics and phylogeny, vector biology, control and epidemiology. The congress provided the scientific depth with a range of plenary and invited talks with a compliment of large number of oral and poster presentations in parallel thematic session. Privilege had also been obtained to felicitate some veteran scientists of West Bengal who contributed immensely in the field of parasitology. The discussion and interactions amongst both seasoned and budding enthusiasts in the area of parasitological research from the diverse section of India was much cheering.

I do feel that the deliberations presented at the congress had encompassed each and every arena of the subject thereby providing a dynamic platform for the vibrant , meaningful, evocative, incomparable and unending exchange of new findings, investigating and enigmatic questions and state of the art techniques between the scientists, researchers, clinicians, faculties, scholars and students as well to usher a score of new avenues for future research projects on parasitology at the national and international level. And, to encounter a number of dreadful diseases inflict on human, domestic animals, aquatic systems and economically important plants to ensure good health for all and self-sufficiency in food thereby benefiting the human society immensely.

The executives of the “Indian society for Parasitology” do strongly believe that organizing such congress in the days ahead would surely deliver educative lessons, hygienic intuitions and general awareness to all concerned especially the poorer and economically marginalized rural populace enormously. To enliven this spirit of our society consistent governmental support and assistance from other benevolent organizations are absolutely supplicated.

I do also feel that our society could emerge as a harbinger in disseminating basic parasitological facts, intrinsic tenets and other insights among the rural, semi-urban and urban populace then we could think of a healthier tomorrow thereby aiming at a huge leap towards developing a disease free world by conquering a number of insurmountable diseases through ingenious parasitological researches to alleviate acute human sufferings.

I like to put on record our sincere thanks to all funding agencies and advertisers for their financial support to the Congress. Thanks are also due to all contributors for providing the manuscripts and for undertaking necessary editorial changes.

I express my heartfelt thanks to the University authorities and all teachers of the Department of Zoology for their kind help, suggestions and active co-operation. I am thankful to all scholars of the Parasitology Laboratory of the department for their active help and co-operation. Last but not be the least, thanks are also due to Sri Dipanjan Mondal, East India Photo Composing Centre, Kolkata-700 006 for printing the proceedings.

Prof. Probir K. Bandyopadhyay
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FOREWORD

Prof. V.P. Sharma
ICMR Chair in Public Health Research
CRDT, IIT-Delhi-110016.

Date : 20.10.2011

Dear Dr. Prabir Bandyopadhyay,

It gives me great pleasure that the 22nd National Congress of Parasitology held at the University of Kalyani from October 30 to November 1, 2010 was a spectacular success. Scientific presentations were of very high quality and discussions enriched the presentation. The plenary lectures, invited talks, memorial & oration lectures, young scientist award lectures and oral and poster presentations provided opportunities for the young scientists to learn and interact on the latest developments in various fields of parasitology. Congress arrangements were all excellent and deserve appreciation. Publication of the Proceedings would provide a valuable material for the researchers to make use of the information in their own work. I wish to congratulate you and your team of Kalyani University for organizing such an excellent event and for publishing the Proceedings.

With best Wishes,

Sincerely Yours,

V. P. Sharma

V.P. Sharma
Editor in Chief
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Malaria in some areas of Hooghly district of West Bengal (2009-2010) : A survey and study of anopheline population

Amit Chattopadhyay, Chiranjib Dey and Pranab Kumar Banerjee

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Abstract : Recent resurgence of malaria in suburban and rural areas of different districts of West Bengal has become a serious health problem. It has been reported that some blocks of Hooghly are malaria prone. Therefore, a systemic investigation has been carried out in some areas of Hooghly (especially Serampore subdivision) in order to know the prevalence of the disease as well as the species diversity of *Anopheles* mosquito. The present study reveals that falciparum malaria is on the rise in urban area in relation to vivax malaria but rural area has followed reverse trend during 2009-2010. Our data also reveals that the Anopheline population includes mainly *Anopheles subpictus* as also *A. anularis* and *A. stephensi* in these malaria prone areas.

Key Words **:** *Anopheles spp.; Falciparum malaria; Prevalence; Resurgence; Vivax malaria*

Introduction

Malaria has been one of the most prominent and an ancient disease which is still a major problem. About 100 countries are malarious. India is reporting about 2 million positive cases per year. In India death caused by *P. falciparum* in last 5 years is as follows 134, 193, 291, and 222 in 2005, 2006, 2007 and 2008 respectively. Fatality rate in West Bengal is 5% (WHO, 2009).

There are 4 sub-divisions and 18 blocks in our Hooghly district. Among these 5 blocks are malaria prone *viz.* Mogra, Singur, Kanaipur, Goghat-II, and Khanakul-II.

The aims and objectives of this study is to know the Slide Positivity Rate (S.P.R.) in different rural blocks of Serampore subdivisions, the seasonal prevalence of malaria, the parasitic variations as well as the Anopheline diversities in the studied areas viz. Kanaipur, Serampore and Seoraphuli of Serampore sub-division, West Bengal.

Materials and methods

A) Survey : The S.P.R. and death records during 2009-2010 of different blocks have been collected. A systemic survey has been carried out in several health centers and hospitals of Serampore and Seoraphuli to know the parasitic variations and seasonal prevalence of this disease in 2009-2010.

B) Collection of mosquitoes and morphological identification : By using manual aspirator *Anopheles* mosquitoes were collected during the month of September and October of 2010. Samples were collected from different cattle sheds just beside the dwelling houses and the time of collection was 6-9 am in morning and 6-8 pm in evening. The sites of collection were Serampore and Seoraphuli as urban and sub- urban areas and Kanaipur as a rural area. Morphological identification was done following the taxonomical keys developed by Christopher (1933) and Nagpal *et al.* (2005).

C) Preparation of polytene chromosome : Polytene chromosome has been prepared from the ovarian nut cell after Saifuddin *et al.* (1978) and Banerjee and Chatterjee (1995).

D) Identification at molecular level : It has been carried out in following way: Isolation of genomic D.N.A. (using phenol –chloroform method) - P.C.R. using ITS2 primer - sequencing for the confirmation of identification.

Result

Table 1 and 2 represents the S.P.R. and malaria death in the blocks of Serampore sub-division during 2009 and 2010(Jan-Dec) respectively. Only death case was reported in the Kanaipur area in 2010. S.P.R. ranges in between 0.01 and 0.23 in 2009 and 2010. Fig.1 represents the seasonal prevalence of Malaria in Sub-urban and Urban areas (Serampore and Seoraphuli) during 2009-2010. Fig.2 shows the variation of parasitic infection during 2009-2010. In the Municipal area occurrence of *P. falciparum*, *P. vivax*, and mixed infections (*P. falciparum* and *P. vivax* both) were reported in 2009 and 2010 as 0, 19 (95%), 01(5%) and 05 (20%), 18 (72%), 02 (8%) respectively. The same in the Rural area was reported as 4 (10%), 36 (90%), 0 and 01 (9%), 10

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(91%), 0 respectively. Table 3 represents the Anopheline diversities in the studied areas *viz*. Kanaipur, Serampore and Seoraphuli. Fig.3 shows inversions in the 2R polytene chromosome of *A. subpictus*. Fig.4 depicts the sequences of ITS2 of some of our collected samples.

Block	S.P.R	Death
Kanaipur	**0.231**	**0**
Jungipara	**0.010**	**0**
Chanditala-I	**0.022**	**0**
Chanditala-II	**0.102**	**0**

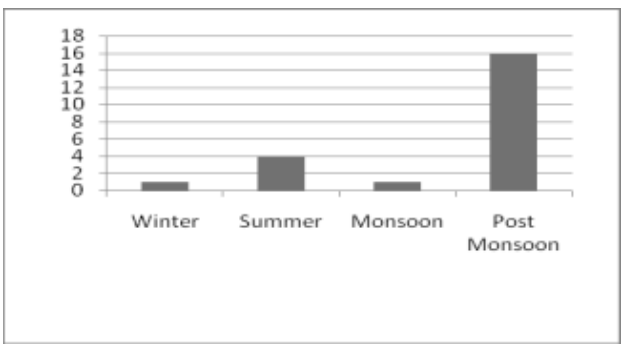
Table 1. S.P.R and Death in the blocks of Serampore sub-division in 2009 (Jan- May)

Block	S.P.R	Death
Kanaipur	**0.069**	**1**
Jungipara	**0**	**0**
Chanditala-I	**0**	**0**
Chanditala-II	**0.115**	**0**

Table 2. S.P.R and Death in the blocks of Serampore sub-division in 2010 (Jan- May)

Place of Mosquito Collection	*Anopheles* Species Found	Number of Occurrence	Month of Sampling
Kanaipur	*Anopheles subpictus*	8	Sept. 2010
	Anopheles annularis	3	
	Anopheles vagus	1	
Serampore	*Anopheles subpictus*	6	Sept. 2010
	Anopheles vagus	2	
Seoraphuli	*Anopheles subpictus*	8	Oct. 2010
	Anopheles vagus	1	
	Anopheles annularis	1	
Serampore	*Anopheles subpictus*	3	Oct. 2010
	Anopheles vagus	1	

Table 3. Anopheline diversities in the studied areas of Kanaipur, Serampore, and Seoraphuli.



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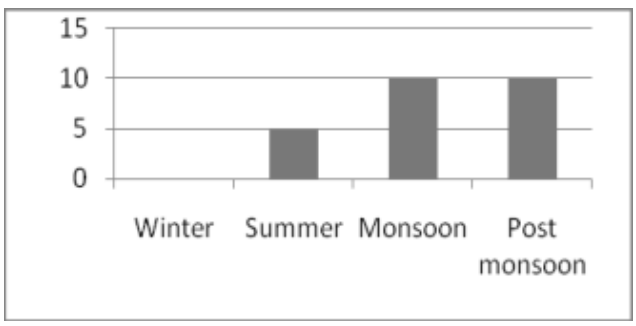

b

Fig. 1. Seasonal Prevalence of Malaria during 2009(a) and 2010(b) (Jan-Dec)

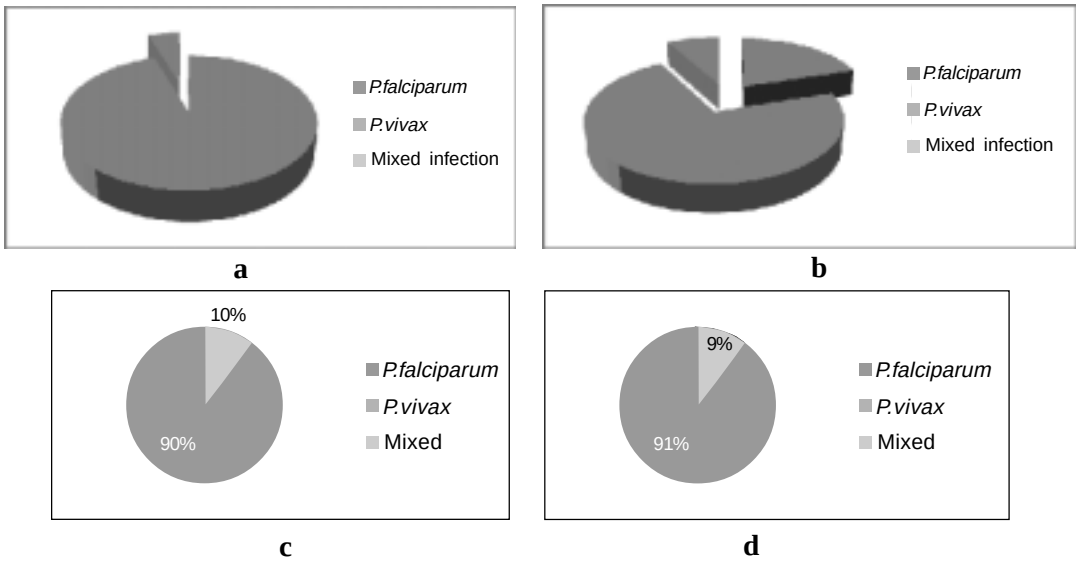

Fig. 2. A comparative account of the parasitic infection during 2009(a) and 2010(b) in municipal areas, and in 2009(c) and 2010(d) in rural blocks.

Fig. 3. Inversion in polytene chromosome of *A. subpictus*

- ***Anpoheles Annularis*; internal transcribed spacer 2**

- ***Anpoheles subpictus*; internal transcribed spacer 2**

- **Fig : Sequences of ITS2 of some of our collected samples.**

Fig. 4. Sequences of ITS2 of some of our collected samples.

Discussion

Survey : Though the S.P.R.s of the blocks of Serampore sub-division is less than 1, a death occurred in Kanaipur in 2010. According to the collected reports from sub-urban and urban areas it can be observed that post-monsoon shows the most prevalence of malaria in 2009 but in 2010, 10 cases have been found in both monsoon and post-monsoon seasons which indicates the increasing trend of malaria in these localities.

Morphological identification : *Anopheles subpictus, A. vagus* and *A. anularis* mosquitoes were identified and reported in the present studied areas. In India, it has been reported that *A. anularis* has role in malaria transmission in the states of Orissa, Assam, West Bengal and Andhra Pradesh (Alam *et al.,* 2007). At selected localities in Kolkata, *P. vivax* sporozoid infection was detected in salivary glands of one *A. anularis* (sporozoid rate 1.5%) (Ghosh *et.al.*, 2010). It has also been reported that *A. subpictus* has role in malaria transmission. As for example sporozoites were detected during summer (2004-2007) in *A. subpictus* in Angul district, Orissa (Kumari *et.al.*, 2009). Again sibling species of *A. subpictus* (fresh water form) has been established as a primary vector of malaria in an area of Tarakeswar, W.B. in India (Chatterjee and Chandra 2000). Therefore, these species might be the causative factors of this disease transmission in our studied areas.

Polytene chromosome : Inversions in the 2R polytene chromosome were observed. It indicates the presence of genetic load in natural population. This mutation is one of the causes of Anopheline variation *i.e.* even in the formation of sibling species which might be a causative factor to make them more potent vector.

Identification at molecular level : Less homology is found in the internal spacer regions (ITS1 and ITS2). So, we have intended to study the ITS2 sequence for the proper identification of species complex.

Therefore, from the foregoing discussion it can be opined that there is a clear signal of re-emergence of malaria in Hooghly district and the Anopheline population in this district should be further studied to reveal their vectorial attributes and hence, identification of sibling species by designing specific primer and detection of the presence of parasite and the type of blood meal within the body of mosquito needs further investigation.

Acknowledgement

We are thankful to Rev. Dr. Lalchungnunga, Principal, Serampore College, Serampore, Hooghly; Dr. R.K.Hazra, Scientist B, Regional Medical Research Centre, Bhubaneswar for their continuous support and encouragement throughout the study.

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Efficacy of botanicals against IIlrd instar larvae of *Henosepilachna vigintioctopunctata* (Coleoptera : Coccinellidae) infesting brinjal crop

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Abstract : The present study has been made to develop eco-friendly management of hadda beetle, *Henosepilachna vigintioctopunctata* using certain botanicals against IIIrd instar larvae. It is a common coleopteran pest infesting brinjal in India and apart and causes economic losses to standing crop right from seedling to harvest. Petroleum ether leaf extracts of *Ageratum conyzoides* and *Nerium indicum* adversely affected the normal development of the beetle. Both the herbal extracts showed a range of mean larval mortality from 33.33 to 83.33% in different concentrations in contrast to 5.56% observed in control. 0.5% concentration of both the extracts negatively affected the feeding behavior of the larvae which resulted in the enhancement of developmental period by 3.1 and 2.2 days in comparison to control where larvae took 12.7 days to complete their life cycle from IIIrd instar to pupae. However, other concentrations did not show any remarkable change in developmental period. A highly significant ($p<0.001$) inhibition in adult emergence was observed from to be 11.1±9.6 to 33.3±9.6% with 0.5 and 1.0% extracts of *A. conyzoides* and *N. indicum* with some morphological deformities. However, a mean of 66.7% adults were successfully emerged from the pupae in 0.1 % extracts of both the plants in comparison with control where 94.4±5.6% emergence was recorded. LC50 values of *A. conyzoides* and *N. indicum* were calculated as 0.216 and 0.238%, respectively.

Keywords **:** *Ageratum conyzoides, Henosepilachna vigintioctopunctata, mortality, Nerium indicum*

Introduction

Henosepilachna vigintioctopunctata (Fabr.) is a polyphagous coccinellid pest which feeds on cucurbitaceous and solanaceous crops including brinjal and causes heavy damage to them. According to Choudhary (1967), it is an important diet which is consumed largely by common men and has medicinal properties also. It is a source of vitamin A and B, carbohydrates, sterols as well as proteins besides minerals, especially iodine. It is a cheaper source of these nutrients and even a common and poor man takes this vegetable in his diet. As larvae and young beetles during the first 5 days after their emergence are the most voracious and harmful and cause a big yield loss in brinjal *(Solanum melongena)* crop by feeding on leaves, flowers, and fruits (Krishnamurti and Appanna, 1951). The fed leaves become the skeleton of veins, dried and finally shed from the plants. The yield losses of brinjal crop often reach upto 25% in an endemic situation and 10% in the zone of low infestation.

Scientists had tried various methods to tackle pest problems among which the most conventional method was the application of pesticides. The management of this notorious pest was also based on chemical pesticides (Jagan Mohan, 1985; Ghosh, 1986; Samanta *et al.*, 1999; Das *et al.*, 2002; Liu *et al.*, 2003). Soon, it was realized that repetitive and indiscriminate use of these pesticides in the fields results into several unwanted ill effects which include health hazards, development of resistance, presence of toxic residues in food, destruction of beneficial insects like honeybees, pollinators, parasites and predators and increase in environmental pollution.

Thus, it is becoming increasingly important to develop and use selective insect control measures that are effective and do not pose hazards for man or the natural environment. Therefore, keeping view in mind, a number of plant products or botanicals with a series of important properties such as; insecticidal, antifeedant, repellent, growth inhibitory, chitin synthesis inhibitor property and environmental friendly nature, attracted the attention of researchers in the direction of pest control programme (Satpathi and Ghatak, 1990; Chitra *et al.*, 1992; Venkataramireddy *et al.*, 1993; Lee *et al.*, 2004). As these botanicals possess more than one active components, there will be less chance of development of resistance and easily bio-degradable in the environment. Thus, the present study has been made to control this coleopteran pest by using the leaves extracts of *Ageratum conyzoides* and *Nerium indicum.*

Materials and methods

Plant material : In search of the effective herbal extract for the control of hadda beetle, the plants were selected from the literature and medicinal information provided by local people. *Ageratum conyzoides* is herbaceous plant commonly known

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as ‘Goat weed’ which comes under the family Compositae and occurs widely in the field having purple coloured bunch of flowers. It contains precocene which shows JH like activity on different insects. *Nerium indicum* is a large evergreen shrub with milky juice producing red or pinkish flowers commonly known as ‘Kaner’ or ‘Pink Kaner’ belonging to the family Apocynaceae. It is an ornamental plant of medicinal importance cultivating in gardens and homes. Its chemical constituents include cardiac glucosides, oleandrin, digitalin, nerientin, folinerin, cornerin etc. It also contains hydrocyanic acid, ursolic acid and sterol.

Organic extraction : For organic extraction, green leaves of *Ageratum conyzoides* (Goat weed) and *Nerium indicum* (kaner) were collected, washed thrice under tap water to remove dust and other particles. The washed leaves were shade dried for 10 days and ground to powder. The powdered material was extracted with petroleum ether (60°-80°C) as solvent in Soxhlet Apparatus for 8 hrs and extracted material was kept in a petridish for overnight to evaporate extra solvent from the crude extract (Mehta *et al*., 1995). From this crude extract, four concentrations viz. 1.0, 0.5, 0.2 and 0.1 % were prepared in distilled water and tested against the IIIrd instar larvae of *H. vigintioctopunctata.*

Rearing and maintenance of test insect, *H. vigintioctopunctata* : Different life stages of *H. vigintioctopunctata* were originally collected from the brinjal fields of Nariawal village of Bareilly and were continuously reared in Pests and Parasites Research Laboratory in the Department of Zoology, Bareilly College, Bareilly. The culture of the test insect was reared and maintained as per the method described by Mehta *et al.* (1995) and Saxena & Sharma (2007). Different stages of the beetle were reared on fresh and tender leaves of brinjal by changing the regularly in plastic jars measuring 12.5cm x 25cm and stalks of leaves were dipped in glass tubes filled with water and corked with thermacole to avoid drying the food material as larvae of hadda beetle do not prefer dried leaves. The glass tubes with leaves were kept in plastic jars covered with muslin cloth. The whole culture jars were placed in Biological Oxygen Demand (BOD) incubator maintained at 28±1°C and 65±5% relative humidity (RH). The culture was continuously maintained to supply different life stages of the insect for *in vitro* evaluation of various herbal extracts.

***In vitro* bioassay :** To evaluate the insecticidal activity of herbal extracts, 2 ml. of each concentration was sprayed on fresh and tender brinjal leaves which were embedded in water filled glass tubes corked with thermacole and fed to newly emerged IIIrd instar larvae of *H. vigintioctopunctata* for 24 hours in plastic jars covered with muslin cloth. After 24 hours feeding, larvae were fed with normal fresh leaves by changing them regularly upto pupation. The control experiment with distilled water was also run simultaneously. The entire experiment was studied at 28±1°C and 65±5% relative humidity in BOD incubator. The experiments were carried out with six IIIrd instar larvae in each concentration and were replicated thrice to collect the data on mortality, developmental period, adult emergence and any other morphological deformity in hadda beetle. Observations on larval mortality were recorded regularly after the treatment.

Statistical analysis : Data were statistically analyzed by Graph Pad Prism 4 software and LC_{50} values of the extracts were determined by probit analysis (Finney 1962).

Results

In the present study, efficacy of two commonly occurring plants, *A. conyzoides* and *N. indicum,* was tested against IIIrd instar larvae of *H. vigintioctopunctata* by feeding treated brinjal leaves are presented in Table-I and revealed that 1.0% concentration of both the plant extracts caused 83.3±9.6% larval mortality which was highly significant at $p<0.001$ level. A

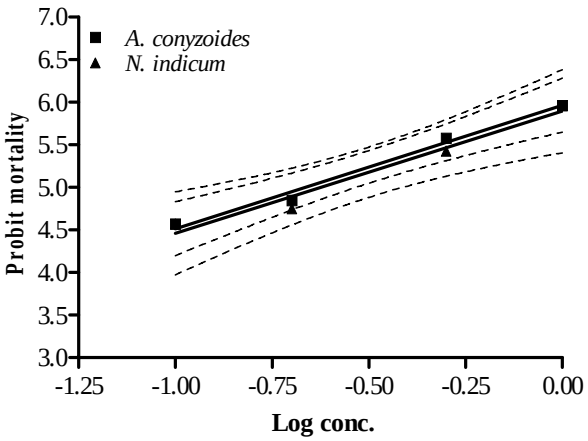

Fig. 1 : Showing probit mortality of treated larvae of *H. vigintioctopunctata* with *A. conyzoides* and *N. indicum* leaf extracts.

significant reduction in larval population was also seen in 0.5% concentration killing 72.2 ± 5.5 and 66.7 ± 9.6% larvae ($p<0.001$) with *A. conyzoides* and *N. indicum* extracts, respectively, as compared to control where 5.6 ± 5.6% larvae could not survive in similar conditions. However, both the extracts could also control the larvae at 0.2% concentration which was significant at $p<0.05$ when compared to control data. Plotting the log-probit mortality graph of transformed values, the LC_{50} of *A. conyzoides* and *N. indicum* against IIIrd instar larvae were calculated as 0.216 (95%CI= 0.131-0.356) and 0.238% (95% CI=0.143-0.395) (Fig. 1).

Data on adult emergence affected by these herbal extracts showed that 1.0 % of *A. conyzoides* and *N. indicum* had shown drastic effect on normal emergence of the adult beetles showing 11.1±9.6 and 16.7±9.6% emergence, respectively which were statistically significant at $p<0.001$ level when compared with control set 94.4 ± 5.6%. However, 0.2 and 0.5% of both the extracts significantly inhibited 22.2 ± 5.5 to 55.6 ± 5.6% adults to hatch from the pupae (Table I). Some of the adults which were emerged from larvae treated with both the extracts were deformed showing abnormal wings and elytra and were unable to fly. It suggests that the plant extracts possess more than one active component which act on target insect variously affecting different physiological and morphological activities of the insect. The effect of the plant extracts were also seen in the form of diapaused pupae. In course of investigation, it was noticed that larvae could not detach their exuviae easily at the time of moulting retaining them longer on their body and later they turned black and died.

Table I : Effect of *A. conyzoides* and *N. indicum* extracts on the development of *H. vigintioctopunctata*.

Extract	Conc. %	Mean developmental period (days)			% Adult Emergence (± SE)	% Mortality (± SE)
		Larva	Pupa	Total		
Ageratum conyzoides	0.1	8.8	5.5	14.3	66.7 ± 9.6	33.3 ± 9.6
	0.2	8.9	4.7	13.6	55.6 ± 5.6[a]	44.4 ± 5.6[a]
	0.5	10.1	5.7	15.8	22.2 ± 5.5[c]	72.2 ± 5.5[c]
	1.0	8.2	5.7	13.9	11.1 ± 9.6[c]	83.3 ± 9.6[c]
Nerium indicum	0.1	8.9	4.5	13.4	66.7 ± 0.0	33.3 ± 0.0
	0.2	8.9	5.0	13.9	55.6 ± 5.6[a]	38.9 ± 5.6[a]
	0.5	9.8	5.1	14.9	33.3 ± 9.6[c]	66.7 ± 9.6[c]
	1.0	7.4	5.7	13.1	16.7 ± 9.6[c]	83.3 ± 9.6[c]
	Control	8.5	4.2	12.7	94.4 ± 5.6	5.6 ± 5.6

[a] significant at $p<0.05$; [b] significant at $p<0.01$; [c] significant at $p<0.001$

The study on developmental period of hadda beetle revealed that only 0.5% extract of *A. conyzoides* increased total developmental period by 3.1 days to complete its emergence. However, lower concentrations of both extracts could not be efficacious to elongate developmental period of *H. vigintioctopunctata*. On the basis of LC_{50} values calculated as 0.216 and 0.238% of *A. conyzoides* and *N. indicum* leaf extract against IIIrd instar larvae, *A. conyzoides* is found more effective than *N. indicum*.

Discussion

Although at present, the emphasis of pest control is based on the minimum application of chemical insecticides and maximum application of other eco-friendly control measures either alone or in integrated form despite that chemical control is still very common practice to the farmers. The herbal extracts could become environmentally safe pest-control agents possessing so many active principles which reduce the possibility of development resistance in targeted pests. In the present study, it was observed that 83.3 ± 9.6% larval mortality of *H. vigintioctopunctata* was recorded with 1.0% concentration of both, *A. conyzoides* and *N. indicum* leaf extracts, while workers like Satpathi and Ghatak (1990) have noted 90% mortality of the same beetle with same concentration of root extract of *N. oleander* which was very close to the present findings and confirm the insecticidal activity of the plant. However, Saxena and Sharma (2005) also reported high insecticidal activity of both the plants when treated to Ist instar larvae of *H. vigintioctopunctata* in dose dependent manner ranging from 16.67-100% mortality in 0.1-1.0% concentration which was similar to the present study as shown in dose-mortality data (Fig. 1). Similarly, Saxena and Sharma (2007) also reported high larvicidal property of seed extract *N. indicum* when IIIrd instar larvae of the same beetle

were fed on treated leaves. Besides, Bai & Koshy (1999) described antifeedant and insecticidal properties of *Thevetia nerifolia* against *H. 28-punctata*, whereas Patil *et al.* (2000) reported anti-feedant and anti-beetle activities of *N. indicum* against stored grain pest, *Callosobruchus chinensis*.

Efficacy data of *A. conyzoides* on adult emergence of hadda beetle documented in the present work were also in corroboration with the results made by Singh and Rao (2000) who observed 59.86% adult emergence in a lepidopteran pest, *Spodoptera litura*, with *A. conyzoides* leaves extract which revealed the presence of active principle in the extract which interferes with chitin formation in the treated insects. Juvenile hormone-like activity of *A. conyzoides* was also reported against *Dysdercus cingulatus* (Fab.) leading to the malformed adults (Srivastava *et al.*, 1985). Here, emergence of some deformed adults and diapaused pupae of hadda beetle also confirm the chitin inhibitor like property of the plants. Similar to the present findings, Gehlot *et al.* (2005) also reported significant inhibition in adult emergence of another coleopteran stored grain pest, *Callosobruchus maculates*, when treated with certain plant extracts including *Eucalyptus globules*.

In the present investigation, 0.5% extract of *A. conyzoides* inhibited the larval growth showing a delay of 3.1 days in developmental period to complete its emergence which is in the agreement with Mehta *et al.* (1999) who also reported the prolonged larval developmental period of hadda beetle by another species, *Ageratum haustonianum*, indicating its growth regulatory activity against *H. vigintioctopunctata*. In contrast to the present findings on *N. indicum* leaf extract, Saxena and Sharma (2007) recorded prolongation in total developmental period with seed extract of the same plant when IIIrd instar larvae of *H. vigintioctopunctata* were treated. Srivastawa *et al.* (1985) reported that *A. conyzoides* extract possesses growth inhibitory and juvenile hormone activities against *Dysdercus cingulatus*. Considering the important properties of the extracts mentioned here, it can be suggested that herbal extracts can play a significant role in integrated pest control management.

Acknowledgement

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Gonotrophic cycle and age gradation of *Phlebotomus argentipes* in West Bengal, India

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Abstract : One of the major challenges in the research on Visceral leishmaniasis is limited knowledge of transmission dynamics of local vector species.Studies on the gonotrophic cycle of vector species *P. argentipes,* was performed in endemic foci of West Bengal, India to establish their physiological age and understand the maximum number of "refeeding", an index factor in the transmission dynamics of *P. argentipes.*

1200 wild caught adult *P.argentipes* were dissected and observed throughout the study year (100/month) for estimation of gonotrophic cycle, of which, 21.92% were nullipara, 38.75% were primigravids, 23% were primipara, 12.42% bipara, 3.5% tripara and 0.4% were quadripara. Availability of *P.argentipes* of greater life span in nature decreased steadily with the attainment of higher parous individuals as 23%, 12.42%, 3.5% and 0.4% primipar, bipar, tripar and quadripar females respectively were found in nature.Presumptive mortality rate from primiparous (P_1) to biparous (P_2) was 46.01, from biparous to triparous (P_3) 71.8, from triparous to quadriparous (P_4) was 88.1.

The distribution of both bi- and triparous females in the rainy and summer seasons respectively were significantly higher than the winter and this disposition in a natural population is of immense epidemiological significance for transmission of the disease, as during these months sandfly population with an average life span of 10/15 days were available in nature. Detection of a few quadriparous females in natural population of *P.argetipes* pointed out that it can undergo four gonotrophic cycles and have a possibility of surviving up to 25 days in nature. Presumptive mortality rate of *P.argentipes* depicted that older the population, the fewer would be its number in nature which explains the availability of a lesser proportion of higher parous females in natural population.The implications of the study has been discussed.

Key words **:** *Visceral leishmaniasis, P.argentipes, Gonotrophic cycle, age grading, transmission dynamics*

Introduction

Visceral leishmaniasis (kala-azar) ranks as the second important protozoan disease next to malaria. The disease, prevalent worldwide, is considered to be endemic in 88 countries,72 of which are developing countries and 13 are among the least developed countries.It is believed that 350 million people are at risk, and 12 million people are affected by leishmaniasis worldwide. Of this, 1.5 - 2 million new cases are esti mated to occur annually of which only 600,000 cases are officially reported (Sinha et al., 2005). Kala-azar has re-emerged from near eradication. The global estimate for the incidence and prevalence of Kala-azar cases per year is 0.5 million and 2.5 million, respectively. However, 90% of the worldwide cases occur in five countries (Bangladesh, Brazil, India, Nepal and Sudan), 60% in well-defined areas of Bangladesh, India and Nepal. (Bora, 1999).

The disease has ravaged parts of India for more than one hundred years with devastating consequences on population and economic development. Indian continent has been ravaged by a series of epidemics of visceral leishmaniasis (VL) since early part of the 19th century (Sengupta, 1947). The endemic states has been mainly North-eastern states, viz. Assam, Bihar & Bengal, with low endemicity in Madras and Gujarat. Kala-azar (KA) mostly proves fatal if untreated. With the resurgence of the disease in seventies in Bihar and Bengal thereafter, it developed renewed interest for epidemiological investigation.

Visceral leishmaniasis is caused by *Leishmania donovani* and its subspecies. It is a vector borne disease. The vector of kala-azar belongs to order Insecta commonly called as "sandflies". Only the members belonging to subfamily

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Phlebotominae are transmitting agents for kala-azar in Bihar and West Bengal. In north-eastern part of India, only one sandfly species i.e. *Phlebotomus argentipes* is the known vector of Visceral Leishmaniasis (Swaminath et al,1942; Dinesh et al,2000) . It is known to occur in well-defined areas in the eastern sectors of the country namely Bihar, West Bengal, eastern districts of Uttar Pradesh, Jharkhand, Assam, foothills of Sikkim and to a lesser extent in Tamil Nadu and Orissa (Marinkelle, 1980).

Resurgence of Kala-azar in India :

As a consequence of withdrawal of DDT spray under NMEP from kala-azar endemic areas in 1963-64, the disease resurgence occurred in late seventies because of slow build up of vector population. In early seventies, kala-azar cases started being reported from many hospitals in north Bihar districts from 1974 onwards. However, by 1977, several districts of Bihar reported fresh cases and then onwards the problem become a regular phenomenon in the entire north districts and some district situated south of the river Ganges in Bihar. The state has witnessed two major epidemic outbreak of kala-azar in the year 1978 and 1992 **(**Kar *et. al.*1999; Ranjan and Bhattacharya, 2002**)** The disease also spread to West Bengal where indigenous transmissions become perceptible in 1980's and Uttar Pradesh in 1990's (Ranjan and Bhattacharya, 2002)

Although spraying of DDT helped control of vector vis-à-vis kala-azar, there are reports of the vector *Phlebotomus argentipes* developing increased tolerance/resistance (Mukhopadhyay et al., 1990, Palit et al., 1994) Again chemotherapeutically the disease presents a clinical dilemma because of its serious complications and rapid development of resistance to first and second line drug of choice i.e. Sodium Antimony Gluconate (SAG) and Pentamidine. Therefore, until a safe and effective vaccine is developed, a combination of sandfly control ably supported by a detailed understanding of its bioecology and treatment of patients will act as a fulcrum for controlling Kala-azar.

Geographical priority& Epidemiological priorities :

According to the WHO scientific working group's report (TDR/SWG/VEC/03.1, 2003) on Insect vectors and public health in respect of leishmaniasis, some of the major challenges in the research on leishmaniasis is limited knowledge of population structure and dynamics of local vector species/populations relevant to endophilic and exophilic transmission.Importance of epidemiological and entomological studies or bioecologic studies together in any focus of visceral leishmaniasis has been highlighted in several earlier studies (Dancesco & Chadli 1982; Ascione et al., 1996; Maroli et al., 2001).

Studies on gonotrophic cycle of *Phlebotomus argentipes* :

Studies on the gonotrophic cycle of *P. argentipes* by means of parity status i.e. number of dilatations of ovariole has not been worked before. This year long experiment was performed to gather the information about their longevity i.e. to establish their physiological age as well as to know maximum number of "refeeding", a single female can undertake during its life, an utmost important factor in the transmission dynamics of *P. argentipes*. This will also give an idea about parous/ nulliparous proportion as a parameter necessary for evaluation of sandfly longevity.

The attempt has been made to evaluate the present position of *P. argentipes* with regard to its gonotrophic cycle and age grading. The study is directly related to bionomics of *P. argentipes* and has never been conducted on such a longitudinal basis before to arrive at a specific and logical conclusion. This study on gonotrophic cycle of *P. argentipes* of India, therefore, is likely to pave the way for addressing effective control measures.

Objectives :

- To obtain the parous/nulliparous proportion as a parameter necessary for the estimation of sandfly longevity.
- To check the number of refeeding of gonotrophic cycles.
- To study the duration of the gonotrophic cycle.
- To establish the physiological age of female P.argentipes (number of batches of eggs laid by a given female sandfly).

Materials & methods

The study was conducted from a period of April 2002 to March 2003. In each month 100 wild caught female flies were dissected for examination. In all 1200 female P. argentipes throughout the study period were examined.

The sandflies were anaesthetized by a few drops of chloroform. Then the insect was held by one wing and the legs were removed one at a time and afterwards the other wing was pulled off. The fly was then returned to the slide and the remaining wing was cut off with the dissecting needle. In order to avoid contamination of the slide by scales caused by wings or part of leg or tiny bristles, these were removed before dissection. The sandfly was then placed on a dry slide and arranged in a more suitable position for dissection.

The method was applied for investigation of sand flies of all physiological stages, viz. unfed, freshly fed, late fed or even gravid females , all wild caught. The ovaries of unfed or freshly fed females with Christopher's stage II were extracted.

The procedure was as follows: (a) The anaesthetised sandfly was placed on a slide. (b) A drop of normal saline was added near the extremity of the abdomen. (c) One dissecting needle was inserted in the thoracic muscle and a small cut was made between the sixth and seventh sternite using the second needle. (d) The ovaries were extracted moving the second needle gently. When ovaries were in stage I – II they come out before malpighian tube and stomach were extracted. (e) The ovaries were separated by cutting the hindgut. (WHO Manual, 1975)

The nulliparous female flies were identified by observing the following aspects, presence of coiled tracheolar skin in the ovary in the Christopher's stage I and early and middle stage II in unfed or fed flies. (ii) Ovary in stage I or early stage II in unfed or fed flies. (iii) Unfertilized females with ovaries in stage I or early II and mid II. (iv) Absence of ovariolar sac or dilatation.

The Parous females were identified by (i) Uncoiled tracheolar skin in the ovary. (ii) Presence of ovariolar sac or dilatation. (iii) Presence of retained eggs. (iv) Ovaries in Christopher's stage II late and Malpighian tubes partially emptied or completely without granules of secretion.

In the ovaries with the ova at a more advanced stage than Christopher's stage III, observations were made for the presence of degenerated follicles by advanced technique.

This time the ovaries were dissected under a high power dissecting microscope in normal saline. The dissection of ovaries on normal saline was carried out as follows: - (i) The wall of the ovary was cut in several places. (ii) The ovarian sheath was detached leaving the ovariole free. (iii) Now the right needle was inserted in the follicle of the eggs and the stalk of the ovariole inserted in the calyx was extended as much as possible to see the number of dilatations or presence of sacs.

Application of the technique :

- **Physiological age :** By the application of the advanced age-grading technique, the physiological age of P.argentipes was established.
- **Calendar age :** The calculation of calendar age was worked out with the help of physiological age more accurately. The calendar age was calculated by multiplying the number of dilatations with the average number of days of the gonotrophic cycle.

Results

This study, as mentioned earlier, was carried out for a yearlong period, from April, 2002 to March, 2003, with wild caught flies captured from the central study village, Karia. In all 1200 wild caught adult *P.argentipes* were dissected for observation of parity status (100 in each month) for estimation of gonotrophic cycle. Out of 1200 *P.argentipes*, 263(21.92%) were nullipara (Fig.1), 465(38.75%) were primigravids (Fig. 2), 276(23%) were primipara (Fig. 3 and 4), 149 (12.42%) bipara (Fig. 5), 42 (3.5%) (Fig.6) tripara and 5(0.4%) were quadripara. Therefore, majority of *P.argentipes* captured in nature were primigravids. It was also clear that 472(39.3%) flies had the opportunity of laying eggs more than once. Availability of *P.argentipes* of greater life span in nature decreased steadily along with the attainment of higher parous individuals as 23%, 12.42%, 3.5% and 0.4% primipar, bipar, tripar and quadripar females respectively were found in nature (Table 1).

Going through the results seasonally, out of 400 females examined in the summer (March'03 and April, May, June' 02), 166 (41.5%) were primigravids, 82(20.5%) nullipars, 81(20.25%) one parous, 52(13%) bipara, 16(4%) tripara and 3(0.75%) were quadripara. Similarly, of 400 females examined in the rainy season (July to October'02), 138(34.5%) were primigravids,86 (21.5%) nullipara, 89 (22.5%) primipara, 65 (16.25%) bipara, 20(5%) tripara and 2(0.5%) quadripara. Dissection of 400 female *P.argentipes*

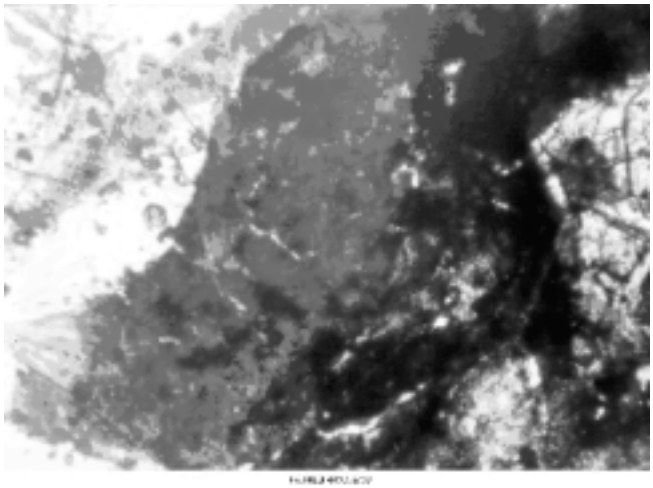

Fig. 1. Nulliparous eggs

Fig. 2. Primigravid eggs

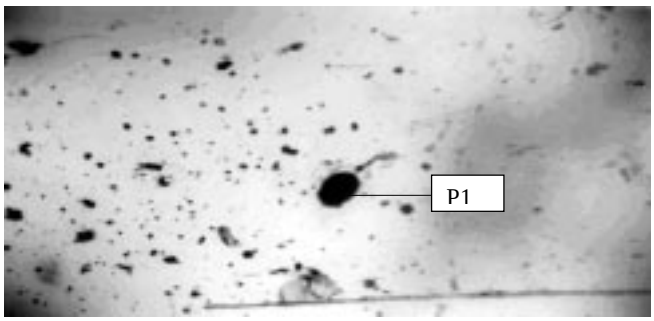

Fig. 3. Primipara (P_1) eggs (Early stage)

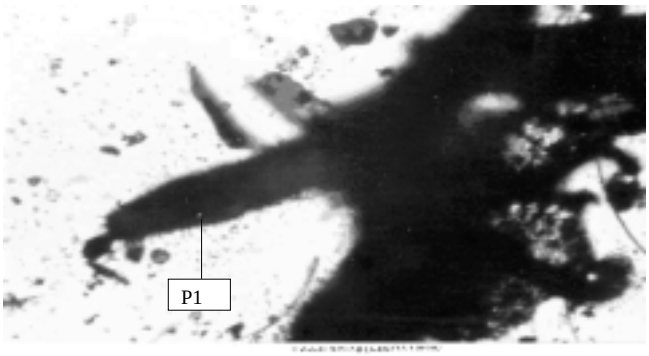

Fig. 4. Primipara (P_1) eggs (Late stage)

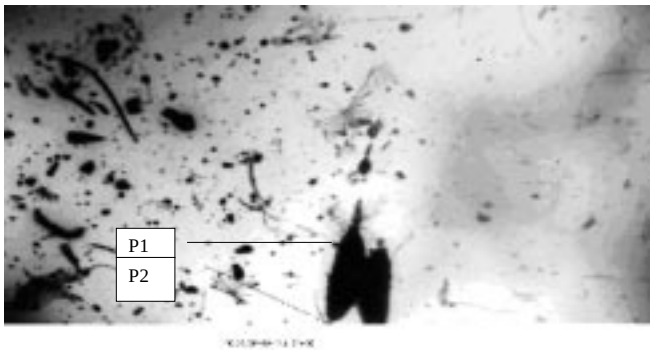

Fig. 5. Bipara (P_2) stage

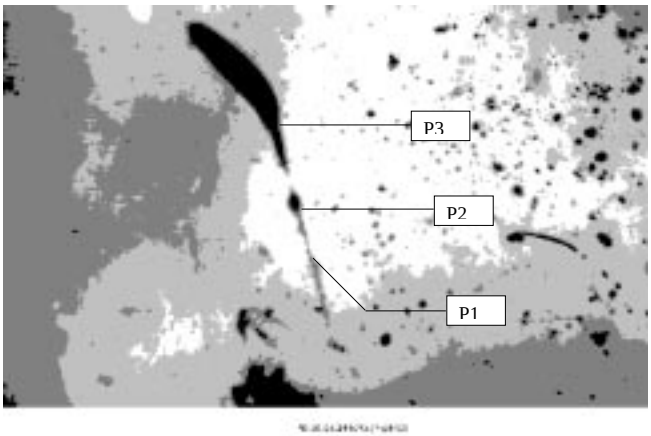

Fig. 6. Tripara (P_3) stage

in the winter (November 2002 to February 2003) showed that 161(40.25%) were primigravids, 95(23.75%) nullipara, 106 (26.5%) primipara, 32(8%) bipara and 6(1.5%) tripara. All these results are presented in Table 2.

Presumptive mortality rate :

Presumptive mortality rate was calculated following the method of Gillies and Wilkes (1965), as applied by them on mosquitoes, *Anopheles funestus* and *A.gambiae*. This rate from primiparous (P_1) to biparous (P_2) was 46.01, from biparous to triparous (P_3) 71.8, from triparous to quadriparous (P_4) was 88.1 (Table 3).

Table 1. Parity status of *P.argentipes*

Month	No. of *P.argentipes* examined	PARITY					
	P*	N	P_1	P_2	P_3	P_4	
April'02	100	45	26	14	11	3	1
May	100	48	21	16	10	5	-
June	100	50	20	10	17	2	1
July	100	40	22	17	14	6	1
August	100	27	25	23	16	9	-
September	100	32	21	26	18	2	1
October	100	39	18	23	17	3	-
November	100	48	15	28	7	2	-
December	100	53	17	24	6	-	-
January'03	100	40	46	7	5	2	-
February	100	20	17	47	14	2	-
March	100	23	15	41	14	6	1
Total	1200	465	263	276	149	42	5
%	100	38.75	21.92	23.0	12.42	3.5	0.4

*P= Primigravid, N= Nulliparous, P_1= Primiparous, P_2= Bipara, P_3=Tripara, P_4=Quadripara.

Table 2. Parity status of *Phlebotomus argentipes* in three seasons (number and percentage-wise)

PARITY	SEASON					
	Summer		Rainy		Winter	
	No.	%	No	%	No.	%
Primigravid	166	41.5	138	34.5	161	40.5
Nulliparous	82	20.5	86	21.5	95	23.75
Primiparous	81	20.25	89	22.25	106	26.5
Bipara	52	13.0	65	16.25	32	8.0
Tripara	16	4.0	20	5.0	6	1.5
Quadripara	3	0.75	2	0.5	-	-

Table. 3. Season wise and year wise mortality of *P.argentipes*

		PARITY						
SEASON		P	N	P_1	P_2	P_3	P_4	TOTAL
Summer	No.	166	82	81	52	16	3	400
	Presumptive mortality			1.2	35.8	69.2	81.25	
Rainy	No.	138	86	89	65	20	2	400
	Presumptive mortality				27.0	69.2	90.0	
Winter	No.	161	95	106	32	6	-	400
	Presumptive mortality				69.8	81.25		
TOTAL	No.	465	263	276	149	42	5	1200
	Presumptive mortality				46.01	71.8	88.1	

In the summer, mortality rate from nulliparous to primiparous was 1.2, from primiparous to biparous 35.8, from biparous to triparous 69.2 and from triparous to quadriparous 81.25. In the rainy season mortality rates from primiparous to biparous, biparous to triparous and triparous to quadriparous were 27, 69.2 and 90 respectively. In the winter again mortality rates from primiparous to biparous and biparous to triparous were 69.8 and 81.25 respectively.

Calendar age :

Calendar age was calculated by multiplying the number of dilatations of the overiolar stalks with the average number of days of the gonotrophic cycle.

In the present study quadriparous females were detected in nature, i.e. with 4 dilatations in their ovariolar stalks. Now it is known that in laboratory condition a fully engorged *P.argentipes* requires 5 days on an average in digesting the blood meal and subsequently laying eggs, there by requiring 5 days to complete a single gonotrophic cycle. Therby female *P.argentipes* can survive upto atleast 20 (twenty) days in nature.

Discussion

The study of the age composition of *P.argentipes* is one of the most important aspects in understanding transmission dynamics in any foci of visceral leishmaniasis in the Indian subcontinent.

In Indian sub-continent, knowledge about the gonotrophic cycle and age-gradation of medically important insects were almost rare and practically no direct attempt was made to understand the age-composition of vector species of sandflies in this area.

Studies on the age composition of *P.argentipes* with the application of advanced age- grading techniques, on such a longitudinal basis, was the first of its kind in India. The only two other preliminary studies for understanding the gonotrophic nature of *Phlebotomus argentipes* was studied in the two endemic states of Bihar and West Bengal by Palit et al. (1990) and Ghosh and Bhattacharya (1992) respectively.

Data analysis from the yearlong study carried out on wild caught *P.argentipes* from the central field station revealed that nulliparous (those which had not yet completed one ovarian cycle) and primigravids (gravid for the first time) constituted the major part of the population (60.7%) in the study area. But at the same time one revelation was that, they almost maintained a steady population throughout the year irrespective of seasonal effects {p= pr($\div^2$ >5.8111/d.f. =2)= 0.05502>0.01}**.** Therefore, a distinct indication was that, in a natural *P.argentipes* population younger females were always in abundance than the older ones {p= pr(Z> 7.39)=very small<< 0.01}.This result is similar to the observations of Scorza & Oviedo (1994) who in their attempt to trace physiological age in *Lutzomyia youngi* populations from an endemic area for cutaneous leishmaniasis, Venezuela, considered that these differences can be used for epidemiological studies as a means of estimating the physiological age of female populations.

The number and proportion of biparous and triparous females, would form the most interesting part, although a few quadriparous females were also detected in nature, since percent of females, which were on subsequent gonotrophic cycles are epidemilogically most important part of population capable of spreading infection (Dergacheva,1979).

Analysis of data revealed that the distribution of both bi- and triparous females in the rainy and summer seasons respectively were significantly higher than those in the winter (when Rainy vs. Winter is analysed, Z value = 4.607 and p <<0.01, whereas in Summer vs. Winter, Z value = 3.128 and p <<0.01), although the highest proportion was found in the rainy season (85 out of 400 i.e.21.25%), followed by the summer (68 out of 400 i.e.17%). This particular result ought to be looked into more carefully. According **to** Dolmatova (1965) vector population had the most epidemiological significance at the time when its density was still high enough and percent of parous females was already high enough and tended to go up. It was observed that percentage of biparous and triparous population of *P.argentipes* in the summer went further up in the rainy season covering more than $1/5^{th}$ of the population. There by it could be opined that the last few summer months (like May and June) and the rainy season (July to October) possibly might be the ideal transmission period for an infective population of *P.argentipes*. So the rainy months, with a higher proportion of biparous and triparous females in the natural population are of immense epidemiological significance for transmission of the disease as vector population with an average life span of 10/ 15 days will be available in nature and as per laboratory observation it is known that, it takes 7/8 days on an average for development of Leishmania in *P. argentipes* prior to its becoming ready for an infective bite/blood meal, thereby requiring a minimum of 2 blood meals. Our finding corroborates with that of Guilvard et al. (1980), that the end of the summer was the period of maximum risk for the transmission of leishmaniasis by *P. ariasi* in a particular focus in France.

Detection of a few quadriparous females in natural population of *P.argetipes* pointed out that they could lay eggs for a fourth time and undergoes four gonotrophic cycles and have a theoretical possibility of fifth blood meal in nature. Guilvard et al., (1980) and Killick-Kendirck & Rioux (2002) in their studies reported thaty in France, *P.ariasi* undergo at least three

gonotrophic cycles. Mukhopadhyay & Ghosh (1999) while studying vector potential of *Phlebotomus duboscqi* and *P. papatasi* observed that *P. duboscqi* females could complete up to eight gonotrophic cycles and in contrast, *P. papatasi* could only complete a maximum of four gonotrophic cycles.

Presumptive mortality rates of *P.argentipes* was calculated following the method applied on mosquitoes by Gillies and Wilkes (1965). It was revealed that mortality rate steadily increased among the female population along with the attainment of higher parity status (Table 3). So the number of gonotrophic cycle of *P.argentipes* would be inversely proportional with the availability of such population in nature. Precisely the older the population, the fewer would be its number in nature. The availability of a lesser proportion of higher parous females in the winter might be explained by the fact that mortality rate was highest during this season.

Rebollar-Tellez et al. (1996) in a similar observation on parity rate of new world sandfly, *Lutzomyia cruciata* in an endemic focus of localized cutaneous leishmaniasis in southern Mexico reported a survival rate per oviposition cycle of 0.68 from the least square regression of parous on total females.

As *P.argentipes* is, in general, gonotrophically concordant (i.e. their ovaries would develop in step with the digestion of a single blood meal and that they would not normally take another blood meal until after oviposition), their calendar age was estimated, which showed that they (quadripars) could live at least up to 20 days in nature. However, quadriparous females caught with a fifth blood-meal in their gut does not rule out the possibility of a few *P.argentipes* surviving up to 25 days in nature. Killick-Kendirck & Rioux (2002) reported that the length of the gonotrophic cycle of *P.ariasi* in nature appeared to be from 6 to 29 days. They viewed that the method appeared to give a clear indication of the number of times female flies had oviposited, and therefore, the number of times they had taken bloodmeals.

However, it could not always be assumed that in nature the cycles conformed to a completely regular pattern. Consequently, the life span of a particular female might be longer than what would be expected. Irrespective of these facts, since estimation of parity status is of utmost importance before implementing control measures against *P. argentipes* in a given focus, the outcome of this study highlighting parity status and life expectancy of a natural population in a given foci, the first of its kind in India, will be of immense help, prior to implementing cost effective, time specific and result oriented spraying operation.

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On a new Cestode *Circumoncobothrium prabhawatinesis* n.sp. (Cestoda : Ptychobothridae) from fresh water fish *Mastacembelus armatus* (Lacepede, 1800) from Parbhani district of Marathwada region, M.S., India

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Abstract : The present communication deals with the study of new species of cestode *Circumoncobothrium* collected from fresh water fish *Mastacembelus armatus* (Lacepede, 1800) from Ahmedpur Dist.Latur Marathwada region M.S., India. The worm under description has in shape the scolex is large in size, spindle shape and broader in the middle region The scolex bears two bothria, the bothria are not equal in size, one is longer and another is broader, The hooks are small in size, 37 in number rod shaped, the hooks are attached quadrant in the centre on both sides, neck is present, The testes are distributed ventro-lateral side of proglottid and 150 – 167 in number, medium in size, oval in shape The vitellaria are granular, distributed anterior to posterior margin of proglottid laterally, The eggs are small in size, oval in shape.

Key Words : *Cestode parasites, fresh water fish, Mastacembelus armatus (Lacepede, 1800) Circumoncobothrium prabhawatinesis n. sp.*

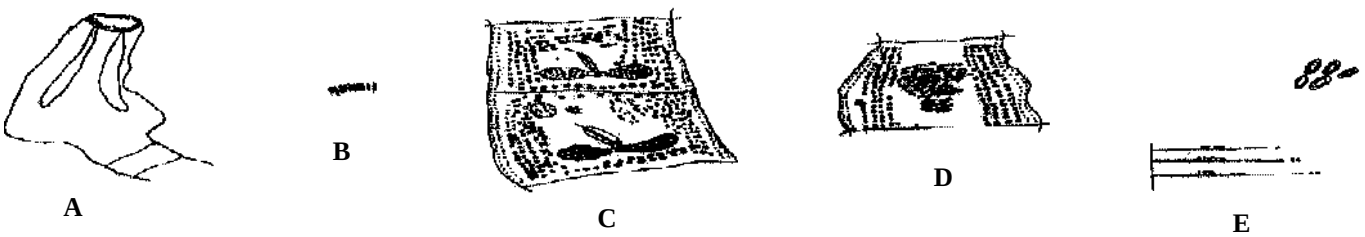
Introduction

Shinde G.B., in 1968 errected the genus *Circumoncobothrium* from the intestine of freshwater fish *Ophiocephalus leucopunctuatus*, as a type species *C.ophiocephali* Chincholikar, 1976 described two new species of the genus as *C. shindei* from fresh water fish, *Mastacembelus armatus* and *C. bagarius* from *Bagarius bagarius*.

In 1977, Shinde added a new species *C.khami* from *Ophiocephalus striataus.* Later on Jadhav and Shinde, 1976 added two new species, under the genus viz.*C.aurangabadnesis* and *C.raoii* from *Mastacembelus armatus* Jadhav and Shinde 1980 described *C.gachuai* from *Ophiocephalus gachua*. Jadhav et.al 1990 described *C.yamaguti* form *Mastacembelus armatus*. Later on Shinde et.al. 1994 added *C.alli* from *Mastacembelus armatus*. Patil et.al 1998, described *C.vadgonesis* from *Mastacembelus armatus*. Jadhav described new species.*C.wangswad* in.1998.Patil and Jadhav also added a new species.*C.vitellariensis* collected from *Mastacembelus armatus* at Varpade Tq.Shindkheda Dist.Dhule in 1999.Tat and Jadhav added by two new species. *C.baimaii* and *C.manjari* from *Mastacembelus armatus* in 2004.

Materials and Methods

Twelve cestode parasites were collected from the *Mastacembelus armatus* (Lacepede, 1800) in the month of January – 2005.They were preserved in 4% formalin stained with Greanchers Alcoholic Borax Carmine, dehydrated through alcoholic grades and mounted in DPX (Yamaguti, 1959 & Wardle et al.,1974) the figures are drawn with aid of camera lucida. All measurements are in millimeters.



Circumoncobothrium prabhawatinesis N.Sp

A. Scolex B. Hooks C. Mature Segment D. Gravid Segment E. Egg

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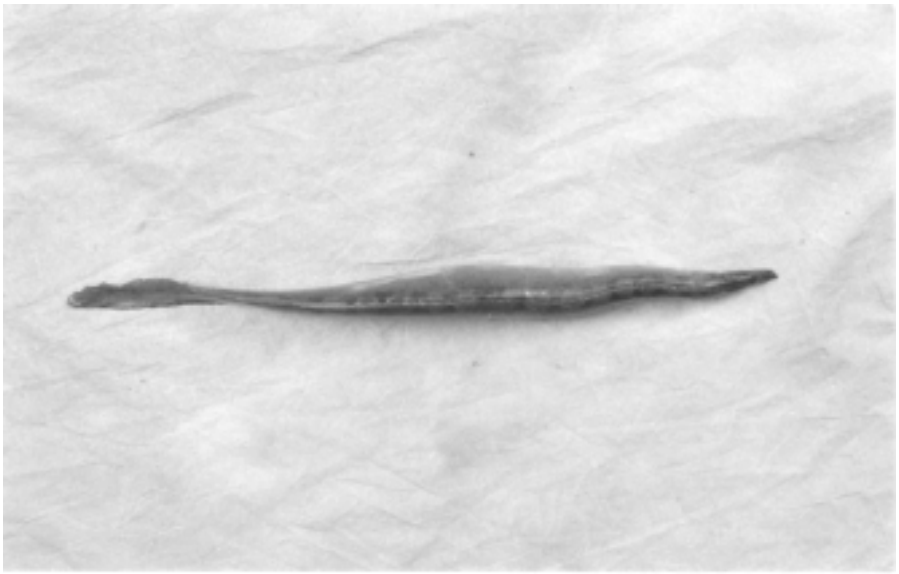
***Mastacembelus armatus* (Lacepede, 1800)**



Description : (Fig. I – A, B, C, D, & E)

On closer observation it is seen that, the worm hashe scolex large in size, spindle shape and broader in the middle region. and measure 1.2135 – 1.5291 in length and 0.3106 – 0.7038 breadth. The scolex bears two bothria, the bothria are not equal in size, one is longer and another is broader, which are attached to the rostellum and measure 0.7038 – 0.7281 in length and 0.0242 – 0.0679 breadth. The rostellum is armed, medium in size, oval in shape. It is present at the apex of scolex region and measure 0.2912 – 0.3155 in length and 0.0776 – 0.1262 breadth.

The hooks are small in size, 37 in number rod shaped, the hooks are attached quadrant in the centre on both sides and measure 0.0128 – 0.6796 in length and 0.0021 – 0.0194 breadth.Neck is present, measure 0.2272 – 0.2184 in length and 0.4924 breadth.

The mature proglottids are large in size and measure 1.1287 – 0.8834 in length and 1.4015 – 0.9466 breadth. The testes are distributed ventro-lateral side of proglottid and 150 – 167 in number, medium in size, oval in shape and measure 0.0530 – 0.0166 in length and 0.0227 – 0.0053 breadth.

The cirrus pouch is medium size; spindle shaped and measures 0.3409 – 0.2669 in length and 0.0530 – 0.0728 breadth. The cirrus is thin straight and measure 0.3409 – 0.2427 in length and 0.0681 – 0.0303 breadth.

The vas deferens is thin, straight and measures 0.0530 – 0.0681 in length and 0.0075 – 0.0227 in length and 0.0075 – 0.0227 in breadth.

The genital pores are medium in size, oval is shape and measures 0.0757 – 0.0909 in length and 0.0227 – 0.0378 in breadth. The ovary is medium in size, oval is shape and each lobe is equal in size and its measure 0.3787 – 0.3939 in length and 0.0681 – 0.1893 breadth. In the ovarian lobes the isthmus is present, which measures 0.0606 – 0.0833 in length and 0.0303 0.0454 breadth.

The vagina is a short tube, thin and it runs posterior region to the ootype and measure 0.0378 – 0.0530 in length and 0.0151 – 0.0227 in breadth. The ootype is small in size, oval in shape and measure 0.0378 – 0.0530 breadth.

The vitellaria are granular, distributed anterior to posterior margin of proglottid laterally and measure about 0.0606 in diameter and are in 3-4 rows.

The uterus is oval in shape and measure 0.2384 – 0.2651 in length and 0.0984 – 0.1742 in breadth. The gravid proglottids are broader and one end is slight curved and measure 1.2121 – 1.25 in length and 0.8333 – 0.02878 in breadth.

The eggs are small in size, oval in shape and measure 0.0149 – 0.1363 in length and 0.0042 – 0.0454 in breadth.

Discussion

The genus *Circumoncobothrium* was errected by Shinde in 1968 as a type species *C. ophiocephali* Later on following species are added to this genus.

1) *C. aurangabadnesis*, Jadhav & Shinde, 1976

2) *C. raoii,* Shinde & Jadhav 1976
3) *C. shindei,* Shinde and Chincholikar 1976
4) *C. bagarius,* Chincholikar and Shinde, 1976
5) *C. khami,* Shinde 1976
6) *C. gachuai,* Jadhav and Shinde 1980
7) *C. yamaguti,* Jadhav et. al. 1994
8) *C. alii,* Shinde et. al. 1994
9) *C. vadgaonesis,* Patil et.al. 1998
10) *C. baimaii,* Wangsawad and Jadhav 1998
11) *C. manjari,*Tat & Jadhav, 2004
12) *C. vitellariensis,* Supugade et.al. 2005

The present cestode differs from *C. ophiocephali*, Shinde in 1968, which is having scolex (Large spindle shape vs. distinct),hooks (37 vs.80), testes (150-167 vs.70-80) in number, ovary (Oval vs. round) in shape, vitellaria (Granular vs. follicular).

The present cestode differs from *C. aurangabadnesis*, Jadhav & Shinde, 1976, which is having scolex (Large spindle shape vs. broad), hooks (37 vs.42), testes (150-167 vs.135-145) in number, ovary (Oval vs. bilobed) in shape.

The present cestode differs from *C. raoii,* Shinde & Jadhav 1976, which is having scolex (Large spindle shape vs. broad), hooks (37 vs.46), testes (150-167 vs.260-275) in number, ovary (Oval vs. bilobed) in shape.

The present cestode differs from *C. bagarius,* Chincholikar and Shinde, 1976,which is having scolex (Large spindle shape vs. narrow), hooks (37 vs.55), neck (Present vs. absent), testes (150-167 vs.275-285), in number, ovary (Oval vs. bilobed) in shape, vitellaria (Granular vs. follicular).

The present cestode differs from *C. khami,* Shinde 1976, which is having scolex (Large spindle shape vs. cylindrical), hooks (37 vs.48), neck (Present vs. absent), testes (150-167 vs.199-200), in number, ovary (Oval vs. bilobed) in shape, vitellaria (Granular vs. follicular).

The present cestode differs *C. gachuai,* Jadhav and Shinde 1980, which is having scolex (Large spindle shape vs. pear shaped), hooks (37vs.46, testes (150-167 vs.375-400), in number, ovary (Oval vs. bilobed) in shape, vitellaria (Granular vs. follicular).

The present cestode differs *C. yamaguti,* Jadhav et al. 1994, which is having scolex (Large spindle shape vs. distinct), hooks (37 vs.56), testes (150-167 vs.130-150), in number, ovary (Oval vs. bilobed) in shape.

The present cestode differs *C. alii,* Shinde et al. 1994, which is having scolex (Large spindle shape vs. triangular), hooks (37 vs.34), testes (150-167 vs.230-240), in number, ovary (Oval vs. distinctly bilobed) in shape.

The present cestode differs *C. vadgaonesis,* Patil et al. 1998, which is having scolex (Large spindle shape vs. broad), hooks (37 vs.56), testes (150-167 vs.490-510), in number, ovary (Oval vs. bilobed) in shape, vitellaria (Granular vs. follicular).

The present cestode differs *C. baimaii,* Wangsawad and Jadhav 1998, which is having scolex (Large spindle shape vs. pear shaped), hooks (37 vs.46-48), testes (150-167 vs.88-100), in number, ovary (Oval vs. compact) in shape.

The present cestode differs *C. manjari,* Tat & Jadhav, 2004, which is having scolex (Large spindle shape vs. triangular), hooks (37 vs.48), testes (150-167 vs.128-145), in number, ovary (Oval vs. bilobed) in shape, vitellaria (Granular vs. follicular)

The present cestode differs *C. vitellariensis,* Supugade et al. 2005, which is having scolex (Large spindle shape vs. triangular), hooks (37 vs.46-48), neck (Present vs. absent), testes (150-167 vs.250-260), in number, ovary (Oval vs. distinctly bilobed) in shape, vitellaria (Granular vs. follicular).

The worm under discussion *Circumoncobothrium prabhawatinesis* n.sp is differing from all other known species of the genus *Circumoncobothrium* Shinde G.B.,1968 The detailed differences are shown in comparative chart. These characters when compared with already known species of genus it is found that these worm has many different differentiating characters which are emerge to accommodate these worm as a new species hence name *Circumoncobothrium prabhawatinesis* n.sp given in the honour of Mother of author, Mr. Munde Anil Vishvanath.

Type Species	*Circumoncobothrium prabhawatinesis* n.sp.
Host	*Mastacembelus armatus* (Lacepede, 1800)
Habitat	Intestine
Locality	Ahmedpur Dist. Latur M.S. India
Date of collection	05th March 2004.

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Epidemiological studies on pathogenic disease malignant tertian malaria in Jodhpur (Rajasthan)

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Abstract : *Plasmodium falciparum*, the pathogenic parasitic continues to be responsible for millions of death per year and the disease caused by it is known as malignant tertian malaria. Thus epidemiological studies have been carried out during March 2008 to June 2008 in summer season to present the epidemiological status and to recognize the risk factors. The desert type climate is prevailing in Jodhpur. A total of 7311 patients (4642 males and 2669 females) were examined in summer season.. About 45 (0.62%) patients were found to be infected with falciparum malaria. The infection percentage was 0.62% and males were more infected than females. Prevalence of parasitemia of *P. falciparum* was observed in 21-30 years of age-group. Heterogeneous parasite species (*P. falciparum* and *P.vivax*) co-exists. Degree of infection were mild 37.78%, moderate 37.78% and no high infection were found. Trophozoite stage of *P. falciparum* were 60%. Trophozoite with gametocyte stage of *P. falciparum* were 15.56%. The gametocyte stage of *P. falciparum* were not found in males and females. The diagnostic test performed widely were the smear test. Fever, headache, bodyache, abdominal pain, loss of apetite, nausea, vomiting were the common symptoms. Common drugs used were chloroquine-phosphate, quinine-sulphate, artesunate, sulphadoxine-pyrimethamine, artemether- lumefantrine, sulphadoxine-pyrimethamine and paracetamol.

Key words **:** *climate, drugs, epidemiology, Jodhpur, P. falciparum.*

Introduction

P. falciparum, the parasitic protozoan continues to be responsible for millions of death per year. It causes malignant tertian malaria. The disease could be fatal if it is not properly diagnosed and treated in time. Thus, epidemiological studies have been carried out to present the current epidemiological status and to recognize the risk factors. So, the methodologies involved are examination of parasite in the host with respect to age and sex, variation of infection in season, areas of incidence, educational status, drugs given, degree of infection have been taken into consideration.

Materials and methods

The age, sex, locality, educational status, drugs given, body temperature and other related symptoms of the patients were recorded. The haemoglobin level, T.L.C., D.L.C., E.S.R., R.B.C. count and platelet count were recorded. The parasitic examination was done either by preparation of thick and thin film with blood collected from finger prick or by Quantitative Buffy Coat Test (Q.B.C.) or by Rapid diagnostic tests.

Results

The infection percentage was 0.62% (males 0.33% and females 0.29%). The infection increases during the month of May. Males were more infected than females (Table-1). The young adults of 21-30 years of age-group represents the high risk group. Prevalence was 0.80% for 0-10 years children (Table-2). Distribution of Initial infection of *P. falciparum* malaria was 57.78% in males and 35.56% in females. No male had reinfection but 06.66% in females had reinfection. Degree of infection were mild 37.78%, moderate 37.78% and no high infection were found during summer season. Trophozoite stage of *P. falciparum* were 26.66% in males and 33.33% in females. Trophozoite with gametocyte stage of *P. falciparum* were 06.66% in males and 08.88% in females. The gametocyte stage of *P. falciparum* were not found in males and females (Table-3). The diagnostic test performed widely were the slide test. The average maximum temperature was 36.92°C and average minimum temperature was 24.2°C. The average maximum relative humidity was 51.25% and average minimum humidity was 26.25%. The average rainfall was 60.92%, average windspeed was 06.82Km/hr and average sunshine was 262.65 hours was observed during the season (Table-4). Total 45 patients were having uncomplicated falciparum malaria and no complicated falciparum malaria was found in this season. The symptoms observed as fever (95.55%), headache (64.44%), abdominal pain (46.66%), loss of apetite (77.77%) and bodyache (60%). Common drugs used were chloroquine-phosphate, quinine-sulphate, artesunate, sulphadoxine-pyrimethamine, artemether-lumefantrine, sulphadoxine-pyrimethamine and paracetamol.

Table No I : Shows total number of host examined and total number of host infected in summer season.

Month	Number of host examined			Number of host infected		
	Male	Female	Total	Male	Female	Total
March	1242	837	2079	03(0.14%)	02(0.09%)	05(0.24%)
April	930	586	1516	07(0.46%)	07(0.46%)	14(0.92%)
May	1119	583	1702	10(0.59%)	06(0.35%)	16(0.94%)
June	1351	663	2014	04(0.20%)	06(0.30%)	10(0.50%)
Total	**4642**	**2669**	**7311**	**24(0.33%)**	**21(0.29%)**	**45(0.62%)**

Table No II : Shows total number of host examined and total number of host infected according to their sex, age group and their percentage of infection in summer season.

Age-group	Number of host examined			Number of host infected			Percentage of infection		
	Male	Female	Total	Male	Female	Total	Male	Female	Total
0-10	842	407	1249	4	6	10	0.48%	1.47%	0.80%
11-20	1109	614	1723	4	6	10	0.36%	0.98%	0.58%
21-30	1159	727	1886	9	5	14	0.78%	0.69%	0.74%
31-40	615	386	1001	4	1	5	0.65%	0.26%	0.50%
41-50	336	211	547	1	2	3	0.30%	0.95%	0.55%
51-60	233	131	364	1	1	2	0.42%	0.76%	0.55%
61-70	182	104	286	-	-	-	-	-	-
71-80	84	51	135	1	-	1	1.19%	-	0.74%
81-90	60	26	86	-	-	-	-	-	-
91-Above	22	12	34	-	-	-	-	-	-
Total	**4642**	**2669**	**7311**	**24**	**21**	**45**	**0.52%**	**0.79%**	**0.62%**

Table No III : Shows the percentage of infection according to areas of incidence, educational status, travelers, mixed infection, occasional cases and stages of infection having *Plasmodium falciparum* in summer season.

Sl. No.	Status	Male	Female	Total
1	Mixed infection	03(06.66%)	03(06.66%)	06(13.33%)
2	Initial infection	26(57.78%)	16(35.56%)	42(93.33%)
3	Reinfection	-	03(06.66%)	03(06.66%)
4	Trophozoite stage	12(26.66%)	15(33.33%)	27(60.00%)
5	Gametocyte stage	-	-	-
6	Trophozoite with gametocyte stage	03(06.66%)	04(08.88%)	07(15.56%)
7	Mild infection	09(20%)	08(17.78%)	17(37.78%)
8	Moderate infection	06(13.33%)	11(24.44%)	17(37.78%)
9	High infection	-	-	-

Table No IV : Shows monthly average climatic conditions in various seasons in Jodhpur, (Raj.) during 2007 to 2008.

Month	Temperature (°C)		Relative Humidity (%)		Rainfall (mm)	Wind Speed (Km/Hrs)	Sunshine (Hours)
	Max. (°C)	Min (°C)	Max. (%)	Min. (%)			
November	33.6	15.1	42	31	-	1.5	276.6
December	25.5	11.6	56	33	-	3.2	254.9
January	23.3	9.8	48	24	-	4.4	222.2
February	26.5	9.5	44	16	-	3.2	267.2
Average	**27.22**	**11.5**	**47.5**	**26**	**-**	**3.07**	**255.22**
March	35.7	19.4	36	14	1.9	3.7	268.5
April	37.8	23.1	38	17	17.9	4.6	299.5
May	37	26.2	64	29	80.4	10.5	278.7
June	37.2	28.1	67	45	143.5	8.5	203.9
Average	**36.92**	**24.2**	**51.25**	**26.25**	**60.92**	**6.82**	**262.65**
July	35.6	27.4	72	50	66.4	8.1	213
August	32.4	25.7	81	64	212.1	5.8	183.4
September	35.5	25.5	73	47	24.9	5.2	252.9
October	37	22.8	49	28	-	2.5	301
Average	**35.12**	**25.35**	**68.75**	**47.25**	**75.85**	**5.4**	**237.57**

Discussion

Sharma *et al*. (2004) showed that parasite prevalence was very low during dry summer season. According to John *et al*. (2005) in areas of highly seasonal *P. falciparum* transmission, the presence of a large reservoir of persistantly infected but asymptomatic individual in the dry season leads to predictable increase in the incidence of clinical malaria in the rainy season. Syafruddin *et al.* (2009) reported that in the wet season *P. falciparum* accounted for 70% of infections; in the dry season *P. falciparum* and *P. vivax* were present in equal proportion. The present epidemiological study shows that falciparum malaria was present throughout the year but seasonal peak of transmission occurred in September during monsoon season and minimum incidence was observed in March in summer season. Males were more susceptible to the disease than females. Sharma *et al*. (2004) concluded all age-groups were affected but the risk of getting clinical attack was twice as high in 5-20 years old than in adults more than 30 years old. Present study concluded that 21-30 years of age-group were more susceptible to the disease. According to Yasinzai *et al*. (2008) the prevalence rate of 52.87% of *P. falciparum* posses a significant health hazard because not only *P. falciparum* infection but infection with *P. vivax* 47.12% may also lead to serious complications. Observations made so far indicated in the present study that mixed species infection of *P. falciparum* with *P. vivax* existed in the desert ecosystem. The co-existence of other *Plasmodium* species with *P. falciparum* were not found. Mixed species infection of *P. falciparum* with *P. vivax* were maximum in the monsoon season (23.86%) and minimum in summer season (6.66%) in males. Adhikari (2000) confirmed that reinfection occurred in a patient at any time after two weeks of the first attack. This may be due to persistant source of infection such as an asymptomatic carrier or persistent malaria in the neighbourhood or household and breeding centres for mosquitoes. Our study shows that in case of females reinfection were highest 6.66% in summer season. Prevalence of initial infection were more than reinfection. However Swarthout *et al*. (2007) concluded the results that though the use of paracheck-*P. falciparum* is a

sensitive as microscopy in detecting true malaria cases a low specificity did present a high frequency of false positive RDT results. But Rakotonirina *et al*. (2008) confirmed that the degree was very high for microscopy (0.99%) and HRP-II based test (0.93%) and high for pLDH based test (0.82%). Gerstl *et al*. (2010) advised that both RDTs were highly sensitive, met WHO standards for the detection of falciparum malaria monoinfections. Our study shows that in summer season smear test were 28.89% applied in males and 17.78% in females. In summer season ICT card test were 15.56% applied in males and 4.44% in females. In summer season Q.B.C. test were 15.56% applied in males and 13.33% in females. In summer season strip test were 4.44% strip test was applied in males. Bousema *et al*. (2004) reported that gametocytes was common in children below five years act as an infection reservoir. In other study Drakeley *et al*. (2006) indicated that the gametocytes that propagate the disease are often neglected. Our study shows that in summer season trophozoite with gametocyte were 6.66% in males and 8.88% in females in females. No gametocytes were observed in summer season in males and females. Bhattacharya *et al*. (2006) concluded that the average relative humidity 55-80% and average temperature 15-30°C remain conductive to malaria transmission. Ye *et al*. (2007) suggested that systematic measurement of local temperature through ground stations and integrations of such data in the routine health information systems could support assessment of malaria transmission risk at the district level for well-targeted control efforts. Our observation shows that the minimum prevalence was observed in summer season when the average maximum temperature was 36.92°C and average minimum temperature was 24.2°C. The average maximum relative humidity was 51.25% and average minimum relative humidity was 26.25%, the average rainfall was 60.92mm, average windspeed was 6.82 km/hr. and average sunshine was 262.65 hours. Reyburn et al. (2009) determined that oral quinine could be used to treat uncomplicated malaria when ACT are not available. Kinzer *et al*. (2010) showed that chloroquine / sulphadoxine-pyrimethamine combination therapy for *P. falciparum* to be highly efficacious. In the present study it was observed that for the treatment of uncomplicated falciparum malaria chloroquine-phosphate, quinine-sulphate,artesunate, sulphadoxine-pyrimethamine, artemether-lumefantrine, paracetamol primaquine etc. were given.

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Effect of homeopathic medicine chelidonium against blood stage infection of rodent malaria parasite

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Abstract : In spite of the global malaria eradication programs, new initiative which aims to halve the burden of malaria that targets to halt the rising incidence of drug resistance is ardently needed. Drug resistant malaria has become a major problem in malaria control. *In vivo* resistance has been reported against almost all antimalarial drugs including artemisinin and its derivatives. Homeopathy is a holistic system of medicine which has been recommended by WHO to ensure adequate global healthcare. Homeopathy intervenes at the level of person's reactive, self curative powers with a goal to bring about a change in the total functioning of the body. Homeopathic drugs have been claimed for their efficacy, no side effects and low cost moreover, they are already used by humans. Thus they could be evaluated for their suitable candidature as potent antimalarials. In the present study the antiplasmodial efficacy of chelidonium mother tincture (□) and its different potencies (6, 30 and 200) was checked against *P. berghei* infection in Balb/C mice. *Chelidonium majus* (chelidonium) is commonly known as the greater celandine or tetterwort. The effect of the fresh herb is of a mild analgesic, antimicrobial, oncostatic and central nervous system sedative. Chelidonium is used mainly for liver conditions (Hepatitis - an enlarged liver, with pain extending to the back and right shoulder blade, and jaundiced, yellow skin which are some of the main pathological conditions in malaria. Six groups of mice having 6 mice each were administered orally with 0.2ml/day/mouse of chelidonium □ and different potencies were administered orally. On day 5 lowest parasitaemia (8.8±4.5%) was observed in mice treated with 30 potency followed by 6, □ and 200 as compared to infected control (26.35±1.9%). The mice were kept for follow up study up to 30 days. Mean survival time of group treated with 30 potency (G5) was maximum (20.5±6.25 days) followed by G3 (19.6±2.63 days), G4 (17.8±2.04 days) and then G6 (16.5±2.25 days).

Keywords **:** *Chelidonium. Homeopathy. Parasitaemia. Plasmodium berghei.*

Introduction

Multidrug resistant parasites are the biggest therapeutic challenge to health care in most malaria-endemic areas (Snow et al. 2001). Drug-resistant malarial parasites present even greater challenges to world health. The WHO has recommended replacement of mono-therapy of antimalarials by synthetic chemical derivatives of *Artemisia annua* in combination with other antimalarials (ACT) (WHO, 2005). We can expect that it won't take long for drug resistance to bed devil ACT. New antimalarial regimens are urgently needed, aiming to cure patients no longer responding to standard therapies.

Homeopathy has been cited by the World Health Organization as one of the systems of traditional medicine that should be integrated with conventional medicine to ensure adequate global healthcare (WHO, 2002). The homeopathic approach may be useful specifically because it does not focus on the cause of the disease but on the teleonomy of the patient's reaction. It is therefore not being considered an alternative approach, but complementary to effective drug use (Bellavite et al. 2007). Thus they could be evaluated for their suitable candidature as potent antimalarials.

In a previous study in our laboratory, the combination of artesunate (100mg/kg) and a homeopathic drug eucalyptus mother tincture (□) has been reported to provide a mutual protection against the blood stage infection of *P. berghei* infected Balb/C mice. The combination also protected the recrudescence up to one month follow up period (Bagai et al. 2008). In another study it was confirmed that 7 days oral administration of homeopathic medicine china (□) and china 30 potency in monotherapy, leads to inhibition of parasite load in *P. berghei* infected Balb/C mice up to a large extent with 50% survival rate of mice till 28th day. Both showed high efficacy when given in combination with artesunate 100mg/kg (Rajan and Bagai, 2009). The present study has been designed to evaluate the antiplasmodial efficacy of (□) and different potencies of homeopathic medicine chelidonium against blood stage infection of *P. berghei* in Balb/C mice.

Chelidonium majus extracts are used mainly in the therapy of biliary and hepatic dysfunctions and it protects hepatotoxicity and shows an established therapeutic safety. They are in accordance with available toxicity data for oral application, which show no hepatotoxic effects and give no indication for extended pharmacovigilance risk limitation (Adler

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et al. 2006). Homeopathic formulation of *Chelidonium* (3X) is also recommended for annually recurring type of malaria (Partington, 2006) but there is no experimental/scientific studies which can support for their efficacy and safety.

Materials and methods

Mouse and parasite strain

White swiss mice *Mus musculus* of Balb/C strain (weighing 22-35g and 4-6 weeks old) of either sex, obtained from the Central animal house, Panjab University, Chandigarh were used as experimental model. They were maintained on a standard pellet diet and water *ad libitum*. Strain of *P. berghei* (NK- 65) was maintained by intraperitoneal inoculation of $1x10^6$ infected RBCs to naïve mice. Parasitaemia was checked by preparing Giemsa stained thin blood smears on glass slides through tail vein incision of infected mice (Santiyanont, 1985)**.**

Ethical Clearance

The treatment of mice was according to the guidelines of committee for the purpose of control and supervision on experiments on animals (Reg No. 45/1999/CPCSEA), Panjab University, Chandigarh.

Drug

Homeopathic mother tincture (□) and different potencies (6, 30 and 200) of chelidonium manufactured by Dr. Reckeweg and Co. GmbH D.64625, Bensheim, Germany, were used in the present study. The medicines were diluted in distilled water (1:2). In homeopathy, potency is measure of dilution of a particular substance. A solution that is more dilute is described as having higher potency and is described by homeopaths to be a stronger and deep acting remedy. Mother tincture (□) of any homeopathic formulation consists of 1 part of drug in 9 parts of nascent alcohol.

Experimental Design

Six groups having 6-8 mice (same sex and age) were used for present study. Groups were designated as G1 to G6 (Table Is). All groups were injected with $1x10^6$ *P. berghei* parasitized red blood cells on D0. Oral dose of various medicines/vehicles (0.2ml/mouse/day) was administered to mice of different groups for 4 days (D0-D3) 1 hour post inoculation. Parasitaemia was checked on day 5 (D4) and mice were kept under observation for follow up studies up to day 28. Smears were prepared weekly i.e. on day 7, day 14, day 21 and day 28. The survival rate of mice in each group was recorded.

Statistical analysis

Data has been presented as mean and standard deviation (SD). Statistical evaluation of differences between the experimental groups was determined by the Student's t-test with the level of significance of $p<0.05$ using GraphPad Software (San Diego, California, USA).

Results

Course of parasitaemia in experimental groups

In infected control (G1) $37.6\pm3.4\%$ infection was observed on day 7 post inoculation after which all the mice died due to heavy infection. In G2 (nascent alcohol) the parasitaemia was observed to be $35.1\pm10.9\%$ on day 5 and all the mice died before day 7 confirming that nascent alcohol in homeopathic drugs doesn't play any role in clearing *P. berghei* infection.

Table I : Dose regimen for various study groups

GROUP	**DRUG** diluted in distilled water (1:2)	**DOSE** OD (D0-D3)
G1(Infected Control)	Diatilled Water	0.2ml/day/mouse
G2 (Placebo Control)	Nascent alcohol	0.2ml/day/mouse
G3	Chelidonium □	0.2ml/day/mouse
G4	Chelidonium 6	0.2ml/day/mouse
G5	Chelidonium 30	0.2ml/day/mouse
G6	Chelidonium 200	0.2ml/day/mouse

Note : All the mice were injected with $1X10^6$ infected RBC on D0

In G3, the infection was observed to decline after cessation of drug regimen up to day 7 ($9.53\pm5.9\%$) but only one mouse survived till day 21. The mouse was highly anaemic and only few infected reticulocytes were observed in the blood smear

of surviving mouse. In G4 and G6 also only one mouse survived with 8.1% and 6.2% parasitaemia respectively on day 21, after which mice died. In G5 lowest parasitaemia (8.8±4.5%) was observed on day 5 which decreased to 6.2±0.2% on day 28 with 30% survival of mice which was extremely statistically significant when compared to other drug treated and control groups (Fig 1).

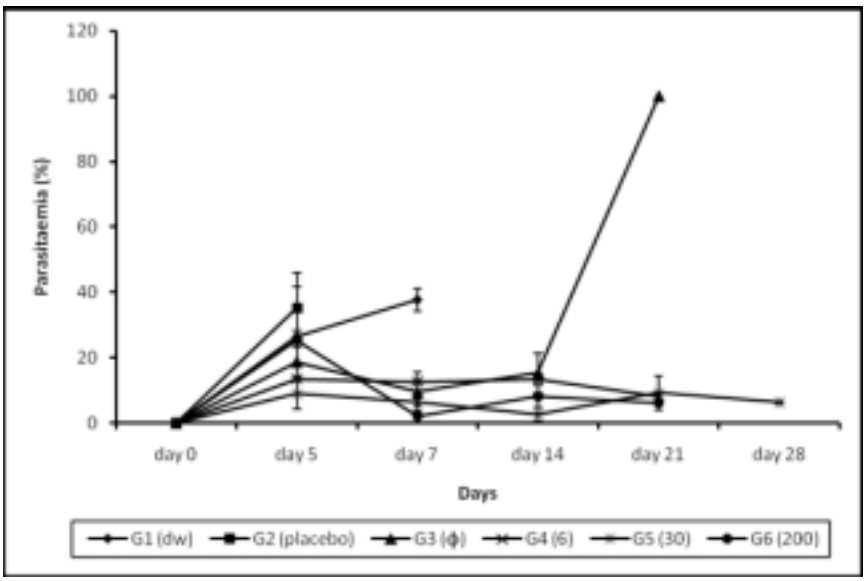

Fig 1 : Graph showing course of parasitaemia in groups treated with chelidonium mother tincture and different potencies including infected control and placebo control on various days of study. Data is in the form of Mean±SD. $p<0.05$ (*considered statistically significant). n is the number of live mice in the group.

Mean survival time

The mean survival time of experimental groups were recorded up to one month follow up period. As all the mice of infected control (G1) and placebo control groups (G2) died within 7 days. The mean survival time was observed to be 6.75±0.5 days and 5±0 days respectively. Among the drug treated groups the lowest mean survival time was recorded in G6 (16.5±2.25 days) followed by G4 (17.8±2.04 days) and G3 (19.16±2.63 days) whereas, maximum mean survival time was observed in G5 (chelidonium 30) i.e. 20.5±6.25 days (Fig 2).

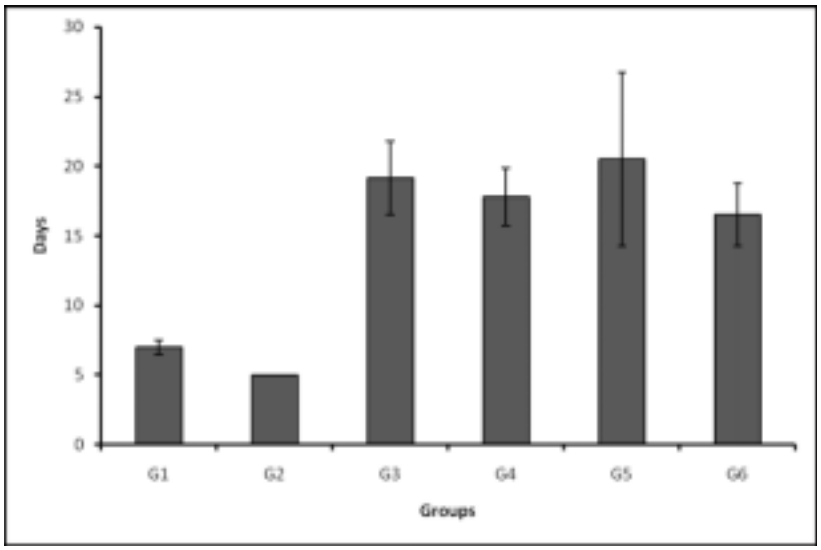

Fig 2 : Histogram showing mean survival time of mice (G1-G6) treated with chelidonium mother tincture and different potencies including infected control and placebo control. Data is in the form of Mean±SD.

Discussion

All the mice when injected with $1X10^6$ *P. berghei* infected red blood cells, died by day 7 post inoculation, because of heavy infection except those in which parasite migrated to reticulocytes. The phenomenon of reticulocytosis in *P. berghei* has been reported earlier too (Miller and Carter, 1976). In the present study also reticulocytosis was observed in G3 (Chelidonium □).

From the present study, it can be concluded that nascent alcohol in homeopathic drugs doesn't play any role in clearing *P. berghei* infection. The infection was observed to increase continuously in vehicle treated group (G2) showing that there is no placebo effect as a result of which all the mice died within 7 days due to heavy infection. A placebo is an inert substance (eg. Sugar pill, saline injection, ethanol) or sham physical/electrical manipulation that is believed to have no chemical, electrical, or physical effect on the patient (Arnstein, 2003). WHO report, also states most of the studies published in the last 40 years have shown homeopathic remedies to be superior to placebo and 'equivalent to conventional medicines in the treatment of illness, in both humans and experimental animals (www.guardian.co.uk/medicine/story/0,11381,1556831,00). For homeopathic research mice (*Mus musculus*) have been used as a model in relation to cytotoxicity, genotoxicity and carcinogenesis as they share a high degree of homology with humans (Khuda-Bukhsh, 2009). Salazar et al. (2006) also demonstrated the effect of homeopathic medicines *Eupatorium perfoliatum* 30CH and *Arsenicum album* 30CH on *Plasmodium berghei* infected mice. In our laboratory also, the effect of homeopathic medicines eucalyptus □ has been checked against rodent malaria parasite in Balb/C mice (Bagai et al. 2008).

The present study also confirms that the homeopathic medicines which are diluted beyond Avogadro's number, show considerable effect on mice model. It was confirmed that homeopathic formulation of *Chelidonium majus* possesses antimalarial potential. Out of □ and different potencies (6, 30 and 200), 30 potency showed maximum parasite inhibition with survival of 30% of mice and mean survival time of 20.5±6.25 days up to one month follow up period when compared to other treated groups. In the homeopathic doctrine of centesimal dilutions, it is generally believed that 'higher the potency (dilutions) stronger the effect (Hahnemann, 1960). The homeopathic mode of treatment often encourages use of drugs at such ultra-low doses and high dilutions that even the physical existence of a single molecule of the original drug substance becomes theoretically impossible (Khuda-Bukhsh, 2003). Present study is in accordance with the homeopathic principle as highest potency (200) showed minimum parasitaemia (6.2%) but only 1 mouse survived up to 21 days which may be due to toxicity caused by higher potencies. The 30 potency of many homeopathic medicines were reported to be more effective than other available potencies (Birch, 2007; Biswas and Khuda-Bukhsh, 2002; Salazar et al. 2006).

The considerable efficacy of chelidonium against rodent malaria parasite in the present study calls for further investigations of its antimalarial potential in terms of its safety and dosage. Chelidonium extracts shows an immune stimulating effect and it is used mainly for liver conditions. Extracts of *C. majus* are traditionally used in various complementary and alternative medicine (CAM) systems including homeopathy mainly in combating diseases of the liver (Taborska et al. 1995). Hepatic damage is one of the main pathological condition during malaria so homeopathic medicine chelidonium can be a potent antimalarial or a suitable partner for combination therapy.

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Study of natural hemagglutinins and their activity from the haemolymph of silkworm, *B. mori* L.

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Abstract : Hemagglutinins or lectins are specific carbohydrate-binding proteins or glycoproteins that have the ability to agglutinate cells with complementary carbohydrates on their surfaces and are present in most organisms. The body fluid or haemolymph of almost all invertebrate species tested accounts for agglutinins and also has been detected in mucus as well as in certain tissues. However, its immunogenic potential is best understood in the haemolymph, and recent studies have shown that purified, haemolymph-derived agglutinins served as opsonins in few insects. Thus, they are implicated in the recognition of non-self and cellular defense and are involved in cell differentiation. The study explored naturally occurring hemagglutinin (HA) in the haemolymph of silkworm, *Bombyx mori* using mammalian and human erythrocytes (RBC) as indicator cells. Highest HA titer of 2^8 = 256 was noticed with sheep RBC followed by titer of 2^7 with cow, pig chicken and human RBCs. An analysis of the physio-chemical properties of the HA showed it to be specifically dependent on the presence of Ca^{2+} for its activity, relatively stable between pH 7 to 9 and showed thermal stability up to 30°C. The potential affinity of natural hemagglutinins from haemolymph of *Bombyx mori* towards sialic acids (NeuAc/NeuGc) was suggested due to their agglutinating activity with human, cow, pig and chicken RBCs.

Keywords **:** *Bombyx mori, carbohydrate-binding proteins, Hemagglutinins, immunity, lectins*

Introduction

Hemagglutinins or lectins are ubiquitous proteins found in plants, animals and microorganisms. In vertebrates, the role of lectins as mediators of nonself recognition in the innate immune response has been well documented. The best studied of these are the mannose-binding lectins, MBLs, which are essential component of vertebrate innate immune system, since MBL-deficient individuals are prone to recurrent infections during infancy (Turner, 1996). In invertebrates due to lack of antibody-based immunity, agglutinins are vital for non-self recognition and clearance of invading microorganisms. Such humoral factors acting as recognition factors have been extensively studied due to their functional similarities to vertebrate antibodies, serving a defensive function (Jayasree, 2001; Jayaraj *et al*., 2008a).

Hemagglutinating proteins have been detected in mucus as well as in certain tissues of invertebrates (Suzuki and Mori, 1991), but its immunogenic potential is best understood in haemolymph, and recent studies have shown that purified, haemolymph-derived agglutinins served as opsonins in few insects (Kawasaki *et al*., 1993). Insect haemolymph contains hemagglutinating activities which agglutinates vertebrate erythrocytes and certain microorganisms and are important for the recognition of non-self and cellular defense and are involved in immune surveillance. Because of the involvement of lectins in the recognition of non-self, a number of studies have concentrated on the role of lectins in parasite-host relationships (Grubhoffer and Matha, 1991; Mohamed *et al*., 1992; Volf *et al*., 1993).

Developmental changes in the hemagglutinating activity of *Bombyx* larval haemolymph have been reported to be under the control of ecdysteroids. Increase in hemagglutinins due to the infection of Cytoplasmic Polyhedrosis Virus (CPV) suggested possible role of these proteins as candidates responsible in the silkworm for immune defense against exogenous pathological agents (Mori *et al*., 1989). Watanabe *et al*., 2006 characterized novel C-type lectin from *B. mori* haemolymph and designated as *B. mori* multibinding protein (BmMBP) as it bound to 5 Gram-negative bacteria, 3 Gram-positive bacteria and 3 yeasts examined under the study. Recently, the detailed specificities of a variety of these proteins towards *N*-linked oligosaccharides like *N*-acetylglucosamine (NeuGc) and *N*-acetylgalactosamine (NeuAc) have been elucidated. The present study was undertaken to explore the natural haemolymph agglutinins form mulberry silkworm, *Bombyx mori* L. and study its carbohydrate specificity

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by studying its RBC binding activities, so as to define their utilization as potent diagnostic tool in identifying diverse epitopes in pathogenic bacteria.

Materials and methods

Experimental animals and maintenance

Silkworm, *Bombyx mori* L. was reared in aseptic conditions at 26 ± 2^{o}C and 75 ± 10% Relative Humidity (RH) at the institute. The feeding, cleaning and sanitation schedule was followed according to Datta Biswas *et al*. (2007)

Preparation of Haemolymph samples

Haemolymph samples were collected from Vth instar Vth day worms by puncturing the abdominal legs and drained into 1.5 ml Eppendorf tubes containing a crystal of phenylthiourea to prevent oxidation of haemolymph and subsequent melanization. The haemolymph was centrifuged at 5400 rpm for 10 min to remove the haemocytes and cell debris and the resultant clear supernatant was used for the hemagglutination titre assay.

Preparation of erythrocyte (RBC) suspension

Human and other mammalian blood samples (viz., sheep, goat, cow, pig and chicken) were obtained by venous or cardiac puncture and collected in sterile AMAB (Modified Alsever's medium containing antibiotics): 0.03M Sodium citrate pH 6.1, 0.077M Sodium Chloride, 0.114M Glucose, 50 mg Neomycin sulphate and 165 mg Chloramphenicol. Prior to use, the RBCs were washed thrice with 0.9% saline with repeated centrifugation at 1600 rpm for 10 min and once with TBS (0.05M Tris-HCl, 0.1M Sodium Chloride and 0.01M Calcium Chloride). The RBC pellet was finally resuspended in TBS as 1.5% suspension (v/v).

Hemagglutination (HA) assay

HA assays were performed in U-bottom microtiter plates by serial two-fold dilution of a 25 µl serum with equal volume of TBS. After dilution, 25 µl of 1.5% RBC suspension was added to each well and incubated for 60 min at room temperature. The HA titers were recorded as the reciprocal of the highest dilution of the sample causing complete agglutination of RBC. Controls for all assays consisted of the substitution of the sample by TBS. All the HA assays were performed in duplicate.

pH and Thermal stability

The stability of haemolymph HA activity (in duplicates) at different pH was examined by dialyzing (24hr, 4^{o}C) 300 µl samples against the following buffers at pH ranging from 3 to 12 (Pearse, 1968): 0.2 M acetate buffer (pH 3 to 6), 0.2 M Tris-HCl buffers (pH 7 to 9) and 0.1 M Glycine-NaOH buffer (pH 10 to 12). The HA titre was determined with sheep RBCs.

To study thermal stability of HA, 300 µl haemolymph samples were held for 30 min at temperatures ranging from 10 to 100^{o}C, centrifuged and tested for HA activity with sheep RBCs.

Results

Haemolymph HA profile

The haemolymph of mulberry silkworm, *Bombyx mori* L. agglutinated human and variety of mammalian (viz., sheep, goat, chicken, cow, pig) RBC types (Table I). Among the various RBC types tested, the highest titre of 2^{8}=256 was observed with sheep erythrocytes. This was followed by a titre of 2^{7}=128 with chicken, cow, pig and human erythrocytes. The hemagglutinins didn't discriminate Human A, B and O RBC types and agglutinated them to same degree. No visible agglutination was observed with Goat RBCs.

Table I : Hemagglutinating (HA) activity of haemolymph from the mulberry silkworm, *Bombyx mori* L. against various mammalian erythrocyte types

RBC types tested	HA titer*
Sheep	256
Chicken	128
Cow	128
Pig	128
Human (O, A, B)	128
Goat	NA[#]

*Based on 20 determinations for each RBC type. All RBC's were collected in modified Alsevier's solution and used within a week after collection. The cells were washed thrice with TBS before assay.

[#] Not Agglutinated

pH and Thermal stability

The haemolymph hemagglutinating activity of mulberry silkworm, *Bombyx mori* L. was tested in the pH range of 3-12 (Figure 1) with sheep RBC showing the highest HA titre. The hemagglutinating activity against sheep RBC was found to increase above pH 3, with maximum stability between pH 7 and 9. The stability reduced above this pH range and completely lost at pH 11 and 12. The activity of haemolymph hemagglutinins against sheep RBC was unaffected up to 30ºC but was considerably reduced at 40ºC and 50ºC and completely inactivated at 60ºC and above (Figure 2).

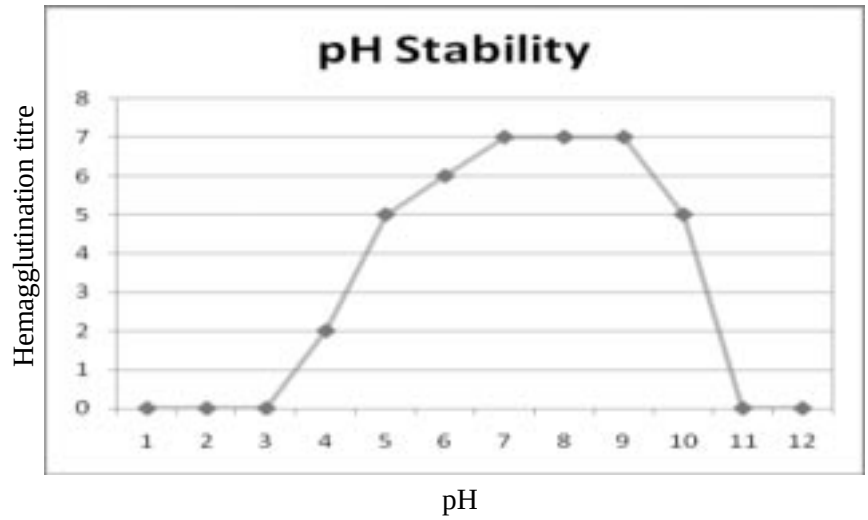

Fig. 1 : pH stability of hemagglutinating activity of haemolymph of *Bombyx mori* L. against sheep RBC

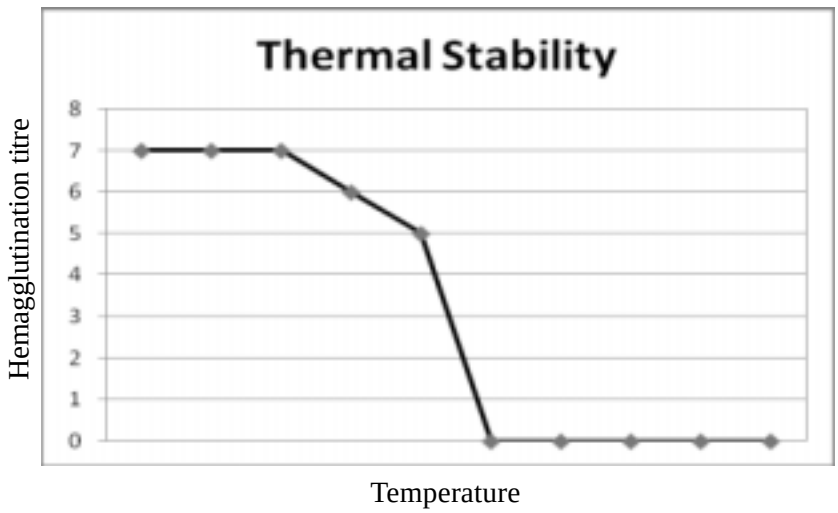

Fig. 2 : Thermal stability of hemagglutinating activity of haemolymph of *Bombyx mori* L. against sheep RBC

Discussion

The haemolymph of mulberry silkworm, *Bombyx mori* L. was found to possess naturally occurring hemagglutinating activity which showed highest reactivity with sheep RBC followed by chicken, cow, pig and human RBCs. These results confirm that the RBC types agglutinated by the haemolymph of *Bombyx mori* L. possibly share a common surface receptor but with qualitative differences in its HA binding sites. The studies are in conformity to the findings of Suzuki and Natori, 1993 having characterized haemolymph lectins from *Bombyx* larvae showing activity against sheep red blood cells with increased activity during larval-larval ecdysis and spinning stage. Previous reports by Mori *et al*., (1989), have indicated the enhance accumulation of hemagglutinating protein in the haemolymph of silkworms infected with Cytoplasmic polyhedrosisvirus (CPV), suggesting its requirement to exclude the virion from haemolymph, derived from their multiplication in the midgut cells or helping scavenge midgut tissue fragments resulting from infection.

Hapner and Jermyn (1981) observed strong hemagglutinating activity (titre 512) towards vertebrate erythrocytes in the haemolymph of both sexes of adult cricket, *Teleogryllus commodus* (Walker). Adeyeye and Cheng, 1997 studied hemagglutinin activity during all developmental stages of corn earworm *Helicoverpa zea*, with highest HA titre during days 3-5 of fifth instar larvae and lowest titre in adult (higher in female than in male) insects and observed highest reactivity with rabbit, sheep, porcine and guinea pig erythrocytes and low agglutinating activity with human, horse and chicken RBCs. Dorrah *et al*., 2009, isolated and characterized three lectins, designated as Sg_1, Sg_2 and Sg_3 from serum of desert locust *Schistocerca gregaria*, highly specific for rabbit RBCs than those of other vertebrates.

The haemolymph agglutinin of silkworm, *Bombyx mori* L. was heat-labile and susceptible to pH extremes. Hapner and Jermyn (1981) showed that *T. commodus* haemolymph hemagglutinating activity was stable at freezing and room temperatures, labile to heating at 56^{o}C and is precipitated with other haemolymph components when dialyzed against water.

The sialic acid affinity of natural agglutinin from haemolymph of silkworm, *Bombyx mori* L. was evident in the present study as their agglutination was highest with sheep, followed by cow, pig, chicken and human erythrocytes (Table II). Common species of sialic acids in Human A, B, O sheep and chicken erythrocytes are N-acetylneuraminic acid (NeuAc) (Jeanloz, 1966; Berman, 1984; Salmon *et al*., 1984) and N-acetylneuraminic acid/N-glycolylneuraminic acid (NeuAc/NeuGc) in pig erythrocytes (Sarris and Palade, 1979). Rat, mouse and horse erythrocytes constitute another group with NeuAc/NeuGc/*O*-acetylated sialic acid (*O*-AcSia) as common species of sialic acids. In rabbit, rat and mice, the position of major *O*-acetyl group was at C-9 and in horse at C-4 position (Ravindranath *et al*., 1985; Mercy and Ravindranath, 1993). This confirms striking correlation between the ability of silkworm hemagglutinin towards agglutination of cells and the absence of *O*-acetylsialic acids. Chinzei *et al*., 1990, reported that the carbohydrate portion of insect haemolymph proteins consists exclusively of mannose and *N*-acetyl glucosamine. Similar studies with crab lectin, exhibited high specificity for both free and glycosidically bound 9-*O*-Ac-NeuAc and 4-*O*-Ac-NeuAc (Ravindranath and Cooper, 1984). It would be interesting to study the reactivity of haemolymph agglutinins from *Bombyx* towards rat, mouse and horse erythrocytes as *O*-AcSia have been associated to cause hindrance to the binding with NeuAc/NeuGc. This also reflects the strong possibility that micro-organisms with NeuAc or NeuGc could be recognized by hemagglutinins of Silkworm, *Bombyx mori* L. and therefore can be used as valuable diagnostic tool.

Table II : Erythrocyte hemagglutination by haemolymph of *Bombyx mori* as compared with the erythrocyte affinity of sialic acid binding haemolymph lectins of other invertebrates [*Melanoplus differentialis* (Hapner, 1983), *Teleogryllus commodus* (Hapner and Jermyn, 1981), *Scylla serrata* (Jayaraj *et al*., 2010*) and Cancer antennarius* (Jeanloz, 1966)].

RBC types	Common species of sialic acids	*Bombyx*	*Melanoplus*	*Teleogryllus*	*Scylla*	*Cancer*
Human A	NeuAc	128	64	32	8	NA
Human B	NeuAc	128	256	32	32	NA
Human O	NeuAc	128	256	32	8	NA
Sheep	NeuAc	256	4	4	2	NA
Chicken	NeuAc	128	4	32	2	NA
Cow	NeuGc/NeuAc	128	4	-	16	NA
Pig	NeuGc/NeuAc	128	-	-	2	NA

NA – Not Agglutinated

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Studies on the biodiversity of aseptate gregarines from the oligochaetes of West Bengal

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Abstract : In a survey of the endoparasitic acephaline gregarines in the North 24 Parganas district of West Bengal, India seminal vesicles of earthworm *Lampito mauritii* (Kinberg) were found to be infested with a new species of the genus *Monocystis* Stein 1848, *Monocystis lampitae* sp. nov. is ovoidal and measures 85.8-155.4 (120.0 ± 18.1) µm x 32.7-69.5 (57.0 ± 10.4) µm. Nucleus rounded, 10.2-20.4 µm (14.1 ± 3.3) µm in diameter. Gametocysts are ovoid and measure 32.7-44.9 (38.6 ± 4.0) µm. Oocysts biconical, measuring 13.8-20.0 (17.2±1.0) x 7.7-9.2 (8.6 ± 0.6) µm

Key words **:** *Acephaline gregarines, earthworm, India, seminal vesicle, Monocystis lampitae sp. nov.*

Introduction

In India research on gregarine protozoan parasites infesting invertebrates has not been carried out with great importance. Gregarines are a group of apicomplexan protozoan parasites with two forms, septate and asepate. Only a few of over 350 species of Indian earthworms have been studied for the occurrence of gregarines, but this research has gained momentum since the 1980ies. It has already resulted in the finding of representatives of the genera *Apolocystis* Cognetti de Martiis, 1923*; Monocystis* Stein, 1848; *Nematocystis* Hesse, 1909; *Stomatophora* Drzewiecki, 1907 and *Zygocystis* Bhatia, 1930 (Hesse, 1909; Ghosh, 1923 – cited by Levine, 1988; Bhatia and Chatterjee, 1925; Bhatia and Setna, 1926; Kar, 1946; Kalavati, 1979; Subbarao et al., 1979; Pradhan and Dasgupta, 1980a, 1980b, 1982, 1983a, 1983b; Roychoudhury and Haldar, 1984; Bandyopadhyay et al., 2001, 2004, 2005, 2006a, 2006b, 2006c, 2006d, 2006e, 2007a, 2007b, 2008; Bandyopadhyay and Mitra, 2004, 2005a, 2005b, 2005c, 2005d, 2006a).

The biodiversity survey of gregarines from earthworms in the North 24 Pargans district of West Bengal revealed a new species of the genus *Lampito mauritii* (Kinberg). This paper presents the description of *Monocystis lampitae* sp. nov., the morphometric comparison with closely related species and the discussion of its taxonomy and systematics.

Material and methods

Samplings were carried out in the sandy soil in the Bagdaha locality of North 24 Parganas district of the state West Bengal. Different species of earthworms were collected during June-July, 2009. The collected individuals were put in soil-filled plastic buckets and taken to the laboratory alive. Some of them were dissected while alive and their seminal vesicles were carefully removed. These were placed on clean glass with a drop of 0.6% NaCl solution. A thin film of seminal fluid was drawn out on a slide covered with a cover slip for examination of living protozoans under a phase contrast microscope (Olympus CX41). After the initial study of living protozoans, the content of the seminal vesicles was semidried and fixed in Schaudin's fluid (20 min). The fixed smears were stored in 70% ethyl alcohol for removal of mercuric chloride. The slides were then passed through a descending series of alcohols (5 min in each) and placed in distilled water. Then they were transferred to a 3% iron alum solution (over night) and stained with Heidenhain's haematoxylin solution (20 min). Differentiation was done with 1% iron alum solution under the low power objective lens of the light microscope. The slides were then washed thoroughly, dehydrated in an ascending series of alcohols, cleared in xylene and mounted in Canada balsam. Drawings of the different stages of gregarines were made using a camera lucida attached to the microscope; photomicrographs were taken under an Olympus phase contrast microscope (× 400 magnification) with an Olympus camera (Model C5060). All measurements are in micrometres; in each case minimum and maximum values are given, followed in parentheses by arithmetic mean, standard deviation and sample size. Plane shapes are described mainly according to Clopton (2004).

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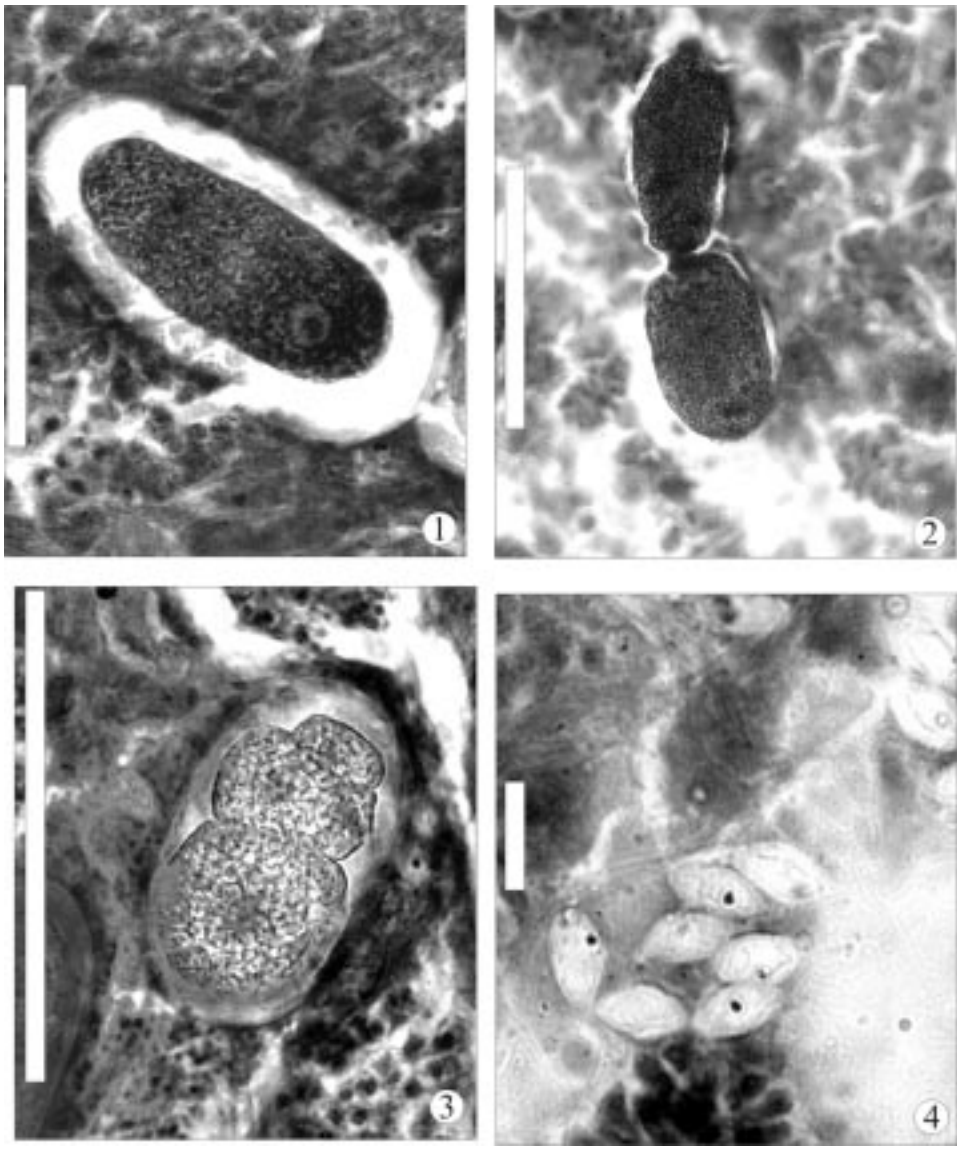
Results

***Monocystis lampitae* sp. nov. (Figs. 1-4, Table 1)**



Figs. 1-4. Photomicrographs of different stages of the life history of *Monocystis lampitae* sp. nov.
1. Gamont; 2. Syzygy; 3. Gametocyst, 4. Oocyst. (Scale bars: 100 µm (1-2), 50 µm (3), 4 (10 µm)

Phylum : Apicomplexa Levine, 1988; Order: Eugregarinorida Léger, 1900; Family: Monocystidae Bütschli, 1882; Subfamily: Monocystinae Bhatia, 1930; Genus: Monocystis Stein, 1848.

Gamont Length (GL) : 85.8-155.4 (120.0 ± 18.1) µm; Gamont Width (GW): 32.7-69.5 (57.0 ± 10.4) µm; Nucleus Diameter (ND): 10.2-20.4 µm (14.1 ± 3.3) µm; Gametocyst Diameter (GD): 32.7-44.9 (38.6 ± 4.0) µm; Oocyst Length (OL): 13.8-20.0 (17.2±1.0) µm; Oocyst Width (WO): 7.7-9.2 (8.6 ± 0.6) µm.

The gamont is ovoidal. The pellicle is smooth. Both ends of the gamont are rounded. Indistinct mucron. The nucleus is round and its position is in the episarc zone. Length and width of the gamont ranges from 85.8-155.4 (120.0±18.1) µm and 32.7-69.5 (57.0 ±10.4) µm respectively. Nucleus measure 10.2-20.4 µm (14.1±3.3) µm. Karyosome present within the nucleus. Width of episarc is 3-5 µm. Cytoplasm is vacuolated in nature.The large and deeply stained vacuoles are found in the endosarc region. Ectosarc is thin and poorly vacuolated. No epicyteal striations are found.

Table 1 : Morphometric comparison of *Monocystis lampitae* sp. nov. with *M. darjeelingensis* Bandyopadhyay and Mitra, 2005 and *M. metaphirae* Bandyopadhyay et. al., 2006

Species→ Characters↓	*Monocystis darjeelingensis* Bandyopadhyay and Mitra, 2005	*Monocystis metaphirae* Bandyopadhyay *et. al.*, 2006	*Monocystis lampitae* sp.nov.
Host	*Amynthas robustsa* (Perrier, 1892)	*Metaphire houleti* (Perrier)	*Lampito mauriti* sp. nov.
Locality	Darjeeling, West Bengal	Barasat, West Bengal	Bagdaha, North 24 Parganas, West Bengal
Site of infection	Coelomic fluid	Seminal vesicles	Seminal vesicles
Gamont	Cylindroid with round extremities. One end slightly wider than the other. Conical or elliptical when young. 210-273 µm x 84-140 µm	Bean shaped, anterior end is always wider than the posterior one. Mucron always present at the anterior end 94.0-151.0 µm x 53.0-81.0 µm	Ovoidal, pellicle is smooth
Size	210-273 µm x 84-140 µm	94.0-151.0 µm x 53.0-81.0 µm	85.8-155.4 µm x 32.7-69.5 µm
Ectosarc	With fine longitidunal striations	Ectosarc is thin , (1-3) µm	Ectosarc thin with poorly vacuolated granules
Endosarc	Granular, size increases with maturation	Endoplasm with evenly distributed granules	Contain deeply stained vacuoles
Nucleus shape	Spherical	Almost rounded	Rounded
Size of Nucleus	8.3-12.5 µm	44.0-16.0 µm	10.2-20.4 µm
Gametocyst shape	Spherical	Ovoid	Ovoidal to elliptical
Gametocyst size	147-163 µm	85.0-102.0 µm	32.7-44.9 µm
Oocyst shape	Navicular	Biconical and symmetrical	Biconical with pointed end
Oocyst size	15.4-18 µm x 8.0-9.0 µm	6.5-11.0 µm x 4.0-7.5 µm	13.8-20.0 µm x 7.7-9.2 µm
References	Bandyopadhyay and Mitra (2005a)	Bandyopadhyay et.al. (2006d)	Present study

Gametocysts are ovoid to elliptical and containing two nearly similar gametocytes. Diameter of the gametocyst measures 32.7-44.9 (38.6±4.0) µm. The large and small gametocytes are semicircular in shape and measures 24.5 – 38.8 (30.5±4.2) µm × 20.4-28.6 (24.6±3.1) µm and 20.4-28.6 (24.3±3.1) µm × 18.4 – 26.5 (21.9±2.1) µm respectively. Nucleus is not so prominent in both gametocytes.

Oocysts are biconical and both the ends of the oocysts are pointed and measuring 13.8-20.0 (17.2±1.0) µm × 7.7–9.2 (8.6±0.6) µm. Some granules are found within the oocyst.

Taxonomic Summary

Type material : *Monocystis lampitae* sp. nov.

Type host : *Lampito mauritii* (Kinberg)

Type of locality : Bagdaha, North 24 Parganas, West Bengal, India.

Symbiotype : LM/01 deposited in the Museum of the Department of Zoology, University of Kalyani, Kalyani-741235, West Bengal, India

Site of infection : Seminal vesicles

Prevalence : 26/78 (33.3%)

Holotype : MSVIII/22 is deposited in the Museum of the Department of Zoology, University of Kalyani, Kalyani 741235, West Bengal, India

Paratype : MSVIII/09 and other slides are in the collection of the Parasitology Laboratory, Department of Zoology, University of Kalyani, Kalyani 741235, West Bengal, India

Etymology : The species name is given after the genus name of the type host *Lampito mauritii* (Kin berg)

Discussion

Cylindroid, solitary with mucron, late syzygy and the parasite of seminal vesicles of oligochate host, earthworm justify its inclusion under the family Monocystidae, sub family Monocystinae and genus *Monocystis* Stein, 1848.

Till now, more than seventy species belonging to the genus have been reported through out the world. Of which sixteen species have been reported from India. Most of the described species are reported from the seminal vesicles of earthworm but some time parasites are also found in the coelom. The present species obtained from seminal vesicles of *Lampito mauritii* (Kinberg), which is considered to be a new one exhibits resemblance to some extent with *Monocystis darjeeligensis* Bandyopadhyay *et al.*, 2005 and *Monocystis metaphire* Bandyopadhyay *et al*., 2005 having cylindroid or ovoidal body with round extremities. In *Monocystis darjeeligensis*, one end is slightly wider than the other which is conical or elliptical in young stage but in the present form it is ovoidal at maturity. Both ends of the present form is round and with vacuolated cytoplasm. The position of the nucleus in the present species is in the anterior end. Karyosome is also found. Syzygy is one of important stage of life history which is not found in both the comparing species. Nature of syzygy is head to head. Gametocyst of the describing species is distinctly differs from the comparable two species. The gametocyte is unequal and ground nut shaped. Size of the gametocyst also differs from that of *Monocystis darjeeligensis* and *M. metaphire* where as it is very small in the present form. So comparing all the morphological features, it may be concluded that no other species of the genus *Monocystis* can be compared with the species obtained from *Lampito mauritii* (Kinberg) and hence it is new to science. Therefore, I propose the species as *Monocystis lampitae* sp. nov. in the present paper.

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Biotechnological diagnostic procedure for rapid detection of microbial pathogens in fish and shell fish

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Abstract : Bacterial, fungal, protozoan and viral infectious diseases are widespread among natural fish population. Various fish species, shrimps, crabs, giant freshwater prawns are being cultivated in different parts of India. Disease becomes evident only when stressful condition occurs in fish. However, the fishes raised in intensive culture system are more vulnerable to disease. Therefore, detection of pathogen from carrier fish is essential for the effective fish disease control. In order to identify pathogen carrier fish, a cost effective, sensitive and specific system are required for surveillance and monitoring fish population. Traditional techniques for diagnosis a disease are time consuming, laborious. With the advent of technological invention in the field of molecular biology and biotechnology, newer DNA based and immunological methods have been developed and applied for rapid detection of many bacterial and viral pathogens of fish and shellfish. Diagnostic tests like ELISA, PCR, *in situ* hybridization and Dot blot hybridization etc have been developed and applied for detection of various bacterial and viral diseases of fish and shell fish like CCV, IHNV, IPNV, VHSV, BKD, WSSV, YHV, MBV, BP Progress in techniques aids epidemiological studies as well as identifying etiology of disease outbreaks or the presence of pathogens in fish and shellfish. This advancement in molecular methods for disease detection is urgently required for sustainable development of aquaculture sector in India. In this paper an attempts are taken to highlight the disease problems of finfish and shellfish and biotechnological methods for disease management in India through effective way.

Introduction

Bacterial, fungal, protozoan and viral infectious diseases are widespread among natural fish population. Various fish species, shrimps, crabs, giant freshwater prawns are being cultivated in different parts of India. Disease becomes evident only when stressful condition occurs. Under intensive aquaculture conditions the risk of stress increases and a significant proportion of the stock may become infected. Therefore, detection of pathogen from carrier fish is essential for the effective fish disease control (Altinok and Kurt, 2003). Therefore, detection of pathogen from carrier fish is essential for the effective fish disease control. In order to identify pathogen carrier fish, a cost effective, sensitive and specific system are required for surveillance and monitoring fish population. Traditional techniques for diagnosis a disease are time consuming, laborious. With the advent of technological invention in the field of molecular biology and biotechnology, newer DNA based and immunological methods have been developed and applied for rapid detection of many bacterial and viral pathogens of fish and shellfish. Molecular techniques are major tools for the analysis of microorganisms from food and other biological substances. The techniques provide ways to screen for a broad range of agents in a single test (Field and Wills, 1998). It has truly come of age and its range of application is perceived to broaden in the near future. This advancement in molecular methods for disease detection is urgently required for sustainable development of aquaculture sector in India. In this paper an attempts are taken to highlight the disease problems of finfish and shellfish and biotechnological methods for disease management in India through effective way.

Important diseases of finfish and shell fish in India

***Vibriosis* :** Bacterial diseases may cause a range of problems ranging from mass mortalities to growth retardation and sporadic mortalities. *Vibrio* spp are the most important bacterial pathogens of shrimp. *Vibrio* spp are aquatic bacteria that are widely distributed in fresh water, estuarine and marine environments. Over 20species are recognized, some of these are human pathogens (eg. *V. cholerae, V. parahaemolyticus* and *V.vulnificus*) while some species are pathogens of aquatic animals including shrimp (eg. *V. harveyi* , *V.spendidus, V. penaecida, V. anguillarum, V.parahaemolyticus, V.vulnificus*). *Vibrio* spp are commonly observed in shrimp hatcheries, grow-outponds and sediments (Otta *et al*. 1999, 2001).

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Luminescent *Vibrio harveyi* is a natural micro flora of marine and costal water bodies and is associated with mortality of larval shrimp in penaeid shrimp hatcheries. It is also known that the bacteriophages occur virtually in all places where their exist. The brooders maturation and spawning facilities in the shrimp hatchery are the main source of luminescent *Vibrio harveyi.* Oakey and Owens (2000) noted that one of the toxin producing strains of *V. harveyi* (VH642) was lysogenic and carried a myovirus like phage (VHML).

***Epizootic Ulcerative Syndrome (EUS) of Fish* :**

Epizootic Ulcerative Syndrome or EUS was first reported in Australia in 1972 and thereafter appeared in India in the Barak Valley region during 1988. Since then it is a scientific puzzle and has been sweeping, in epidemic form, amongst fresh and brackish water fish in the Asian Pacific region including Indian Sub-continent. Natural outbreaks of EUS in more than 100 fish species had been recorded. Initially, the disease is marked by few red spots on the skin surface, which progress in size and eventually causes circular deep haemorrhagic ulcers, exposing the skeletal musculature. Various pathogens, including bacteria, fungus and virus have been reported to be isolated from naturally infected fish. The most predominant microflora of EUS infected fish were reported as *Aeromonas hydrophilla*, *Enterobacter* sp., *Vibrio* sp., *Pseudomonas* sp. *Escherichia coli*, *Aphanomyces invadans and Aspergillus* sp. A highly invasive fungus *Aphanomyces invadans* has been consistently associated with Epizootic Ulcerative Syndrome (EUS) in Indian fishes. It is presumed that a diverse group of microbial agents such as viruses, bacteria and cutaneous ectoparasites may initiate skin lesions, which is subsequently colonized by Aphanomyces *invadans* and ultimately lead to EUS. The fungus invades and proliferates in tissues away from the site of dermal ulcers and even penetrates across the spinal cord and enters muscles and bones. The fungus readily invades the body cavity and produces mycotic granulomas in all the visceral organs (Santiago *et al*.2008).

***Viral nervous necrosis (VNN*).**

The Viral encephalopathy and retinopathy (VER) is an emerging viral disease of marine fishes in Asiatic region. VNN has been associated with high mortalities in several marine fish species of economic importance in Europe, Asia, Japan and Australia. Due to its affinity to neuronal cells and its ability to cause marked histopathological alterations in nervous tissue, the disease condition is referred as viral encephalopathy and retinopathy (VER) , viral nervous necrosis(VNN) or fish encephalitis. The disease is characterized by abnormal swimming behavior and vacuolating lesions within the brain and retinal tissue of affected fish. The causative agent of the disease is fish nodavirus which is a non enveloped, icosahedral RNA virus belonging to genus betanodavirurses of nodaviridae family. VNN has been reported from 40 species of fishes with the greatest impact being in Asian and European Seabass, grouper, jack and parrotfish. The tissue lesions in the brain and the spinal cord of Asian Seabass with history of mass mortalities have confirmed presence of viral nervous necrosis (VNN) by RT –PCR and histopathology (Santiago *et al*.2008).

***White spot syndrome virus* :**

White spot syndrome is a viral disease of shrimp that has vitiated the sustainability and viability of shrimp culture worldwide. First report on white spot viral disease in shrimps from the state of Andhra Pradesh, India was reported in 1994. With its ability to cause 100% mortality within 5-10 days the disease has caused major economic losses in shrimp culture industry. The disease is caused by White spot syndrome virus (WSSV) which is a double stranded DNA virus of genus *Whispovirus* belonging to the family *Nimaviridae*. WSSV virions are enveloped and are ellipsoid to bacilliform in shape. WSSV is known to affect most commercially important species of penaeid shrimp including *P. monodon, P.japonicus, P. indicus, P. chinensis, P.merguiensis, P.aztecus, P. stylirostris, P. vannamei, P. duorarum* and*P. setiferus* (Lightner 1996). WSSV infects most tissues originating from both ectoderm and mesoderm. These include subcuticular epithelium, gills, lymphoid organs, antennal gland, hematopoietic tissues, connective tissue, ovary and the ventral nerve cord (Wongteerasupaya *et al*. 1995,Wang *et al*. 1998).

***Monodon baculovirus (MBV)* :**

From 1994 to 1999 the Indian shrimp farms on east and west coast experienced repeated outbreaks of Monodon baculovirus (MBV). It is a nuclear polyhedrosis virus (NPV) of the family Baculoviridae (Lightner and Redman 1981). As with all NPVs, it has a double stranded circular DNA genome of 80-100 x 106 Da within a rod shaped, enveloped particle often found occluded within proteinaceous bodies (Rohrmann 1986). Gross signs of MBV are pale bluish grey black coloration of the body, reduced feeding and growth rate, increased epibiotic and epicommensal fouling on the surface of the larvae which leads to sluggish and inactive swimming behavior and appearance of white midgut line which can be seen throughout the abdomen in severely affected larvae and post larvae.

Hepatopancreatic paravovirus (HPV)

Hepatopancreatic parvovirus infects various types of penaeid shrimps and is distributed around the world. Shrimps affected by HPV usually show non-specific gross signs, including atrophy of the hepatopancreas, anorexia, poor growth rate, reduced preening activities and as a consequence increased tendency for surface and gill fouling by epicommensal organisms (Lightner and Redman 1985).

Baculovirus penaei(BP)

The BP was first detected in 1974 in the pink shrimp,*Penaeus duorarum* (Couch 1974).BP has beenreported to cause significant mortalities in the larval,post-larval and early juvenile stages of *P.aztecus, P.stylirostris, P.vannamei* and *P.penicillatus* (Couch1991, Lightner and Redman 1991, 1992).BP infects only the hepatopancreas and midgut epithelial cells, and it is transmitted from shrimp to shrimp.

Biotechnological Diagnostics

Polymerase Chain Reaction:

Polymerase chain reaction is a technique for amplifying a specific region of DNA, defined by a set of two "primers" at which DNA synthesis is initiated by a thermostable DNA polymerase. Usually, at least a million-fold increase of a specific section of a DNA molecule can be realized and the PCR product can be detected by gel electrophoresis. The regions amplified are usually between 150-3,000 base pairs (bp) in length. Primer design is important to obtain greatest possible sensitivity and specificity. Therefore, the primers should be sufficiently long to allow a high annealing temperature and reduce the opportunity for nonspecific primer annealing, but primers that are too long may facilitate nonspecific annealing even to regions of DNA that are not perfectly complementary to the primer sequence. The reaction includes template DNA that may be in various forms, from a simple tissue lysate to purified DNA, primers, polymerase enzyme to catalyze creation of new copies of DNA, and nucleotides to form the new copies. During each round of the thermocycling reaction, the template DNA is denatured, primers anneal to their complementary regions and polymerase enzyme catalyses the addition of nucleotides to the end of each primer, thus creating new copies of the target region in each round. Theoretically, the increase in amount of product after each round will be geometric (Altinok and Kurt, 2003). PCR is the most popular diagnostic technique for the detection of WSSV. Several primers have been designed for the detection of WSSV. Nested PCR has afforded greater sensitivity than single step PCR. Lo *et al.* (1996) described a set of PCR primers for detection of WSSV. Wang *et al.*(1996)described PCR based detection of BP in post larvae. Central Institute of Brackish water Aquaculture (CIBA) has developed a nested PCR kit that is being marketed by Genei, Bangalore. Similar PCR based diagnostic kits have already been developed by Mangalore Fishery College, Central Marine Fishery Research Institute (CMFRI) which brought down the cost of the PCR test and rendered a great service to shrimp industry.

Antibody based tests :

A variety of antibody-based tests and molecular tests have been developed to detect mainly bacterial and viral fish pathogens, although tests have also recently been reported for parasites and fungal agents. The antibody-based tests include slide agglutination, co-agglutination/latex agglutination, immunodiffusion, direct and indirect fluorescent antibody tests (FAT and IFAT),immunohistochemistry (IHC) and enzyme linked immune sorbent assay (ELISA), dot blot/dip stick and western blot (WB) (reviewed by Adams, 1999). The antibody-based test selected for the identification of pathogens depends on a variety of factors since each method has its merits and disadvantages. Although such methods are useful for the detection of pathogens in pure culture or/and in infected fish tissue, their sensitivity thresholds limit use in environmental samples, especially where pathogen levels are extremely low. DNA detection methods, however, such as polymerase chain reaction (PCR) and *in situ* hybridisation are ideally suited (Adams 1999).

Hybridoma technology :

Monoclonal antibodies (MAbs) against several aquatic pathogens such as *Aeromonas hydrophila, Aphanomyces invadans* of EUS , white spot virus and against immunoglobulins of (IgM) of Indian Major carp rohu have been developed and employed in antigen characterization, pathology, diagnosis and epidemiology.

Nucleic Acid probe :

Nucleic acid probes are segments of DNA or RNA that have been labeled with enzymes, antigenic substrates and chemiluminescent moieties or radio isotopes. They can bind with high specificity to complementary sequences of nucleic acid.

Probes can be directed to either DNA or RNA targets and can be twenty to thousand bases long. Oligonucleotide probes have the advantage of hybridizing more rapidly to the target molecules and can be chemically synthesized and purified by instruments available commercially. Commercial kits for labeling probes are available with affinity labels such as biotin and digoxigenin (Karunasagar *et al* 1999). Nucleic acid probes like *in situ* hybridization and *dot blot* hybridization have been applied for fish and shell fish disease diagnosis by numerous scientists.

Conclusion

Molecular tools are increasingly relevant to fish diseases. The sequencing of the complete genomes of pathogens is allowing great advances in studying the biology, and improving diagnosis and control of pathogens. Using nucleic acid as targets, and new methods of analyzing polymorphism in this nucleic acid, can improve specificity, sensitivity, and speed of diagnosis and offer means of examining the relationships between genotype and phenotype of various pathogens. Progress in techniques aids epidemiological studies as well as identifying causes of disease outbreaks or the presence of pathogens in future.

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Quantification of the role of parasitic nematode *Hexamermis* sp in controlling brown planthopper in farmer's rice field in eastern India with special reference in West Bengal

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Abstract : Onfarm adaptive trials were carried out under World Bank aided National Agricultural Technology Project (NATP) and National Agricultural Innovative Project (NAIP) at Rainfed rice growing areas of West Bengal during Kharif 2003 – 04 and boro 2008 – 09. Each trial comprised three treatments viz. Natural Biological Control (NBC) with no pesticide application ,Schedule Treatment (ST) involving application of insecticide based on schedule commonly applied by farmers and Need Based Protection (NBP) judged by periodic monitoring of *Hexamermis* sp population and locally recommended economic threshold. The results revealed that NBC resulted in maintaining highest level of parasitism of Brown Planthopper by this parasitc nematode as compared to NBP and ST.

Introduction

India is the second largest producer of rice in the world. Rice being the principal crop occupies 66% of the gross cropped area of Eastern India particularly in West Bengal. Brown planthopper *Nilaparavata lugens* Stål has been recognised as the major biotic stress responsible for significant reduction in yield of rice in West Bengal (Satpathi et al 2005, Katti et al 2006). About 30% of the natural control of brown planthopper in Eastern India are due to parasitic nematode (Satpathi et al 2008). Farmers of Eastern India have been relying mostly on chemical pesticides for the management of brown planthopper, however their indiscriminate use has led to the destruction of this parasite. Hence on-farm trials were conducted by National Agricultural Technology Project (NATP) and National Agricultural Innovative project (NAIP) during kharif 2003 & 04 and boro 2008 & 09 to generate the information on the effect of different treatments on *Hexamermis* sp population in rainfed rice of West Bengal.

Materials and Methods

Field trials were conducted in 2 villages spread across Birbhum and Bankura districts of West Bengal during kharif season of 2004 & 05 and boro seasons of 2008 & 09. In each village, 3 hectare field was selected, where there were three treatment blocks *viz* (i) Natural Biological Control (NBC) – with no pesticide application throughout the crop season (ii) Need based protection (NBP) – judged by periodic monitoring of brown planthopper population and (iii) Schedule treatment(ST) – involving application of insecticides based on a schedule commonly adopted by farmer. The details of the treatments are given in Table -1. Except for insecticidal application all the other agronomic practices are common to three treatments and followed as per recommended package of practices.

The population of brown planthoppers were recorded randomly from 50 hills in each subplot at weekly interval. For estimation of parasitism the mummified adult and nymphs were collected at regular interval and released on potted plant for laboratory study (Fig-1).

The infected hopper pests were split horizontally and kept in containers with small quantity of water at 20 to 25^0c to enable the nematode to emerge. After 20 to 24 hours of incubation the water was collected and retained for examination.

Results and Discussions

At Birbhum, the percent of parasitisation was low 1.61 to 5.0 during the kharif season of 2008 at 60 DAT and there is a significant difference among the treatments. However the percentage of parasitism was recorded up to 30.00% in NBC treatment showed significantly higher parasitisation in the range of 16.00 to 30.00% compared to 7.49 to 4.25% in schedule treatment (ST) and 3.22 to 9.67% in Need Based Protection (NBP) during 70 to 130 DAT.

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Table 1 : Detail of treatment Kharif 2003 – 04 and boro 2008-09

Location	Birbhum	Bankura
	2003 & 2004	2008 & 2009
Season	Kharif	Boro
Natural Biological Control (NBC) Schedule treatment (ST)	No insecticide application throughout the crop growth ● Seed treatment with Thiram (fungicide) @2g/kg of seed ● Application of Butachlor 5G (herbicide) @ 650 g ai/ha during land preparation ● Application of Carbofuran 3G @ 1.0 kg ai/ha at 25 to 35 DAT ● Spray application of Chlorpyriphos 20% EC (insecticide) @ 500 gm ai/hec and Hexaconazole 5% EC (Fungicide) @ 100 gm ai/ha at 50 DAT ● Spray application of Chlorpyriphos 50% EC + Cypermethrin 5% EC (insecticide) @ 825gm ai/ha and Propiconazole 25% EC (Fungicide) @ 250 gm ai/ha at 70 to 80 DAT ● Spray application of Imidacloprid 20%SL @75 gm ai/ha at 80-90 DAT	No insecticide application throughout the crop growth ● Seed treatment with Thiram (fungicide) @2g/kg of seed ● Application of Carbofuran 3G @ 1.0 kg ai/ha at 25 to 35 DAT ● Spray application of Chlorpyriphos 50%EC + Cypermethrin 5% EC(insecticide) @ 825 gm ai/ha and Hexaconazole 5 EC (Fungicide) @ 100 gm ai/ha at 50 DAT ● Spray application of Triazophos 40% EC (insecticide) @ 600 gm ai/ha and Tricyclazole 75 WP (Fungicide) @ 750 gm ai/ha at 90 DAT
Need based protection (NBC)	● Seed treatment with 2g/kg seed (Fungicide) ● Seed bed treatment with Carbofuran 3 G @ 1kg ai/ha at 2 days before transplanting ● Application of Butachlor 5G (herbicide) @ 650 gm ai/ha during land preparation ● Spray application of Propiconazole 25 EC (Fungicise) @ 250 gm ai/ha at 30 DAT ● Spray application of Cartap hydrochloride 500gm ai/ha at 65 DAT	● Seed treatment with Thiram 2g/ kg seed (Fungicide) ● Seed bed treatment with Carbofuran 3 G @ 1kg ai/ha at 2 days before transplanting ● Application of Butachlor 5G (herbicide) @ 650 gm ai/ha during land preparatio ● Spray application of Triazophos 40 CE (insecticide) @ 600 gm/ha and Tricyclazole 75 WP @ 750 gm ai.ha at 60 DAT(Fungicide)

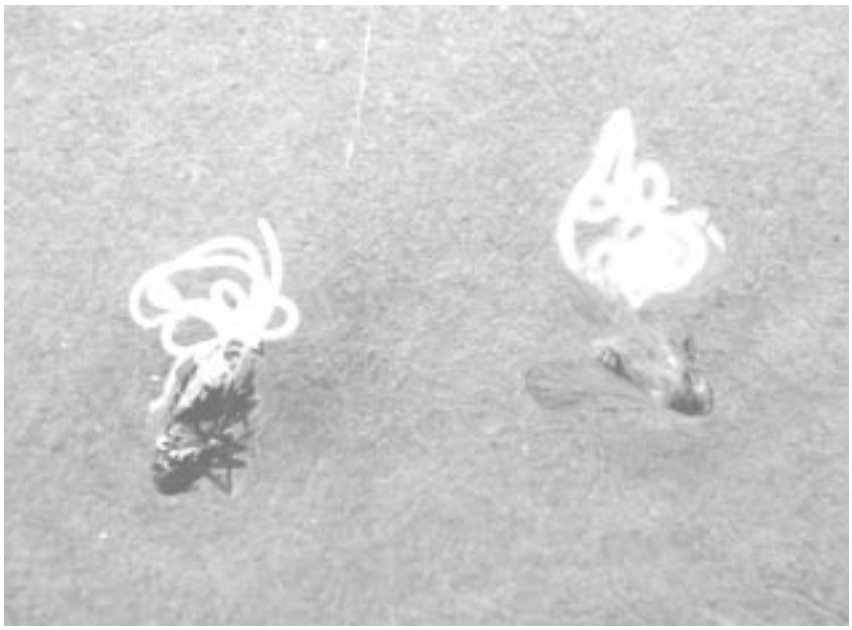

Fig-1. BPH (Male & Female) Parasitised by *Hexamermis* Sp

Although the percentage of parasitism was low at 60 DAT during kharif season 2004 but the higher parasitism in the range of 12.00 to 34.00%, 1.62 to 4.50%, 3.84 to 10.24% were recorded in NBC, ST and NBC during 70 to 130 DAT respectively (Table-2).

At Bankura the percentage of parasitism was very low in the range of 2.40 to 2.56 %in NBC treatment during boro season 2008 showed significantly higher as compare 0.212 to 0.264% in ST and 0.78 to 0.80 in NBP during 100 to 110 DAT. There was also significant differences among the three treatments in 2009, ST recorded lowest parasitism of 0.250 to 0.293% followed by NBPshowing 0.689 to 0.768% while the range was 2.12 to 2.40% in NBC during 100 to 110 DAT.

Table 2 : Percentage of parasitism of brown planthopper by *Hexamermis* sp. during kharif 2003 - 04

Birbhum Kharif 2003								
Treatment	Days after transplanting							
	60	70	80	90	100	110	120	130
NBP	1.61	3.22	6.45	5.8	9.67	9.03	5.1	5.32
ST	0.7	1.44	2.83	2.56	4.25	3.97	2.24	2.34
NBC	5.6	10	20	18	30	28	16	16.5
CV%	6.72	2.58	12.78	14.32	8.5	9.11	15.37	1.57

Birbhum Kharif 2004								
Treatment	Days after transplanting							
	60	70	80	90	100	110	120	130
NBP	1.41	3.84	6.72	5.68	10.24	10	5.76	5.6
ST	0.62	1.62	2.95	2.49	4.5	4.4	2.53	2.46
NBC	5.07	12	21	17.75	34	32	18	17.5
CV%	8.4	21.44	12.22	0.29	7.95	8.76	14.22	1.97

Table 3 : Percentage of parasitism of brown planthopper by *Hexamermis* sp during boro 2008 & 09

Bankura Boro - 2008							
Treatment	Days after transplanting						
	60	70	80	90	100	110	120
NBP	-	-	-	-	0.8	0.78	-
ST	-	-	-	-	0.264	0.212	-
NBC	-	-	-	-	2.56	2.4	-
CV%				1.47	1.33		

Bankura Boro - 2009							
Treatment	Days after transplanting						
	60	70	80	90	100	110	120
NBP	-	-	-	-	0.768	0.689	-
ST	-	-	-	-	0.25	0.293	-
NBC	-	-	-	-	2.4	2.12	-
CV%					1.79	0.32	

In the present study, it is thus evident that Natural Biological Control (NBC) has shown the optimum result by exhibiting higher parasitism as compare with that of schedule treatment (ST) (SBP) and Need Based Protection (NBP) due to the considerable reduction in number of applications of insecticide. The need based protection resulted in optimum build up of *Hexamermis* sp population akin to that natural biological control (NBC) making it more environmental friendly.

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Mitochondrial- DNA and host parasitic interaction

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Abstract : DNA bar coding is based on a relatively simple concept. Most eukaryote cells contain mitochondria, and mitochondrial DNA (mtDNA) has a relatively fast mutation rate, which results in significant variation in mtDNA sequences between species and, in principle, a comparatively small variance within species. A 648-bp region of the mitochondrial cytochrome c oxidase subunit I (COI) gene was proposed as a potential 'barcode'. DNA barcoding has met with spirited reaction from scientists, especially systematists, ranging from enthusiastic endorsement to vociferous opposition. For example, many stress the fact that DNA bar coding does not provide reliable information above the species level, while others indicate that it is inapplicable at the species level, but may still have merit for higher-level groups. Others resent what they see as a gross oversimplification of the science of taxonomy. And, more practically, some suggest that recently diverged species might not be distinguishable on the basis of their COI sequences. Due to various phenomena, it was found that some 23% of animal species are polyphyletic if their mtDNA data are accurate, indicating that using an mtDNA barcode to assign a species name to an animal will be ambiguous or erroneous some 23% of the time. Studies with insects suggest an equal or even greater error rate, due to the frequent lack of correlation between the mitochondrial genome and the nuclear genome or the lack of a barcoding gap. Problems with mtDNA arising from male-killing microorganisms and cytoplasmic incompatibility-inducing symbionts are also particularly common among insects. Given that insects represent over 75% of all known organisms, this suggests that while mtDNA bar coding may work for vertebrates, it may not be effective for the majority of known organisms.

Key words **:** *Co –speciation, DNA barcode, Mitochondrial DNA, Parasite, Symbionts.*

Introduction

The study of evolution frequently requires understanding the history of the population, species or clade under study. In population genetics, a recent history of population bottlenecks may restrict genetic variation and thus constrain the speed of adaptation. In examining diversification over space, we need to have detailed knowledge of the different populations' histories of colonization and the gene flow between them. In comparative analyses of processes of adaptation or molecular evolution, and in studies of historical biogeography, we require resolution of the relationships between species. Biotrophic parasitism is a common mode of life that has arisen independently many times in the course of evolution. Depending on the definition used, as many as half of all animals have at least one parasitic phase in their life cycles (Price, P.W. 1980) and it is also frequent in plants and fungi. Moreover, almost all free-living animals are host to one or more parasite taxa. (Price, P.W. 1980). As a result of host defenses, some parasites evolve adaptations that are specific to a particular host taxon and specialize to the point where they infect only a single species. Such narrow host specificity can be costly over evolutionary time, however, if the host species becomes extinct. Thus, many parasites are capable of infecting a variety of host species that are more or less closely related, with varying success. In some cases, a parasite species may coevolve with its host taxa. In theory, long-term coevolution should lead to a relatively stable relationship tending to commensalism or mutualism, in that it is in the evolutionary interest of the parasite that its host thrives. A parasite may evolve to become less harmful for its host or a host may evolve to cope with the unavoidable presence of a parasite to the point that the parasite's absence causes the host harm. For example, although animals infected with parasitic worms are often clearly harmed, and therefore parasitized, such infections may also reduce the prevalence and effects of autoimmune disorders in animal hosts, including humans. (Rook, G.A.W. 2007). The presumption of a shared evolutionary history between parasites and hosts can sometimes elucidate how host taxa are related. For instance, there has been dispute about whether flamingos are more closely related to the storks and their allies, or to ducks, geese and their relatives. The fact that flamingos share parasites with ducks and geese is evidence these groups may be more closely related to each other than either is to storks. In rare cases, a parasite may even undergo co-speciation with its host. One particularly remarkable example of co-speciation exists between the simian foamy virus (SFV) and its primate hosts. In one study, the phylogenies of SFV polymerase and the mitochondrial cytochrome oxidase subunit II from African

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and Asian primates were compared. (Switzer WM et.al.2005) Surprisingly, the phylogenetic trees were very congruent in branching order and divergence times. Thus, the simian foamy viruses may have co-speciated with Old World primates for at least 30 million years.

Co-speciation

A central question in the evolutionary study of any host–parasite assemblage is the relative antiquity of the host–parasite association; is the parasite a recently acquired ‘‘souvenir’’ or an ‘‘heirloom’’ inherited from the host’s ancestor (Sprent, 1970) In the latter case, if a parasite is specific to a single host lineage then, as that host lineage speciates, populations of the parasite on the descendant host species may themselves speciate. If parasites only speciate when the hosts speciate, and never go extinct independently of their hosts, this parallel cladogenesis or ‘‘cospeciation’’ (Brooks, 1988) will yield host and parasite phylogenies that are mirror images of each other. However, if other processes such host switching and extinction occur then the relationship between the evolutionary trees for the two members of the association will be more complex and hence more difficult to unravel (Lyal, 1986; Page, 1994b) Parasites may speciate independently of their hosts, go extinct, or switch to different hosts. These processes can result in more than one lineage of closely related parasites on the same host. This is termed a ‘‘duplication’’ by analogy with gene duplications If the two descendant parasite lineages subsequently cospeciate with the host then the host clade will harbor two parallel sets of parasites Following Fitch’s (1970) terminology for genes, the two sets of parasites are called as ‘‘paralogous’’; members of the same lineage of parasites are ‘‘orthologous’’. Recent advances in molecular systematics facilitate rigorous analysis of the history of host–parasite assemblages, in part by providing mutually independent phylogenies for host and parasite (Page et.al. 1998). Because cospeciation is inferred when the topologies of host and parasite phylogenetic trees are more similar than would be expected by chance (Hafner and Nadler, 1990) it is vital that host and parasite evolutionary histories are inferred independently. The use of molecular data can reduce the extent to which knowledge of the classification of one member of the association influences that of the other, as well as minimizing the possibility that the phylogenetic analysis is misled by host effects on parasite morphology (Downes, 1990) Molecular data are also attractive because they provide a common yardstick for comparing evolutionary character change (anagenesis) in taxonomically (and morphologically) disparate groups (Page, 1993c). Homologous genes can be readily found in most taxa, whereas homologous morphological features may be extremely difficult to find in, say, a vertebrate and its insect parasite. Furthermore, if the molecular divergence in host and parasite is approximately clock-like then specific hypotheses about the relative antiquity of a particular host–parasite association can be tested (Page *et al.,* 1996). At the same time, cospeciating host–parasite assemblages provide a unique opportunity to compare the evolutionary rates of dissimilar organisms that havev lived in intimate association over long periods of time (Hafner and Page, 1995; Page and Hafner, 1996). Calibrating the absolute rate of evolution in a single clade of organisms can be problematic if the fossil record of that clade is poor. However, if that clade has cospeciated with a clade that does have a fossil record, then those fossils can also be used to calibrate the rate of evolution in the clade that lacks fossils of its own. This is because if a pair of host and parasite lineages has cospeciated then those lineages are of the same age. This approach of calibrating one lineage using another, associated, lineage has been applied to endosymbiotic bacteria of aphids (Moran *et al.,* 1993) and cockroaches (Bandi *et al.,* 1995) and alpha herpes viruses of vertebrates (McGeoch *et al.,* 1995). Even if neither member of a cospeciating association has an adequate fossil record we can still obtain a measure of the relative rate of evolution in the two lineages because cospeciation implies that there are events of the same age in the two clades, even if we do not know the absolute age of those cospeciation events. Relative rates of molecular evolution have been measured in this way between chewing lice and mammals (Hafner *et al.,* 1994) and endosymbiotic bacteria and aphids (Moran *et al.,* 1995).Calibrating rates of evolution in hosts and parasites depends crucially on the presence of cospeciation and on correctly identifying which events in the two clades represent cospeciation. It is not enough simply to compare divergence in pairs of hosts and their parasites. If the parasites are older than their hosts, then greater divergence between a pair of parasites may reflect greater antiquity relative to their hosts, rather than a difference in rate of evolution. This is analogous to the mistake of using divergence between paralogous genes as a measure of time of divergence between organisms; valid comparisons of host and parasite divergence must use ‘‘orthologous’’ parasites (Page, 1993b). Conversely, if the parasites have not cospeciated with their hosts but instead have switched hosts then the parasite pair may be much younger than their hosts. In this study we use molecular phylogenies to determine whether there is evidence for cospeciation between a group of birds and their lice and to investigate the relative rates of evolution in these two taxonomically distant organisms.

mtDNA- an useful tool

The most widely used molecular tool of these animals has been variation in the mitochondrial DNA (mtDNA) sequence. Mitochondria have their own genome of about 16,500 bp that exists outside of the cell nucleus. Each contains 13 protein coding

genes, 22 tRNAs and 2 rRNAs. They are present in large numbers in each cell, so fewer samples is required. They have a higher rate of substitution (mutations where one nucleotide is replaced with another) than nuclear DNA making it easier to resolve differences between closely related individuals. They are inherited only from the mother, which allows tracing of a direct genetic line. They don't recombine. The process of recombination in nuclear DNA (except the Y chromosome) mixes sections of DNA from the mother and the father creating a garbled genetic history. So this choice is well reasoned. mtDNA can be easily amplified from a variety of taxa and, because it is haploid, the sequence can be obtained without cloning. Because it has a high evolutionary rate and an effective population size approximately one-quarter that of nuclear markers, it allows a chance of recovering the pattern and tempo of recent historical events without an extensive sequencing effort. Thirdly, as an area of at least low recombination, the whole molecule can be assumed to have the same genealogical history. In contrast, while stringent efforts have been made to develop and amplify nuclear markers, this often involves the refinement of primers for the target species, the sampling of several genes before one with an appropriate evolutionary rate is discovered and the separation of alleles from heterozygous individuals via cloning before sequencing. While these problems can be overcome outside 'model' taxa where genomic sequence data are available, they increase the effort needed to gain the required information. Mitochondrial DNA has therefore remained the marker of choice in many population, biogeographic and phylogenetic studies. Roderic D. M. Page et.al. (1997) studied the phylogeny for the lice (Insecta: Phthiraptera: genus *Dennyus*) parasitic on swiftlets (Aves: Collocalliinae) was constructed based on mitochondrial cytochrome *b* DNA sequences. This phylogeny is congruent with previous phenetic analyses of morphometric data for the lice. Comparison with a previously obtained phylogeny for the hosts indicates some degree of cospeciation. These cospeciation events are used to compare relative rates of evolution in the birds and their lice for the same segment of the cytochrome *b* gene. Cytochrome *b* is evolving two to three times more rapidly in lice than in birds, and louse cytochrome *b* is highly divergent compared to that of most other insects. Although generation time has been suggested as an explanation for the disparity in evolutionary rates between lice and their hosts, and the small effective population sizes of lice coupled with founder events occurring during transmission to new host individuals may be an important factor The use of it has also been recommended in taxonomic studies, with the proposal that all described species are given an mtDNA sequence tag or bar-code (Hebert et al. 2003). Indeed, the use of mtDNA differentiation in defining taxonomic units has been suggested. While mtDNA is a very useful marker, its use is not without complication. It was recognized at the outset that mtDNA was strictly a marker for historical processes in females; should male and female history differ in a species, then this marker would not reflect the history of the species as a whole but that of the female portion Further, there have been technical issues arising from the presence of nuclear integrations of mtDNA sequence. mtDNA integrated into the nuclear genome may still amplify with conserved primers aimed at mitochondrial copies, complicating or confounding analysis (Bensasson et al. 2001). Further to these known problems, Ballard & Whitlock (2004) have recently argued that mtDNA evolution is non-neutral with sufficient regularity to question its utility as a marker for genomic history. Direct selection (selection Proc. R. Soc. B (2005) 272, 1525–1534 on mtDNA itself) and indirect selection (selection arising from disequilibrium with other maternally transmitted genes) is sufficiently common to make inferences from mtDNA data unreliable.

The effects of inherited symbionts

It has long been recognized that many arthropods carry passenger micro-organisms: microbes that exist inside the cells of their host and pass from a female to her progeny through the egg. These micro-organisms can broadly be classified into two kinds: beneficial to the host and parasitic. In the case of the former, treatment of the arthropod host with antibiotics produces a decrease in host fitness (and commonly infertility or death). In the case of the latter, the drive that produces the spread of the micro-organism is frequently the manipulation of host reproduction towards the survival of infected females and the daughters of infected females. Beneficial symbionts are found widely in arthropods and other invertebrates. The majority of aphid species, for instance, depend on the presence of the bacterium Buchnera to be able to synthesize essential amino acids. Cockroaches, termites and a variety of Hemiptera (e.g. white fly), Diptera (e.g. tsetse fly), Hymenoptera (e.g. carpenter ants) and Coleoptera (e.g. weevils) likewise rely on beneficial symbionts. Feeding on depauperate diet (blood throughout life; phloem; nitrogen-poor wood) are strong predisposing factors for the occurrence of these beneficial micro-organisms. The associations between beneficial symbionts and their host can be very long, as indicated by cocladogenesis of symbiont and host (e.g. Bandi et al. 1998). The observation that they have genomes that have shrunk massively over the course of symbiosis indicates that these beneficial symbionts undergo repeated selective sweeps associated with genomic rearrangements during the early parts of their association with the host (Wernegreen 2002). Parasitic passenger micro-organisms are found even more widely. These micro-organisms show a variety of phenotypes associated with promoting the production and survival of infected daughters (which can transmit the maternally transmitted micro-organism) via negative effects on the production and

survival of infected males and uninfected females (which cannot). In the simplest case, this involves creating a female bias in the host sex ratio. This is represented by cases of parthenogenesis induction in haplodiploid species and feminization in Crustacea. In a twist to this manipulation, killing of male hosts during embryogenesis is common in insects. Here, the advantage derives from removing any negative effects that male hosts may have on their sisters. Examples are found in Hemiptera, Hymenoptera, Diptera, Coleoptera and Lepidoptera. Even in Drosophila, which for ecological reasons are not the most likely to bear male-killers, there are 14 records of male-killing bacteria, indicating these parasites are widely present (Hurst and Majerus 1993). The incidence is certainly higher in other species, such as the coccinellid beetles. The most commonly observed form of parasitism is cytoplasmic incompatibility, which differs subtly in logic from sex ratio distortion. In this manipulation, zygotes formed following fertilization of an uninfected egg with sperm from an infected male die during early embryogenesis. This behaviour produces the spread of the infection in structured populations, as the bacterial phenotype is essentially one of selectively killing uninfected individuals. Cytoplasmic incompatibility has been described in insects, mites and crustaceans, and is probably very common (although cryptic, as frequently all individuals within a population are infected; (Stouthamer et al. 1999). Two micro-organisms, Wolbachia and Cardinium, are known to induce it (Breeuwer et al. 1992; Hunter et al. 2003). In total, these parasitic interactions are common. In large surveys, repeatable over geographical regions and across arthropod groups, Wolbachia alone infects in excess of 20% of insect species at any point in time (Werren et al.1995;Werren & Windsor 2000; Jiggins et al. 2001; Hurst and Jiggins 2005) and, in a limited survey, just over 50% of spiders (Rowley et al.2004). The bacterium Cardinium infects around 7% of arthropods (Weeks et al. 2003).The interactions between parasitic symbionts and their hosts are relatively short-lived in comparison to beneficial symbioses. While cocladogenesis of host and Wolbachia is sometimes observed (e.g. Marshall 2004), studies on focused host clades have indicated it is rather rare (Shoemaker et al. 2002). The mean lifespan of any particular interaction is therefore generally less than the mean time to speciation. This conclusion is reinforced by the observation of three actively spreading Wolbachia infections in natural populations (Turelli and Hoffmann 1991; Hoshizaki and Shimada 1995; Riegler and Stauffer 2002). Thus, we can conclude that the 20% incidence reflects a pattern where new infections must arise relatively commonly. As a final complicating feature, it should be noted that a single population may be infected with more than one strain or species of parasitic inherited microorganism and that different populations may show different infection statuses Finally, recent study has revealed the widespread presence of ‘secondary symbionts’. These are vertically transmitted micro-organisms that are not essential but appear to be locally beneficial (e.g. enhancing resistance to parasitoids and pathogens or adaptation to growth on a particular species of host plant; (Oliver et al. 2003; Ferrari et al. 2004; Tsuchida et al. 2004). These can be common and it is known that their frequency varies geographically (Tsuchida et al. 2002). What is uncertain is the extent of disequilibrium with mtDNA, which will be inversely related to the rate of these symbionts’ horizontal transmission in the field.

Passenger microorganisms

Inherited micro-organisms will influence the population genetics of the host’s mtDNA if they are cotransmitted and therefore in linkage disequilibrium. However, linkage disequilibrium will break down if either the symbiont or mitochondria are paternally or horizontally (infectiously) transmitted with sufficient frequency (Turelli et al. 1992).This process is equivalent to recombination breaking down the linkage between nuclear genes. Horizontal or paternal transmission of both symbionts and mtDNA has been documented in insects. Despite this, symbionts in natural populations are typically found in linkage disequilibrium with mtDNA (table 1), suggesting that such transmission is so infrequent as to be unimportant. For example, paternal transmission of both Wolbachia and mtDNA has been recorded in laboratory populations of Drosophila simulans but it is sufficiently rare that they remain in linkage disequilibrium in the field (Hoffmann and Turelli 1988; Kondo et al. 1990; Turelli et al. 1992). However, there are some species where infectious transmission of symbionts is so common that there is unlikely to be any association between the microbe and host mtDNA (Huigens et al. 2000).Provided the assumption of linkage disequilibrium is met, if a population becomes infected with a symbiont that has sufficient drive to spread, the mtDNA type associated with the initial infection will hitchhike through the population (‘indirect selection’ on the mtDNA). This process has been recreated in laboratory populations of the mosquito Aedes albopictus infected with a Wolbachia strain that induces cytoplasmic incompatibility (Kambhampati et al. 1992). The most remarkable example, however, comes from Californian populations of D. simulans. These were originally uninfected, but during the 1980s they were invaded by a Wolbachia strain that induced cytoplasmic incompatibility (Turelli and Hoffmann 1991). This infection rapidly spread to a high prevalence and carried with it a mtDNA haplotype that was previously rare or absent in uninfected Californian populations (Turelli et al. 1992). Once the infection neared fixation, the original mtDNA haplotype was completely replaced by the haplotype linked to the symbiont. The sweeps of mtDNA seen in California D. simulans are probably regular events in insects. Despite being transient events, the spread of a new Wolbachia strain over space has also been documented in the delphacid bug Laodelphax striatellus

and the fly Rhagoletis cerasi (Hoshizaki and Shimada 1995; Riegler and Stauffer 2002).These observations, together with the lack of cocladogenesis between hosts and symbionts discussed above, indicate that new interactions (which must involve symbiont spread) occur during the lifespan of many species. It is clear from these studies that mtDNA within a population is affected by symbiont spread. Further, the ability of symbionts to spread between populations by occasional movement of hosts, and the ability of different host populations to maintain different symbiont strains, can also confound interpretation attempts to reconstruct the phylogeography of a species. The drive associated with symbiont manipulation can even result in the spread of the symbiont into a new species following an occasional hybridization event. This will homogenize the mtDNA of different species. We now review the effects that symbionts may have on patterns within populations, between populations and between species variation in mtDNA.

Recently diverged species

At first sight, passenger micro-organisms are expected to affect the dynamics of mtDNA within populations but not the branching pattern of mtDNA on a phylogeny. However, two case studies indicate this is not necessarily the case (Hurst and Jiggins 2005). First, there is the A. encedon and A. encedana species pair. These both bear a male-killing Wolbachia infection, and evidence from the Wolbachia sequence indicates they are very closely related strains (note that A. encedon also has a second, more distantly related, infection). When the phylogeny of individuals of the different species is constructed based on mtDNA, A. encedon and A. encedana individuals that bear the same Wolbachia infection bear identical mtDNA sequences, distinct from that found in uninfected.A. encedon individuals. While the species clearly are distinct on morphological grounds, on the grounds of nuclear DNA sequences and in terms of genetic isolation, they appear identical on the basis of the mtDNA sequences of the infected individuals. The most likely explanation for this is that rare hybridization events, although producing very little in the way of flow of nuclear genes, can produce the transfer of the male-killer and associated mitotype from species to species. This male-killer has a drive mechanism that results in its increasing in frequency, despite initially being in poorly adapted hybrid individuals, and the infection and associated mitotype spreads into the new species. This transfer and fixation of mtDNA following hybridization would appear at first sight to be a rare event. However, it has also been observed in Drosophila. In Drosophila, mtDNA has introgressed between D. simulans and D. mauritiana, associated with Wolbachia infection (Rousset and Solignac 1995; Ballard 2000b). Anecdotally, three out of four cases we have studied in our laboratories have shown evidence of symbiont-induced mtDNA introgression confounding phylogenetic estimation from an mtDNA sequence.It is possible that symbiont-driven introgression may explain some recent case studies where mtDNA phylogenies conflict with those obtained from nuclear DNA. (Shaw 2002), for instance, observes that while certain crickets of the genus Laupala in Hawaii do not differ in mtDNA sequence, they do exhibit substantial differentiation at nuclear loci. This incongruence points to selection causing introgression of the mtDNA following hybridization. A good hypothesis for this observation is that hybridization carries novel symbiont infections. The spread of the introgressed symbiont would then be associated with the spread of introgressed mtDNA, homogenizing mtDNA variation across the species boundary despite a high genetic integrity of the species as recorded on nuclear markers.

Conclusion

One recent and contentious use of mtDNA sequence is in DNA bar-coding (Hebert et al. 2003; 2004a). In DNA bar-coding, the sequence of the mitochondrial 648-bp region cytochrome oxidase subunit 1 (COI) gene is used for the purpose of taxonomic identification and assessment of biodiversity, with the philosophy that for each species there is one bar-code (and reciprocally, one bar-code indicates a given species). DNA bar-coding relies on there being low levels of mtDNA variation within a species compared with differentiation between species and monophyly of mtDNA within species. While there may generally be low mtDNA diversity within a species, and species may frequently be delineable by mtDNA, this pattern can be disrupted. While a review of species polyphyly on the basis of mtDNA suggested 23% of species may not be monophyletic for mtDNA sequences (Funk and Omland 2003), bar-coding tests have not revealed this pattern (Hebert et al. 2003; 2004a). However, with notable exceptions (Hebert et al., 2004b), past tests have tended not to explicitly test the ability to discriminate a range of closely related sibling species but rather a range of congenerics, many of which are relatively distantly related. Further, the sample size used when testing intraspecific variation has often been limited, with one or two extra individuals within known species obtained and found to possess very similar COI sequences to those previously found. Indeed, where they have been found to carry divergent sequences, the inference has been made that the process has revealed cryptic species, rather than that mtDNA can act as a poor marker. mtDNA alone cannot be used to reliably infer population history or the history of closely related species as there is a very high probability of an incorrect conclusion due to indirect selection arising from the presence of a symbiont. Reciprocally, it is also problematic to infer symbiont history from mtDNA alone. There are several records of

inherited bacteria being present in nematodes (Adams and Eichenmuller 1963; Marti et al. 1995; Sironi et al. 1995) and also records of inherited bacteria in disequilibrium with mtDNA in mollusks (Hurtado et al. 2003). It should not be assumed that taxa lack symbionts until careful surveys have been carried out because recent history indicates that even well studied groups (e.g. filarial nematodes) can in fact be covertly infected with inherited symbionts (Sironi et al. 1995).

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Parasitic adaptation : trends since Darwin

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Abstract : Parasitic adaptation in Darwin's speculation was a 'degenerate'. The morphological and biochemical reductions found in adult parasites can be considered as an economy of effort rather than a degenerate condition. Parasitic adaptations are all responses to particular features of the parasite's environment, and with the exception of the host's immune response and parasitic virulence the same features are also found in free-living environments. In order for a parasite to continue its mode of life it or its offspring must find a new host the attribute is called 'infectivity'. Hosts evolve to decrease the effectiveness of their parasites' adaptation**s**, and parasites evolve to decrease the effectiveness of their hosts' defenses. Virulence inevitable in parasitism is the decrease in fitness of a standard host caused by association with a parasite. Increase in host's fitness via increased resistance usually invites decrease in parasite's fitness, whereas tolerance is the mode where host gets fitness without necessarily reducing parasite fitness. Parasite should evolve into a less virulent because death of a host should have negative effect on parasite's survival. Parasitic adaptation and host parasite coevolution in terms of two attributes-infectivity and virulence have been discussed in the present communication.

Keywords **:** *coevolution, infectivity, symbiosis, virulence*

Introduction

Evolution of parasitism and parasitic adaptation are the topic of discussion since a long time. Several views are available even in the literature of early nineteenth and several reports on morphological, physiological, biochemical and also immunological aspects have been dealt with due consideration but moot-point on the evolution of sickness due to parasite's virulence has emerged rather recently. In a parasitic association the negative effects on the host's fitness may vary not only between host species, genotypes and individuals but even between ecological conditions and subpopulations (Dybdahl and Storfer, 2003). This reduction in fitness is principally concerned with the characteristic variable called virulence. Because the long term persistence of a pathogenic species entwined with host survival it requires balancing conflict that is selective forces at the individual and population level as well as trade-off between pathogen and host evolutionary strategies (Price, 2008). Parasitic adaptation in Darwin's speculation was a 'degenerate'. Darwinism lies in the very notion of symbiosis itself, quite apart from the 'cooperative' nature of symbiotic associations. There is no empirical evidence that leads to suppose that natural selection will as a general rule lead to anything that may sensibly called 'progressive'. During transition from prokaryotes to eukaryotes symbiosis played a crucial role. The morphological and biochemical reductions found in adult parasites either in protozoa or helminthes can be considered as an economy of effort rather than a degenerate condition. The concept that parasitism evolves towards becoming harmless to each of the partners has now opened the way to further analysis. Adaptation to physical environment differs from that of the parasitic organisms which practically explore in living environment. In the later, adaptation of species rather induces reciprocal biochemical and genetic response, as the other species itself evolves in specific ways to alleviate those evolutionary changes. Infectivity and virulence appears also to play momentous role in parasitic adaptation.

Discussion

In order for a parasite to continue its mode of life it or its offspring must find a new host. The host finding capacity associated with the susceptibility and resistance of the host is referred to as infectivity. Apparently it seems to be a difficult environment for invasion but those organisms that have done successfully so have often been very successful both in terms of number of individuals and also in number of species. This attribute is expressed as :

Infectivity or **I**= host finding capacity of the parasite + susceptibility of host/ resistance of host.

Infectivity of parasites may be compared with the capacity of the free living organisms to get distribution and dispersion, failure of which may cause hindrance in adaptation as well as survivality.

On the other hand the extent of damage on host by the parasite i.e.

Pathogenecity is expressed as: **P= I** x number of parasites in host x damage to host per parasite.

Virulence : The most common definition of virulence is the mortality caused by a parasite in a standard host. Virulence is the decrease in fitness of a standard host caused by the association with a parasite. Though there remains no scale to quantify the negative effects exerted by the parasite on the host it is related to the virulence of the parasite. No doubt direct mortality reduces the fitness. On the other hand, reduced fecundity due to shift in nutritional and behavioural status or interaction with competition or predation, parasitic castration where fecundity is zero are added to the mortality attribute. Virulence has also more to do with host specificity and generality. There are evidences that a simple mutation in the genetic constitution of the parasite may lead to virulence. *Yersinia pestis* the causative agent of human virulent plague differs in having two mutations than the mild pathogenic form *Y. pseudotuberculosis*.

Resistance and Tolerance

Hosts in the interacting association bears opposite force against parasitic virulence. While virulence is the harm on a standard host, resistance on the other hand measures the harm or reduction in harm done to a host by a standard parasite. In addition to the immunological strategies, a single mutation in the host causes resistance to parasites infectivity as in the cases where sickle cell anemic or Duffy negative blood group people are shield from the infection of *Plasmodium*.

If the parasite induced mortality level drops by evolutionary time there remains no reason to think that virulence has decreased. Another important aspect in host parasite association is the evolution of tolerance. Tolerance ensures the reduced level of immunopathology.

Increase in host fitness via increased resistance usually invites decrease in parasite's fitness, where as tolerance is the mode where host gets fitness without necessarily reducing parasite fitness.

In the early phase of association the host and the parasite are not going to be well adapted with each other. This is expected that recently evolved parasites to be virulent, but gradually the host would be more tolerant of the parasite and the obvious question arises: Acute? Chronic? Mutualism?

One paradigm states that parasite should evolve into less virulent mainly because death of a host should have a negative effect on parasite's survival (Clayton et al, 1999).

The emerging concepts on virulence are as followed :

1. **Red Queen's Hypothesis and Evolutionary arms race :** Literary Red Queen's race is an incident that appears in Lewis Carroll's *"Through the looking glass"* and involves the Red queen a representation of a Queen in chess and Alice constantly running but remaining in the same spot. This term has also been used by Isaac Asimov to illustrate the concept of predestination of paradox.

For an evolutionary system, continuing development is needed just in order to maintain its fitness relative to the system it is co-evolving with (http://pespmcl.vub.ac.).This hypothesis intends to explain:

1. *the advantage of sexual reproduction at the level of individuals and*
2. *constant evolutionary arms race between competing or interacting species*.

The first segment is a micro evolutionary version while the second one is macro evolutionary that tends to explain the probability of extinction of groups of organisms is hypothesized to be constant within the group and random among the groups.

The Red Queen's Hypothesis was formulated by Valen (1973) as an explanatory tangent to his proposed Law of Extinction. The hypothesis also provides a conceptual underpinning to discussion of evolutionary arms race. Literally arms race refers to the fact of fighting between two to attain supremacy by each. From the point of evolution, an evolutionary arms race is an evolutionary struggle between competing sets of co-evolving genes that develop adaptations and counter adaptation against each other. Co-evolving gene sets of the interacting species may vary as in a prey-predator or parasite-host association (Vermeji,1987).

Arms Race and Virulence : Coevolution itself is not necessarily an arms race. In parasitism rather an asymmetrical arms race involves opposite selection pressures where parasite is driven to enhance infectivity and the host develops resistance (Dawkins and Krebs, 1979). Further Combes (2000) referred to evolution of parasite virulence and host resistance as

evolutionary arms race. Observation of Jaekel *et al*. (2001) supports this contention. Virulence of *Sarcocystis singaporensis* in wild rat is relatively constant and intermediate with maximal production of bradyzoites. Highly virulent strains may be developed in laboratory mice and immune reaction of these mice reduces the production of bradyzoites (lessen infectivity).

2. Conventional Wisdom : Conventional wisdom (May and Anderson, 1983) explains that well adapted parasite harms its host a little. The evolutionary trajectories of a parasite host association is towards a mutualistic or at least a commensal symbiosis. Less harm a parasite does better the host does. Virulence was considered as an artifact of recent host parasite association (Dubos, 1965; Burnet and White, 1972). Thus-

i. Individual hosts are healthier and survive a longer

ii. Host populations are larger providing more opportunities for parasite's offspring and

iii. Host may evolve weaker parasite defenses.

The logic is really pleasant to human sensibilities (Levin, 1999).

Evidence supporting the trajectories :

- Syphilis (*Treponema pallidum*) epidemic in Europe during 1493 – 1510 appeared to have less virulent in time. In contrast HIV is transmitted between humans and its association with Homo sapiens is almost universally considered recent (Essex and Kanki, 1988).
- Some virulent pathogens like *Shigella* and *Neisseria gonorrhoeae* humans act as the dominant host as well as the vector for the infectious transmission (16). Lethal pathogen of tuberculosis have had a long history in human population and also been transmitted solely by humans (Levin, 1999).

Is conventional wisdom is over simplified? Conventional wisdom is not based on the hypothesis that can be readily tested or rejected. Short term individual selection will often lead to higher virulence that may eventually lead to extinction of the host population and the parasite lineage along with it. On the other hand evolving and becoming prudent in treating their hosts require some form of group level or kin selection (Frank, 1996). Similarly the question arises how a host evolutionary response unilaterally converts an otherwise virulent parasite into a commensal. Conventional wisdom theory does not account for the actual mechanisms responsible for the evolution of benign association between parasites and their hosts.

3. Tradeoff and the enlightenment : This is based on the epidemiologic model referred to the "enlightenment" (Levin and Sanborg, 1990). The theory of the evolution of virulence has taken the approach of arguing evolutionary changes in virulence are a result of tradeoff between virulence and transmission of diseases between the hosts. According to this view natural selection could favour the evolution and maintenance of virulence in commensal as well as in other symbiotic associations. In other words, virulence could be the primitive stage of the association . Direction of natural selection in a given situation depends on the epidemiology and ecology of the parasite and in particular, the relationship between its virulence and its rate of infectious transmission in the host population. While the existence of the parasite mediated selection and evolution in the host population are acknowledged, the enlightenment view has concentrated on the changes in the parasite population (Levin, 1999). At any given host density the fitness of the parasite in the host population is directly proportional to its transmissibility.

This agrees with the "Conventional wisdom" but from an individual selection perspective :

- Being less virulent just means that parasite's own host will survive longer
- The parasite will be getting longer time to reproduce.

This has been postulated that natural selection would favour highly transmissible, incurable commensals. On the other hand if the transmission and virulence in were positively coupled, natural selection could favour the evolution and maintenance of some level of virulence in the parasite population. At present much of the research on the evolution of virulence has focused on the association between two components:

i. The rate at which the parasite is transmitted between hosts and

ii. The rate of parasite mediated mortality in individual infected hosts.

If these relationships are positive then some level of virulence may be favoured (Levin 1999).

Experimental evidence : Evidence of host parasite coevolution comes from the experiment on myxoma virus. Myxoma viruses are the members of the genus *Leporipoxvirus* and native to specific species of rabbit of the genus *Salvilagus* found in California and extending south to South America. A South American strain of myxoma virus was intentionally introduced in pernicious feral European rabbit (*Oryctolagus cunniculus*) population in Australia (1950) and was also released with

forethought into the native rabbit population of Europe. The massive mortalities of European rabbits that ensued in both continents are land mark events in discussion of long term host species dynamic modification by a viral agent (Regnery, 2007). The viruses recovered from the then decimated and sometimes more resistant wild rabbit populations were less virulent (with lower level of disease induced mortality) on control laboratory rabbits. Viruses from the wild but attenuated were less active than that which could be achieved in the first stage of the experiment. This was interpreted as evidence for a positive coupling between the rates of infectious transmission and rates of virus induced mortality- a tradeoff between virulence and transmission. Highly virulent forms had disadvantage because the killed the rabbits too quickly and thus reduced the time available for the pathogen to be picked up by the flea or mosquito vectors required for the transmission. Viruses that were too attenuated had the disadvantage because they generated fewer skin lesions and had lower densities of circulating virions. This also reduced the rate at which they would be picked up by the vectors and the magnitude of virions to b e picked up at any given bite. Thus where there is a positive coupling between a parasite's virulence and its infectivity natural selection could favour and maintain the virulence of the parasite. A parasite's virulence is constrained solely by the need to keep the host active to facilitate its transmission to new host. Though myxoma virus experiment is a model to explain the tradeoff hypothesis substantive experimental data are still wanting to demonstrate that the increase in virulence generated during a passage experiment is also reflected as increased transmissibility as is necessary for the tradeoff interpretation. Indeed it may well be that an increase in the case of mortality rate of a parasite will be reflected as a reduction in its natural transmissibility.

Though Myxoma virus provides elegant evidence that parasite transmitted at a higher rate in the host population has a selective advantage over less transmissible forms, there is no reason to assume that in general a parasite's rate of transmission will be positively associated with its virulence. Moreover, while there remains no relationship or a negative relationship between transmission and virulence there are some alternative ways by which natural selection can lead to the evolution and maintenance of virulence. These are :

i. **Coincidental evolution (Gould and Lewontin, 1979)**

ii. **Shortsighted within host selection (Levin and Bull, 1994)**

Oincidental evolution : The factors responsible for the virulence of a parasite in a host may have evolved for some purpose other than to provide the parasite an advantage of transmission (Levin, 1999). An analogy is that, the spandrels of a gothic church may frame the frescos and paintings within, but this is not the reason for the existence of the sprendels, rather these are s architectural constraints (Gould and Lewontin, 1979). *Clostridium tetanae* though can proliferate in humans and also produce toxin they are soil bacteria and the effect of toxin may not contribute to their capacity to colonize, proliferate and be maintained in humans to its capacity to be transmitted between human hosts. E. coli a commensal of human gut occasionally held responsible for the morbidity of urinary tract infection and adhesin produced by the bacterium generates painful symptoms. This may confer no advantage for *E. coli* and may in fact leads to clearance of the bacteria from the site infection (Levin and Sanborg, 1990). It is necessary to test the hypothesis that the morbidity and mortality generated by the expression of a specific virulence provides neither a within or between host (infectious transmission) advantage to the parasite.

Shortsighted evolution : This hypothesis states that the morbidity or mortality caused by a parasite infection could be the result of the within host evolution that is shortsighted. In such case virulence actually reduces the rate at which that parasite is transmitted to other host. Out of several known examples the case of Polio virus causing Poliomyelitis is being cited here. Polio viruses normally replicate in the mucosal cells of the mouth, throat and intestine and is transmitted by oral faecal route. Viruses invading and proliferating in the central nervous system (causing pathogenesis) would almost certainly not be transmitted. The evidence in support of shortsighted (myopic) evolution for the virulence of these specific parasitesis mostly circumstantial (Levin and Bull, 1994). On the other hand, shortsighted evolution for the virulence of specific parasite is a hypothesis that can be tested. If the hypothesis is valid the parasite responsible for the symptoms would be genetically different from their ancestors that infected the host and better adapted for proliferation in the sites of the symptoms than the ancestors themselves.

Concluding comments : Results of recent studies by the evolutionary and population biologists predict at least the following :

i. There is a positive relation between the parasite's virulence and rate of infectious transmission – Direct selection

ii. Parasite virulence is due to the character(s) favoured and maintained by natural selection for some other function and the expression of virulence by the parasite in an infected host does not confer to the net advantage or disadvantage in the parasite population at large— Coincidental evolution.

iii. The parasites responsible for the morbidity and mortality of an infection are selected for within the host because of local advantage and evolution reduces the rate at which that locally adapted parasite is transmitted between the hosts — Shortsighted evolution.

Views on evolution of parasitism and adaptation thereof are based on hypotheses only a few of which have been tested or may be tested. Evolution occurs spontaneously in nature and it is still a burning question how much the laboratory or field experiments may be correlated with those spontaneously occurring in nature. It is imperative that biologists would be much cautious before drawing conclusion based on speculations.

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Prevalence and seasonal abundance of protozoan parasites in penaeid shrimp *Penaeus monodon* in high saline bheries of West Bengal

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Abstract : Prevalence and seasonal abundance of protozoan parasites in penaeid shrimp (*Penaeus monodon*) of high saline bheries of West Bengal was carried out for a period of 20 months from February 2000 to November 2001 covering two successive cropping seasons. The water quality parameters like temperature, hardness and salinity influenced the prevalence and distribution of parasites. These variables have definite impact individually or as a whole on the prevalence of parasites of shrimp. The protozoan ectoparasites identified commonly were *Zoothamnium sp*, *Vorticella sp*, *Epistylis sp* and *Acineta sp*. The only endoparasite found was microsporidians (*Nosema sp*) with low Parasitic Frequency Index (PFI). Monsoon was found to be the most favorable season for almost all the parasites (*Zoothamnium sp*, *Vorticella sp*, *Epistylis sp*, *Nosema sp*) except *Acineta sp*, which showed it's proliferation in summer season.

Keywords **:** *Bheries, Protozoan parasites, Penaeus monodon*

Introduction

Bheries are the unique ecosystem that sustains the world's largest wetland, and it happens to be one of the oldest practices of integrated resource recovery, since beginning of the last century (Saha, 1970; Mondol, 1996 and Jana, 1998). They form a large spectrum in the aquaculture field of West Bengal ,both freshwater and brackish water. The present study covers a close surveillance of the incidences of various infectious and non-infectious diseases in the culture shrimps which have more than a considerable impact on the economic statistics of shrimp aquaculture. Poor water quality ,health management, irregular feeding practices and host specificity or preference are some of the key factors that lead to stressful conditions and subsequent parasitic infection of the host organism.

The study was conducted in five traditional shrimp farms, locally called as bheries, randomly selected in high saline region (Latitude 22°10 'N and Longitude 88° 1' E) of West Bengal .Regular monthly samplings of water and shrimps were made from the bheries for period of 20 months between February 2000 and November 2001,covering two successive cropping seasons (C_1 and C_2).The water quality parameters were measured as described in APHA (1998).

Materials and methods

The methods of collection and preservation of samples for ecto and endoprotozoan parasites were followed as described by Mandal and Nandi (1980). The samples from gills, body surfaces, appendages and telson region and also from internal organs such as muscle, intestine and hepatopancreas of live and freshly killed normal as well as diseased or abnormal shrimps, were taken separately. The parasites were observed under Light microscope and identified based on their individual features (Kudo, 1977). Parasitic Frequency Index PFI (%) was calculated by taking the percentage of number of hosts infected by an individual parasite against the total number of hosts examined. The water quality parameters were measured as described in APHA (1998). Correlations for the water quality parameters with parasites were done for different years (Crop -1 and 2) separately (Fisher and Yates, 1963).

Results

The average values of water quality parameters recorded in 5 bheries of high saline region of West Bengal are presented in Table-I. The temperature ranged from 27.5 to 31.5^{0}C. The salinity varied from 19.5 to 21.5 ppt (C_1) and 20.2 to 21.5 ppt (C_2). The lowest pH observed was 7.2 and the highest was 8.1 in crop-1, while the range of pH was 7.4 - 8.1 for crop-2. The ammoniacal nitrogen varied from 0.55 to 0.61 ppm for crop-1 and 0.57 - 0.62 ppm for crop-2. The nitrite nitrogen ranged from 0.004 to 0.017ppm for crop 1 and 0.003-0.016 ppm for crop-2. The lowest transparency recorded was 14.3 cm and the highest 18.0 cm for crop-1 and for crop-2 the range was 13.3-18.0 cm. The hardness varied from 390.0- 485.5 ppm from crop-1 and from

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370 – 475.5 ppm for crop-2. The dissolved organic matter varied from 44.5-58.8 ppm in crop-1 and 46.0-56.8ppm for crop-2. The dissolved oxygen varied from 4.60 to 4.95 ppm for both the crops. The average PFI of *Zoothamnium sp* varies from 33.33 to 32.75% in summer season in crop-1 & 2, where as its prevalence were more in rainy season (49.99 to 50.11 %) in crop-1 & 2 compared to winter. *Vorticella sp* and *Epistylis sp* were found more in rainy season in both the crops, where as Acinetids were dominant in summer season only. *Nosema sp* were more in summer season in both the crops. (Table-II)

Table I : Water quality parameters of the bheries in high saline zone in two cropping seasons [L_3, C_1 & C_2] (Average of 10 Months)

	Station	Temper-ature (ºC)	Salinity (ppt)	pH	Ammoniacal-N (ppm)	Nitrite-N (ppm)	Trans-parency (cm)	Hardness as $CaCO_3$ (ppm)	DOM (ppm)	DO (ppm)
C_1	S_1	28.8	21.2	7.6	0.55	0.011	14.3	461.5	50.1	4.95
	S_2	29	21.5	7.9	0.57	0.006	17	430	58.8	4.71
	S_3	30.5	19.5	7.4	0.59	0.017	18	390	47	4.8
	S_4	29.8	20.5	7.2	0.61	0.004	15.5	485.5	48	4.6
	S_5	31	21.5	8.1	0.58	0.005	17	435	44.5	4.9
C_2	S_1	28	20.2	7.8	0.58	0.012	13.3	453.5	51.1	4.95
	S_2	27.5	20.5	7.6	0.59	0.005	18	435	56.8	4.71
	S_3	31.5	21.5	7.4	0.62	0.016	17	370	46	4.8
	S_4	30.8	20.5	7.7	0.61	0.007	16.5	475.5	49	4.6
	S_5	31	21	8.1	0.57	0.003	18	445	48.5	4.9

Table II : Seasonal abundance 'PFI' (%) of parasites in the penaeid shrimp (*P. monodon*) of high saline bheries in two cropping seasons (L_3, C_1 & C_2) (Average of 5 stations)

	Parasite	Summer	Rainy	Winter
C_1	Z	33.33	49.99	48.09
	V	19.75	39.75	37.73
	E	42.37	44.04	35.23
	A	61.11	56.31	60.85
	N	7.83	3.33	7.37
C_2	Z	32.75	50.11	47.55
	V	20.12	40	38.85
	E	41.62	43.95	36.11
	A	61.23	52.9	60.05
	N	8.12	3.85	7.56

Z-*Zoothamnium*, V–*Vorticella*, E–*Epistylis*, A–*Acineta*, N–*Nosema*

Discussion

The observations indicated that temperature was positively correlated with *Nosema sp*, and *Acineta sp* for both the crops. This implicated that higher temperature favour the growth of *Acineta sp* at higher saline region. In the higher saline bheries salinity was negatively correlated with *Zoothamnium sp* and *Vorticella sp* for both the cropping seasons and *Nosema sp* for crop-2 only. It was observed that *Zoothamnium sp and Vorticella sp* showed their preference towards lower salinity and *Acineta sp* seems to prefer higher salinity. The results corroborated with the work of Kudo (1977). In high saline bheries *Acineta sp* and *Nosema sp* showed their positive correlation for crop-1 and 2, respectively.

Table III : Correlation matrix (r) of protozoan parasites verses variables of high saline bheries. (L_3, C_1 & C_2)

CROP	Water Quality Parameters									
	Parasite	Temp (ºC)	Salinity (ppt)	pH	Ammo-N (ppm)	Nitrite-N (ppm)	Trans (cm)	Hardness (as $CaCO_3$) (ppm)	DOM (ppm)	DO (ppm)
C_1	*Zooth*.T	-0.003	-0.548*	-0.012	-0.061	0.383*	-0.055	-0.772*	-0.786*	0.739*
	Vort.T	0.209	-0.544*	-0.192	0.013	0.340*	-0.191	-0.791*	-0.696*	0.570*
	Epis.T	0.022	-0.138	0.195	-0.132	0.287*	0.097	-0.392*	-0.558*	0.343*
	Acin.T	0.490*	0.116	0.512*	-0.627*	0.155	0.357*	0.120	-0.297*	0.091
	Nose.T	0.494*	-0.247	-0.373*	0.404*	-0.088	-0.499*	-0.278	-0.124	0.002
C_2	*Zooth*.T	-0.001	-0.495*	-0.015	-0.124	0.216	0.130	-0.775*	-0.796*	0.556*
	Vort.T	0.163	-0.487*	-0.175	-0.021	0.208	0.058	-0.816*	-0.727*	0.448*
	Epis.T	0.053	-0.083	0.189	-0.090	0.171	0.148	-0.382*	-0.493*	0.273
	Acin.T	0.468*	0.184	0.530*	-0.691*	0.120	0.418*	0.130	-0.321	-0.034
	Nose.T	0.433*	-0.302*	-0.504*	0.409*	-0.195	-0.326*	-0.314*	-0.242	0.192

Degree of Freedom = 48; n=50 *; P<0.05; T-Sin arc transformed value, (n-2)

The low pH favours the growth of microorganisms. The average low pH values may be attributed due to sewage dilution with bhery water, which supports the growth of peritrich ciliates like *Zoothamnium sp* and *Epistylis sp.* The pH and temperature of water regulate the portion of total ammonia, which occurs in unionized form. In high saline zone ammonical-N was negatively correlated with *Acineta sp* and positively with *Nosema sp* (Table-III) for both the crops. Also, nitrate-N is toxic to crustaceans particularly to *P. monodon*. Nitrite-N has also been found to oxidize the respiratory pigments and cause hypoxia to penaeid shrimps (Lightner, 1983). The mean values varied from 0 to 0.05 ppm in both the crops, which was much less as compared to recommended safe concentration (Law, 1988). In high saline zone nitrite-N was positively correlated with *Zoothamnium sp, Vorticella sp and Epistylis sp* in 1st crop only (Table- III).

The sewage mixed water in bheries resulted in heavy plankton growth, thereby reduced the transparency level. Transparency was positively correlated with *Acineta sp* and negatively correlated with *Nosema sp* for both the crops (Table-III). Hardness was found to change according to the fluctuations of pH in culture ponds. However, the mean values varied from 370.0 to 485.5 ppm, for both the crops, which was higher than the recommended range. Though high hardness acts as prophylactic means to different types of diseases, Romero and Jimenez (1997) opined that the high hardness level (>300 ppm) enhanced the growth of ciliates, which partly supports the present observations. In this zone, the hardness was negatively correlated with *Zoothamnium sp, Vorticella sp and Epistylis sp*. While *Acineta sp* showed positive correlation for both the crops. This indicated *Acineta sp* only preferred high hardness level. However, *Nosema sp* showed a negative correlation in crop-2 only. These observations were similar with the earlier works (Kudo, 1977). *Acineta sp* was positively correlated with salinity, hardness, pH, ammonia and dissolved organic matter and negatively correlated with nitrite and transparency. The high PFI value for both the crops confirmed the positive correlation with salinity and coincided with the description of Camacho and Chinchilla (1989) about their preference towards saline environment. The higher hardness and salinity along with the high PFI in respective location and crop indicated their preference for growth and proliferation.

The present record of investigation with high PFI value of *Acineta sp* coincided with low PFI value of *Vorticella sp and Epistylis sp*. in the respective location and crop confirmed the description of Camacho (1987). This may be due to the preference of Acinetids towards higher hardness and salinity followed by its enormous appetite. They feed on their relatives, more particularly ciliates like *Vorticella sp and Epistylis sp. and Zoothamnium sp.* Dissolved organic matter was positively correlated with *Zoothamnium sp* and *Vorticella sp* in both the crops and *Epistylis sp* in crop-1 only in high saline environment. According to Romero and Jimenez (1997) the poor water quality condition might allow the proliferation of large number of fouling organisms mostly peritriches ciliates. Generally, high nutrient rich water commonly found in culture situations tend to favour

the proliferation of these organisms, which feed by filtering nutrients or microorganisms from the water. The intensity of peritrich ciliates was correlated with water quality and organic detritus as described by Lightner (1983), which also supports the present observation. The results of study on these bheries also showed the increased dissolved organic matter with increased salinity and hardness. In higher saline zone dissolved oxygen was positively correlated with *Zoothamnium sp* and *Vorticella sp* for both the crops and *Epistylis sp* for 1st crop only (Table-III). High dissolved oxygen levels greatly reduce stress factors, so that growth and propagation of fungi and parasites can be kept down. The lowest value observed was much higher than the value (2.5 ppm) (Lightner, 1983), which ruled out the lethality of shrimp due to *Zoothamnium sp.* association.

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Invitro studies on antihelminthic activity of seeds of *Butea frondosa* on the metacercaria of *Euclenostomum heterostomum* a parasite of fresh water fish *Channa punctatus*

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Abstract : The present study is to prove the effect of *Butea frondosa* seeds as antihelminthic in the progentic metacercaria of *Euclenostomum heterostomum*. The progenetic metacercaria of *Euclenostomum heterostomum* is a parasite in the liver, kidney and viscera of fresh water fish *Channa punctatus*. 3 different concentrations were prepared by adding 10ml of Tyode solution to 10µM,100µM,300µM extract.Glycogen content in presence and absence of glucose was estimated. Decreased levels of glucose and glycogen and increased levels of lactic acid was observed. These results indicates the blockage of uptake of glucose which leads to energy crisis. Increased tissue lactic acid levels implies that either malate pathway is inhibited or excreation of lactic acid from parasite is blocked which leads to the death of the parasite.

Introduction

Helminthic infestation undoubtedly constitutes a major medical,veternary and public health problem all over the tropical and sub tropical regions of the world, particularly in developing countries. *Channa punctatus* is economically important and abundantly available fish. The heavily parasitized fish looked pale in colour and lethargic in behavior which will finally lead to mortality. The intensity of infection is more during warmer months,attaining peak in the month of june. To curtail the disease several chaemotherapeutic drugs were used, which were costly beyond the reach of farmers and their toxicity to the host are posing serious threat. Alchohalic extract of *Butea frondosa* seeds showed significant antihelminthic effect. The trees are found in greater parts of India. The seed oil contain glycerides of palmitic, stearic, lingoceric and linolic acids. seeds of *Butea frondosa* have been used as a dependent antihelmintic agent in indigenous systems of medicine (Kartikar and Basu., 1933, Mukerji and Chopra 1950; Chopra et al., 1956; Dasturi, 1962 ; Mehta and Praskar 1966; Rao et al., 1977 and Satyanarayana and Krishnaiah, 1982).

The present study is therefore undertaken, to elucidate the possible biochemical mechanisms of seeds of *Butea frondosa* in vitro, taking mebendazole as a reference drug. The study may also be helpful in developing better and cheaper indigenous antihelminthic drugs.

Materials & methods

Glycogen content in presence of glucose, absence of glucose were studied in the metacercaria of Euclinostomum heterostomum. The seeds were collected from the fruit, washed thoroughly. 50gms of dried seeds were grounded by using mortar and pestle. The aqueous extract of the seeds was prepared with sterilized distilled water and filtered through a muslin cloth. The filtrate was allowed to boil at 50°C for 2 hours to make it more concentred. The metacercaria were collected and kept in seven tissue baths with Tyrode solution. Ten metacercaria in each tissue bath.One tissue bath as control. *Butea frondosa* seed extract was added in three tissue baths 10 µM, 100µM, 300 µM concentrations. And incubated for 2 hours. Mebendazole is taken in remaining 3 tissue baths 3 µM,10 µM and 100 µM were incubated for 2 hours. After incubation biochemical analysis was done by taking 10µM of standard drug mebendazole to compare the results and 5% of homogenate was used for biochemical studies. Glucose uptake studies were undertaken following Van den Bossche (1972), and Ahmed and Nizami (1987).

After incubation the amount of glucose was estimated by the method of Holtman(1959), glucose by using Hassid et al., (1957) and Holtman (1959), lactic acid by using Barker and Summersan, (1942) method.

Results

The effect of *Butea frondosa* seed extract and mebendazole on glucose uptake is presented in the Table I. *Butea frondosa* extract at 100µM and mebendazole at 10 µM significantly inhibited the glucose uptake of *Euclinostomum heterostomum*, and

significantly decreased the tissue glycogen content. While the decrease in tissue glycogen with mebendazole 10 µM was comparable to 100 µM of *Butea frondosa* extract ($P < 0.05$). The lactic acid levels are increased significantly (P, 0.001).

Table 1 : Invitro Effect of Mebendazole and seed extract of *Butea Frondosa* on glucose uptake and glycogen content and lactic acid production in the metacercaria of *Euclinostomum heterostomum.*

	Control	Mebendazole 10uM	Butea seed extract		
			10uM	100uM	300uM
Glucose uptake (mg%) n=6	4.5+0.20	2.62+0.16	3.18+0.14	2.81+0.19	1.65+1.50
Glycogen content (mg%) in the presence of glucose in incubation medium n=6	3.18+1.3	1.32+0.20	2.14+0.13	1.47+0.15	1.14+0.20
Glycogen content (mg%) in glucose free medium n=6	4.23+0.7	1.22+1.40	2.01+0.13	1.54+3.31	1.18+1.20
Lactic Acid (ug/g) n=6	218+1.6	308+1.60	402+2.50	496+2.60	510+2.50

Significant as control to the untreated control $P<0.05$ in case of lactic acid $P<0.001$.

Discussion

The metabolic pathways particularly the carbohydrate metabolism is the major target site for the action of antihelmintic drugs. These targets include, inhibition of glucose uptake, inhibition of glycogen metabolism, inhibition of fumarate reductase and uncoupling of phosphorylation (Van den Bossche,1980; Wang,1984 and Sharma 1987).

Keeping this in view, the present study was conducted to delineate the mechanism of action of seed extract of *Butea frondosa*, with respect to the vital invitro parameters such as glucose uptake, glycogen depletion and parasite tissue lactic acid content. MBZ known to inhibit gloucose uptake and glycogen depletion (Van den Bossche, 1972; Rahman et al., 1977 and Sharma,1987) was included as a reference standard drug to compare the activities of butea seed extract. Mebendazole is also known to increase the lactic acid production (Rahman et al., 1977; and Pampori et al., 1984).

Although, on the basis of molar concentration, MBZ was more potent in inhibiting the glucose uptake invitro in the metacercaria of *Euchinostomum heterostomum.* seeds extract of *Butea frondosa* also was equipotent in inhibiting the glucose uptake. These results suggest that seed extract can give rise to energy crisis consequent to the blockage of the uptake of glucose, a major source of energy for the parasite. However, the parasite can still metabolize its own glycogen source for the production of ATP (Mc Manus,1987). In view of this possibility, the effect of *Butea frondosa* seed extract was studied on the tissue glycogen level of *E.heterostomum*.

In the presence of glucose in the incubation medium, MBZ, *Butea Frondosa* extract at equimolar concentrations, caused significant depletion of tissue glycogen. The mechanism of action of seed extract of *Butea frondosa* appears to be similar to that of MBZ which has been reported to cause glycogen depletion invitro in the presence of glucose in the medium (Ahmed et al., 1987). Because of the presence of glucose in the medium, it is difficult to assess the effect of the drugs directly on the glycogen metabolism for the fact that glycogen depletion might result consequent inhibition of glucose uptake from the medium to generate ATP to maintain normal physiological functions of the parasite. To eliminate such a possibility, the experiment was designed to study the effect of these drugs on the tissue glycogen level in the glucose free medium. Intrestingly, mebendazole and *Butea frondosa* seed extract failed to alter the glycogen levels of the metacercariae of *Euclinostomum heterostomum* in the absence of glucose.

The increased tissue lactic acid level implies that either the malate pathway is inhibited or the excreation of lactic acid from the parasite is blocked. (Rahman et al., 1977 and Pompori et al., 1984). The possibility of either of the mechanisms can not be ruled out based on the results of the present study. Thus the antihelmintic action of mebendazole and *Butea frondosa* seed extract may result from an inhibition of ATP production (Rahman, et al., 1977; Van den Bossche, 1980; Pompori etal.,1984 and Wang 1984) because of main effect on malate succinate pathway or due to the deleterious effect of accumulated lacticacid.

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Recent advances and application of technologies for diagnosis, treatment, prevention and control of malaria and kala-azar in endemic areas of Bangladesh

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Abstract : The present study was carried out in 13 Upazillas of 13 endemic districts for Malaria and 15 upazillas of 3 endemic districts for kala-azar in Bangladesh to assess the current application of techniques for Diagnosis, Treatment, Prevention for malaria and kala-azar by the professionals.

For Malaria, 300 professionals were interviewed of Upazilla Thana Complexes, they apply Examination of peripheral blood film (40.8%) and Rapid Diagnostic test (38.50%) for diagnosis of Malaria and also tests CBC and MP in small range. About 85% professionals use Quinine as an appropriate drug for treating Malaria, drugs like Chloroquinine, Pyremethamine, Artemisinin and Malaone are also in use.

For Kala-azar, out of 205 professionals interviewed, about 80% practice rK39 dipstick test for diagnosis of Kala-azar while, DAT, AT, Bone marrow examination and Spleen puncture are rare. About 73.3% professionals use SAG, 85.7% Miltefosine and 62.9% AmphotericinB as appropriate drug for Kala-azar.

Professionals believe in use of mosquito nets, awareness campaign, insecticide spray, early detection and treatment of kala-azar and malaria. These modern technologies are widely used world-wide and in Bangladesh for diagnosis, treatment and prevention of malaria and kala-azar, which are safe, have less side-effect and reliable. So, Bangladesh is not behind from the advances in world.

Key words **:** *Technologies, diagnosis, treatment, kala-azar, malaria.*

Introduction

Considerable progress has been made towards three key objectives in tropical infectious diseases in recent years,: new approaches to overall clinical management. Inadequate vector control; poor nutrition, sanitation, and drinking water; civil war; and bare bones health budgets continue to present obstacles to preventing and controlling epidemics. Early scientific results include progress in chemotherapy for Malaria and Kala-azar; in the developing the fundamental knowledge required to develop a vaccine against malaria; and in simple and accurate diagnostic field tests for malaria and kala-azar. In addition, institution strengthening and training support, awarded exclusively to institutions and scientists of developing endemic countries, has increased rapidly. Over 1 billion people are infected with one or more of the 14 Neglected Tropical Diseases (NTD) defined by WHO are most common and living on less than $2 a day Those affected are often marginalized and forgotten by Government, left to suffer in silence. NTD are diverse but all cause severe disability or death, bring a major economic burden on endemic countries (WHO, 2005a).

NTDs are also known as “poverty-related” or “tropical” diseases, are sometimes fatal and inflict severe and permanent disabilities and deformities on almost 1 billion people around the world, especially among the poorest populations in developing countries (Mondal *et al.* 2007, Mondal *et al*. 2008). The neglected diseases impose an enormous economic burden on affected communities due to lost productivity and other issues. While there are some drugs and vaccines for neglected diseases, these interventions do not always reach those who need them - even when the drugs and vaccines are donated.

Drug resistance is most commonly seen in *P. falciparum*. Resistance to chloroquine is most prevalent, while resistances to most other antimalarials like pyrimethamine, quinine, mefloquine, artemesin and quinoline compounds have also been reported. These developments further justify the cause and urgency for formulating an effective vaccine against malaria. The vaccine fulfilling this extreme requirement is the type 2 vaccine. This extreme approach to *Malaria Vaccine- Development* does not take into account specifically populations affected by malaria that fall between these extremes, such as individuals in endemic regions at high risk of *P. vivax* infections (.Akhter *et al.* 2000a)

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Malaria is one of the major public health problem in Bangladesh. Out of 64 districts, malaria is highly endemic in 13 districts and 10.9 million people are at risk of malaria. Three hill tract districts (Banderban, Khagrachori and Rangamati) and Cox's Bazar districts report more than 80% of the malaria cases and deaths every year. In Bangladesh, Both *falciparum* and *vivax* malaria are prevalent in the country of which the number of f*alciparum* cases are 75% of the total cases in recent years due to increasing drug resistance. The first line drug Chloroquinine has been replaces by Artemisinin based Combination therapy. (ACT) for treatment of falciparum malaria cases in 2004. *Anopheles dirus, An. minimus,* and *An. phillipensis* are the principal vectors and all are susceptible to malathion and synthetic pyrethroid. Promotion and use of ITNs/LLINs, selective IRS for containment of outbreaks and intensive IEC for increasing awareness of the people are the main components for the vector control (WHO, 2008). Although diagnosis by microscopic examination of blood films remains standard, rapid immuno-chromatographic detection of circulating parasite antigen has entered the clinical practice. These dipstick strip tests are specific, almost as sensitive as thick blood films, and simple to perform. Polymerase chain reaction testing for plasmodium antigen is most sensitive but is a research tool. (Jima *et al*. 2005, WHO, 2010).

Visceral leishmaniasis is caused by an intracellular protozoan parasite *Leishmania donovani* of reticulo-endothelia system of vertebrates. Kala-azar affects the spleen, liver, bone marrow, etc. and the shin becomes black. PKDL is also prevalent there. Bangladesh has 12 endemic districts for kala-azar and prevalent in 46 districts (Haque et al. 2005, Khanum et al. 2008). .. .

Bangladesh is one of the endemic countries for visceral leishmaniasis in the world, second and largest parasitic killer and also a major impediment to socio-economic development (Khanum and Musa, 1999). In last ten years, a total of 69000 cases were reported in Bangladesh of which 42,780 (62%) cases were from Mymenshingh district. In 2008, 73.6% and in 2009, 71.1% of the kala-azar cases of the country were reported from Mymenshingh MPDC UNIT, DGHS (1963-97), (Malaria and Parasitic Disease Control Unit, Directorate General of Health Services, Mohakhali, unpub.)

The objectives of the present investigation were to assess the knowledge, attitude and practices of technologies for diagnosis, treatment, prevention control for kala-azar and malaria endemic and intervention areas of Bangladesh by the Formal Health care Providers.

Materials and methods

1. Design of the study : A Cross-sectional study was conducted in selected endemic areas of Kala-azar and malaria in Bangladesh. The study was completed during the period from 8th June, 2009 to 13th March, 2010.

2. Study sites : 28 Upazillas have been be selected, 13 for malaria and 15 for kala-azar.

For Malaria The present study was conducted in areas where malaria had been reportedly a problem for many years. The KAP study was conducted among 300 professionals of 13 Upazillas of Thana Complexes and NGOs are working in the same Upazillas are: Kurigram, Moulvibazar, Chittagong, Khagrachari, Rangamati, Bandarban, Cox's Bazar, Sherpur, Mymenshingh, Netrokona, Sunamganj, Sylhet and Habiganj.

For Kala-azar A total of 255 professionals and health service providers were_interrogated. The present study was carried out in 3 districts of Bangladesh comprising 15 upazillas. Intervention areas in Bangladesh, under Mymenshingh district, folloing 8 upazillas are found as kala-azar intervention areas (Narail, Haluaghat, Muktagacha, Gafargaon, Bhaluka, Trishal, Fulbaria and Ishwarganj) and Under Jamalpur district (Shorishabari, Melandah, Madarganj, Dewanganj, Islampur and Sadar Jamalpur) and under Tangail district (Shakhirpur).

3. Study population : Formal health service providers of kala-azar and malaria endemic areas of Bangladesh. Total seven categories of professionals were interviewed from each upazillas, namely UHFPO, RMO, MO, Senior Nurse, SACMO, Medical Assistant of UHC, and Medical Assistant of one of the Union Sub-centers, Health Assistant, Health Inspector, Assistant health Inspector, Different NGOs are working in the selected epidemic/endemic areas of Bangladesh. NGO Worker and NGO Field workers from the NGOs are working for vector borne disease control and treatment of patients.

Data analysis : The analysis of the collected data was done through SPSS version 15.

Results

In the present investigation, it was observed that, out of 300 professionals interrogated, about 40.8% of the professionals have knowledge and practice for **Examination of Peripheral blood film** as the single most important blood test for diagnosis of Malaria and which is also recommended by malaria elimination program. (Table-I) Professionals (38.5%) also recommended Rapid Diagnostic Test for malaria diagnosis.

Table I : The percentage of the professionals regarding the practice and knowledge about blood test for diagnosis of Malaria.

Blood test	Frequency	Percent	Valid Percent	Cumulative Percent
CBC	35	11.5	11.5	11.5
Examination of Valid peripheral blood film	122	40.8	40.8	52.3
Rapid Diagnostic test	116	38.5	38.5	90.8
MP test	28	9.2	9.2	100.0
Total	300	100.0	100.0	

Table II : Percentage of professionals and their knowledge regarding the drug are used for treatment of Malaria.

Drug	Valid		Missing		Total	
	N	Percent	N	Percent	N	Percent
Quinine	255	85	45	16		100
Chloroquinine	69	22.9	231	77.1		100
Pyremethamine	11	3.8	289	96.2	300	100
Artemisinin	189	62.9	111	37.1		100
Malarone	9	2.9	291	97.1		100

Regarding the drugs are used for treating Malaria, majority of the professionals and service provider selected Quinine (85%), Artemisinin (62.9%) Chloroquinine (22.9%), Pyremethamine (62.9%), and Malarone (2.9%) (Table II). For the prevention of malaria from the community, it was observed that, considering the risk factors for malaria infection, the professionals believe the use of mosquito nets, Awareness campaign, Insecticide spray, early detection and treatment of malaria, etc. (Table-III).

In the present investigation, to assess the practice, knowledge and attitude of the professionals and service providers regarding the diagnostic tests, drugs and prevention of kala-azar, it was observed that, out of 255, about 80% of professionals have knowledge and practice of the rk39 dipstick test as the single most important blood test for diagnosis of Kala-azar and which is also recommended by kala-azar elimination program. (Table-IV)

Table III : The Percentage and the knowledge regarding the measures can be taken to prevent Malaria.

	Cases					
	Valid		Missing		Total	
	N	Percent	N	Percent	N	Percent
Drinking clean water	15	5	285	95		100.0%
Use of mosquito net	297	99	3	1		100.0%
Awareness campaign	243	81	57	19	300	100.0%
Insecticide spray inside the house and its surroundings	267	89	33	11		100.0%
Ensure that no crack and crevices remain on muddy walls	246	82	54	18		100.0%
Early detection and treatment of Malaria cases	240	80	60	20		100.0%

Table IV : The percentage of the professionals and their knowledge about the single most important blood test for diagnosis of Kala-azar

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Examination of peripheral blood film	2	1.0	1.0	1.0
	Bone marrow Examination	2	1.0	1.0	1.9
	rK39 dipstick test	164	80.0	80.8	82.7
	DAT	18	8.6	8.7	91.3
	AT	14	6.7	6.7	98.1
	Splenic puncture	4	1.9	1.9	100.0
	Total	203	99.0	100.0	
Missing	0	2	1.0		
Total		205	100.0		

Regarding the drugs are used for treatment of Kala-azar, 73.3% of all interviewed professionals selected, SAG, 85.7% Miltefosine and 62.9% selected Amphotericin B for treatment of Kala-azar.(Table-V).

Table V : Percentage of professionals and their knowledge regarding the drug are used for treatment of Kala-azar.

Drugs	Cases					
	Valid		Missing		Total	
	N	Percent	N	Percent	N	Percent
SAG	150	73.3%	55	26.7%		100.0%
Cephalosporin	6	2.9%	199	97.1%		100.0%
Parmomycin	47	22.9%	158	77.1%	205	100.0%
Eiethyl carbamazine	8	3.8%	197	96.2%		100.0%
Miltefosine	176	85.7%	29	14.3%		100.0%
Amphotericin B	129	62.9%	76	37.1%		100.0%

To assess the attitude and knowledge of the professionals regarding the prevention of Kala-azar from the community, it was observed that the professionals believe the use of mosquito nets (86.7%), awareness campaign (79%), Insecticide spray inside the house and its surroundings (84.8%), Ensure that no crack and crevices remain on muddy wall (96.2%) and early detection and treatment of kala-azar cases etc as preventive measures for Kala-azar elimination. (Table-VI)

Table VI : Assessment of knowledge of professionals regarding the measures can be taken to prevent Kala-azar.

Preventive measures	Cases					
	Valid		Missing		Total	
	N	Percent	N	Percent	N	Percent
Drinking clean water	8	3.8%	197	96.2%		100.0%
Use of mosquito net	178	86.7%	27	13.3%		100.0%
Awareness campaign	162	79.0%	43	21.0%		100.0%
Insecticide spray inside the house and its surroundings	174	84.8%	31	15.2%	205	100.0%
Ensure that no crack and crevices remain on muddy walls	197	96.2%	8	3.8%		100.0%

Discussion

During the ‘Malaria Eradication program‘ through DDT spraying controlled all the transmission of vector borne diseases like malaria, kala-azar, filarial and dengue. In 1970s kala-azar re-emerged sporadically. During 1981-85 only 8 upazillas reported kala-azar which increased to 105 upazillas in 2008. The current estimated total cases are 45000. Annually average 10,000 cases are treated by control program but the cases treated by private clinics, practitioners and NGOs are not always reported. WHO, 2010, Harith *et al.* 1988 and Khanum *et al.* 2008).].

The studies of Akhter *et al.* (2000a) reported a entomological surveillance on vector in a malaria endemic areas of Bangladesh. Akhter *et al* (2000) also assessed the effectiveness of different drug regimen according to the nature of malaria cases in some selected areas of Bangladesh. The studies (Akhter *et al*. 2001, Ahmed *et al.* 2009) on effectiveness and impact of Deltamethrin impregnated bed nets on malaria transmission in some rural areas of Bangladesh.

Chloroquinine resistant *P. vivax* is now being encountered, and *P. falciparum* continues to develop resistance to newly introduced drugs. The two most important groups of drugs for malaria treatment are still based on quinine or artemisinin.. A separate combination, atovaquone plus proguanil (Malarone), is active against malaria around the world. In complicated severe malaria, parenteral administration of artemether and artesunate is as effective as intravenous quinine or quinidine, active against quinine resistant parasites, and often easier to use. Chemoprophylaxis may soon be advanced if Malarone, primaquine, or Tafenaquine (a new 8-aminoquinoline) become licensed for this indication. Malarone or primaquine are taken daily, but Tafenaquine will probably need to be taken only once a week. In contrast to current drugs which target the blood stage of infection these drugs act on the liver stage so will probably require just a few days of treatment after departure from a malarious area (Ahmed *et al.* 2009).

Insecticide impregnated bed nets appreciably reduce mortality in children in Africa, and major efforts are under way to widely deploy them in endemic regions. Despite a firm immunological rationale and tremendous effort and promise, a licensed vaccine is not anticipated in the next five years. Publication of the DNA sequence of chromosomes 2 and 3 of *P. falciparum* has raised hopes that sequencing of the entire genome will lead to new, clinically useful directions in vaccine development as well as in diagnosis, treatment, and overall control of malaria. The WHO's new Roll Back Malaria campaign is aimed at expanding prevention and treatment to substantially reduce morbidity and mortality in the next five to 10 years. (WHO, 2010).

Although a number of separate epidemiological studies have been carried out, there is no specific data or report on the basic knowledge, practice and the professionals who are handling Kala-azar and the Malaria patients in the endemic/ epidemic areas. The present study has been done to understand the basic concept and knowledge about the diseases which are creating an extra load on the rural public health of Bangladesh. Thus leading to an economic loss of the country, for protective measures to betaken against kala-azar and malaria, it is essential to know the current status and comparative epidemiological study of different communities (Ben et al. 2005, Oshi *et al*, 2008, Brooker *et al.* 2004 and Haque *et al.* 2005).

The World Malaria Report (WHO, 2004) and WHO (2004) reveals that the tide may be beginning to turn against malaria as control and prevention programmes start to take effect. According to the World Malaria Report half of the world's population is at risk of malaria and an estimated 243 million cases led to nearly 863 000 deaths in 2008. The advent of long-lasting insecticidal nets and artemisinin-based combination therapy, plus a revival of support for indoor residual spraying of insecticide, presents a new opportunity for large-scale malaria control.

Kala-azar is a vector borne parasitic disease that affects the cells of the mononuclear phagocyte system of the body of human., caused by *Leishmania donovani* and is transmitted by female phlebotomine Sandfly, belonging to the species and sub-species of *Phlebotomus* and *Lutzomyia* are the proven vector of *Leishmania* (Alam *et al.* 2009)... *Phlebotomus argentpes* is the common species found thought the country,. The environment management methods of control are generally directed at the elimination of the breeding site in and around houses and cattle shed should be kept clean and plastered at regular intervals. The cattle shed and lose soil and organic materials should be remove daily. The situation of kala-azar tends to be epidemic in multiple foci in Bangladesh. It has a high mortality rate and gives rise to important public health problem (Haque *et al.* 2005 and Amal *et al.* 2009).

Conclusion

It is expected that, the finding of the present study will contribute to future planning for development of knowledge, needs for training of the professionals for prevention and control of kala-azar and malaria in endemic areas of Bangladesh. There is an urgent need for scientists and researchers to investigate further the potential impact of climate changes and risk factors on the transmission of specially NTD

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Studies on digenian trematodes of *Channa punctatus* (Bloch) from Awangsoi Lake, Manipur related to months

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Abstract : The present communication was a preliminary study on digenian trematodes of *Channa punctatus* (Bloch) from Awangsoi Lake, Manipur. During the present investigation four species of trematode, *Clinostomum complanatum* Rudolphi, 1819, *Allocreadium handiai* Pande 1937, *A. fasciatusi* Kakaji, 1969 and *Genarcopsis goppo* Ozaki, 1925 were recorded. In the present study there were concurrent infections by two or more parasite species. Seasonal variation in the occurrence of these parasites may be attributed to ecological conditions, particularly distribution of intermediate hosts and also the age of the host and the life cycle of the parasite species. The prevalence of infection among different months was significant at $p<0.05$ level.

Key words **:** *Awangsoi Lake, Channa punctatus, digenian trematodes, Manipur, months.*

Introduction

Fish diversity of NE India hotspot region have been worked out by many workers like Viswanath, 2002; Kar *et al*., 2005; Kar and Sen, 2007; Kar, 2010 and so on. In India population dynamics of helminth parasites have studied by many workers like Gupta *et al.,* 1984; Niyogi *et al.,* 1982; Sinha and Sinha, 1994, 1996. Many works have been done in relation to seasonal occurrence of helminth parasites of fresh water fishes in many countries. Chubb, 1977, 1979, 1980, 1982 illustrated the studies of seasonal occurrence of helminthes in fresh water fishes of different climatic zones of the world. Pal, 1963; Madhavi, 1979; Jha *et al.,* 1992; Shomorendra *et al.,* 2005 worked on the occurrence of helminth parasites of fresh water fishes in India. However population dynamics of helminth parasites of fishes of Awangsoi Lake, Manipur has not yet been studied by any worker. It was, therefore decided to study the population dynamics of helminths infecting the freshwater fish *Channa punctatus*. The present study reveals the prevalence and intensity of infection of digenetic trematodes in *Channa punctatus* from Awangsoi Lake, Manipur during different months at different water temperature. Water temperature has been taken into account as it is one of the most important factor to emerge, which affects the life cycles of intermediate and definitive host and parasites, exerting a direct effect on ectoparasites and an indirect effect, through their poikilothermic host, on adult parasites in fish and larval stages in the intermediate host.

Materials and Methods

A total of 362 indeviduals of *Channa punctatus* ranging from 18-38 specimens each month were examined from June'08 to May'09. The fishes were collected from the study site and brought to the laboratory in the polythene bags containing water of the same locality. The fishes were killed by severing the spinal cord. The external body organs as well as the internal body organs were thoroughly examined for the parasites. The parasites collected, upon being fully relaxed, were fixed in the fixatives prescribed for different helminthic groups. The trematodes were fixed in AFA (alcohol-formalin-acetic-acid) solution and stored in 70% alcohol. To facilitate identification of the worms the trematodes were stained in alum carmine and mounted in canada balsam following Bylund *et al*., 1980. The ecological terms used in the present work are based on the description of Margolis *et al.,* 1982.

Statistcal analysis

Total numbers of parasites were determined directly by numerical count. The number of fish sampled, prevalence, intensity and standard deviation values are given in the table. One way ANOVA and Post Hoc Test were use to compare the means among different months at the level of 0.05. Statistical analysis of data was carried out using SPSS 17 package programs.

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Table I : Prevalence and intensity of digenian trematodes in *Channa punctatus* from Awangsoi Lake, Manipur from June'08 to May'09.

Months	June '08	July	Aug	Sept	Oct	Nov	Dec	Jan'09	Feb	March	April	May
Temp. (°C)	28	29	31	28	27	22	20	16	18	20	28	28
N	n=38	n=36	n=34	n=29	n=26	n=26	n=25	n=28	n=18	n=33	n=32	n=37
Clinostomum complanatum												
Prevalence	0	0	5.88	6.89	0	0	8	3.57	0	0	0	0
Intensity	0	0	4.5	5	0	0	20.5	9	0	0	0	0
SD	0	0	23.52	25.33	0	0	27.12	18.55	0	0	0	0
Allocreadium handiai												
Prevalence	5.26	2.77	0	0	0	7.69	4	0	0	0	9.37	0
Intensity	6	9	0	0	0	4	6	0	0	0	1.66	0
SD	22.32	16.43	0	0	0	26.64	19.59	0	0	0	29.14	0
Allocreadium fasciatusi												
Prevalence	0	0	0	3.44	0	3.84	0	0	11.11	3.03	0	10.8
Intensity	0	0	0	2	0	2	0	0	4.5	3	0	1.5
SD	0	0	0	18.24	0	19.23	0	0	31.42	17.14	0	31.05
Genarcopsis goppo												
Prevalence	0	2.77	0	0	7.69	11.53	0	0	0	0	3.12	8.1
Intensity	0	5	0	0	6.5	1	0	0	0	0	4	1.66
SD	0	16.43	0	0	26.64	31.94	0	0	0	0	17.39	27.29

N= Number of fish examined

Results

During the study period four species of digenetic trematodes were recovered comprising *Clinostomum complanatum* Rudolphi, 1819, *Allocreadium handiai* Pande 1937, *Allocreadium fasciatusi* Kakaji, 1969 and *Genarcopsis goppo* Ozaki, 1925. Out of 362 fish individuals examined, 144 fishes were infected with these parasites. Total prevalence and intensity of infection were 39.77 and 1.99 respectively. In the present study no digenetic trematode showed any regularity in their occurrence all through the months.

The trematode, *Clinostomum complanatum* showed maximum prevalence in December'08 and minimum in January'09. Intensity of infection was also highest in the month of December'08 and lowest in August'08. This trematode was found in liver of the fish host.

Allocreadium handiai showed maximum prevalence in April'09 and minimum in July'08. Intensity of infection was maximum in July'08 and minimum in April'09.This trematode was found to infect in body cavity of *C. punctatus*.

Allocreadium fasciatusi was also found to infect in body cavity of the host. It showed maximum prevalence in February'09 and minimum in March'09. Intensity of infection was maximum in February'09 and minimum in May'09.

In case of *Genarcopsis goppo* the prevalence of infection was maximum in November'08 and minimum in July'08. The intensity of infection was highest in October'08 and lowest in November'08. This worm was also recovered from the body cavity of the host.

Discussion

The present findings conclude that there is a great diversity of digenian trematodes infecting *C. punctatus in Awangsoi Lake*. In the present study there was concurrent infection by two or more helminth parasites. Gupta *et al*., 1984; Niyogi *et al*., 1982; Sinha and Sinha, 1994, 1996; Amin, 1987 have also reported concurrent infection of helminth. Concurrent infection causes niche segregation and reduction in the number of helminth parasites in fish. It seems to be no clear cut impact of water temperature on the occurrence of *digenian trematodes*. Jha *et al*., 1992 also showed that water temperature did not play an important role in the seasonal occurrence of helminth parasites. The prevalence of infection among different months was found to be significant at $p<0.05$ level. Occurrence of helminth parasites may be attributed to ecological conditions and particularly distribution of intermediate hosts. Ecological factors have been held widely responsible for the occurrence of adult digenetic trematodes by Halvorsen, 1972 quoted from Chubb, 1979; Madhavi, 1978.

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Two new and one already known species of *Myxobolus* (Myxozoa : Myxosporea : Bivalvulida) infecting gill lamellae of Indian major carp fishes in Ropar and Harike wetlands (Punjab)

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Abstract : A survey of parasites of freshwater fishes in Ropar and Harike wetland of Punjab (India) revealed the presence of two new and one already known myxosporean species belonging to the genus *Myxobolus* Butschli, 1882 parasitizing mucous membrane around gill lamellae. Spores of the first species, *M. catli* sp. nov. from *Catla catla* (Ham.) (Cypriniformis: Cyprinidae) vern. thail measure 8.3x4.7µm, oval to egg shape in valvular view having blunt anterior end and rounded posterior end. Parietal folds are absent. Polar capsules are two, equal, measure 3.3x1.67µm, elongately oval with bluntly pointed anterior end and rounded posterior end. Both polar capsules are placed posteriorly from the tip of the spore and are parallel to each other in the spore body cavity. An intercapsular process is absent. Spores of the second species, *Myxobolus kalmani* sp. nov. parasitizing *Cirrhina reba* vern. kursa measure 10.0x4.7µm and elongately oval to ellipsoidal in valvular view. Parietal folds are absent. Polar capsules are two, equal, measure 3.4x1.67µm, placed anteriorly and slightly converge toward the anterior end. Spores of the third species, *M. moli* Fomena *et al.* (1985) (Revised diagnosis and new nomenclature *M.* sp. 4 Fomena *et al.,* 1985) measure 9.2x6.2µm are elliptical in valvular view having blunt anterior end with a conspicuous pore and rounded posterior end. Parietal folds are absent. Polar capsules are two, equal, measure 4.75x1.7 µm are anterior in position and converge slightly towards the anterior end of the spore. Polar capsules are broadly pyriform to elongately oval with bluntly pointed anterior end and rounded posterior end.

Key words **:** *Harike wetland, mucous membrane, plasmodia, polar capsules*

Introduction

The wetlands of Punjab (included in the Ramsar List of wetlands of International Importance), with their vast expanse of water bodies, have a rich freshwater fish fauna and are the major source of food fish in North India. Due to the growing economic value of this fish, it is important to know various parasitic infections affecting them. One particular group of parasites, the myxozoan, is well known for the diseases they cause in commercially important fish host. Up till now, Phylum Myxozoa include 4 malacosporean and 2,180 myxosporean species to a total of 62 genera (Lom and Dykova, 2006). Recently a new genus *Thelohanelloid bengalensis* gen. nov. sp. nov. from gall bladder of *Arius sagor* (a marine fish in Bay of Bengal) has been described by Sarkar (2009). Today more than 2,180 myxosporean species have been described, among which *Myxobolus* is predominant having more than 744 species belonging to this genus (Eiras et al 2005). Kalavati and Nandi (2007) have reported the existence of 104 myxobolid species from Indian species. During the present study on the fishes of Ropar and Harike wetlands of Punjab (India), a total number of 73 fishes belonging to *Catla catla*, *Cirrhina reba* and *Amblypharyngodon mola* were examined. The present communication describes two new species *M. catli* sp. nov. and *M. kalmani* sp. nov. and one already known species *M. moli* collected from gill lamellae of 3 different genera of catfishes. The description has been prepared in accordance with the guidelines of Lom and Arhtur (1989)

Materials and methods

Fishes collected from Ropar and Harike wetlands were brought to the laboratory and examined for myxozoan infections. Plasmodia when found were removed and teased on slide and covered with cover slip and examined under the oil immersion for the presence of myxospores. Fresh spores were treated with 8% KOH solution for the extrusion of polar filaments. For permanent preparation, air-dried smears were stained with Ziehl-Neelsen and Iron-haematoxylin. Drawings were made from stained material with the aid of camera lucida. Measurements of spores were done with the aid of a calibrated ocular micrometer. All measurements are presented in µm as range values followed by mean ± SD in parentheses. The abbreviations used in the

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paper are as follows: -LS: Length of spore; WS: Width of spore; LPC: Length of polar capsule; WPC: Width of polar capsule; ICP: Intercapsular process; NC: Number of coils of polar filaments; SD: Standard deviation.

Results and Discussion

***M. catli* sp. nov.**

Plasmodia

Minute, present in the mucous membrane around gill lamellae. 5-7 spores are present per plasmodium.

Spore description (Figs. 1, 2, Table I)

(Measurements based on 4-5 spores in frontal view)

The spores are histozoic, measure 8.3x4.7µm, oval to egg shape in valvular view having blunt anterior end and rounded posterior end. Shell valves are thin, smooth, symmetrical and measure 0.4µm in thickness. Sutural ridge is straight in lateral view. Parietal folds are absent. Polar capsules are two, equal, measure 3.3x1.67µm, elongately oval with bluntly pointed anterior end and rounded posterior end. Both polar capsules are placed posteriorly from the tip of the spore and are parallel to each other in the spore body cavity. Polar filaments form 5-7 coils arranged perpendicular to the polar capsule axis. Polar filaments are equal, thread-like, measure 25.0µm in length and extrude independently. An intercapsular process is absent. One capsulogenic nucleus is present in between polar capsules measuring 0.06 µm in diameter. Sporoplasm is agranular, homogenous, half moon shaped and contain two sporoplasmic nuclei measuring 0.8-1.0(0.9±0.14) µm in diameter. An iodinophilous vacuole is absent.

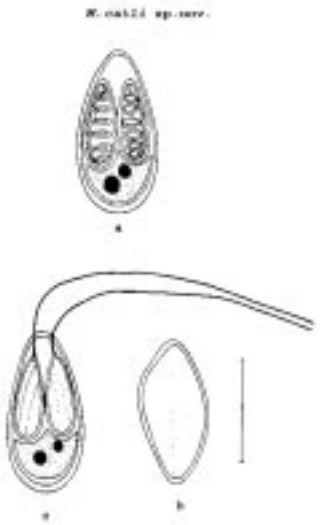

Fig. 1. : a spore stained in Ziehl-Neelsen (valvular view)
b Spore in side view
c Spore stained in Iron-haematoxylin (extruded polar filaments) Scale bar = 0.005 mm

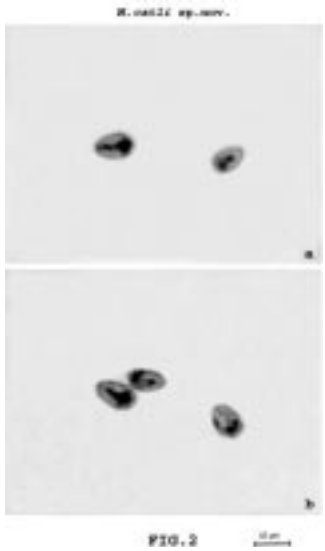

Fig. 2. : a, b spores stained in Iron-haematoxylin

Table I. Measurements (in µm) and ratio of *M. catli* sp. nov.

Characters	Range	Mean Values	SD
LS	7.9-8.7	8.3	0.56
WS	4.2-5.2	4.7	0.70
LPC	2.8-3.8	3.3	0.70
WPC	1.27-2.07	1.67	0.56
Ratio: LS/WS		1.7	
ICP		absent	
NC		5-7	
Parietal Folds		absent	

Taxonomic summary of *M. catli* sp. nov.

Type host	:	*Catla catla* (Ham.) vern. thail
Type locality	:	Ropar wetland, Punjab, India
Type specimen	:	Paratypes are spores attained in Ziehl Neelsen and Iron-haematoxylin, deposited in the museum of department of Zoology, Punjabi University, Patiala, India. Slide no CC/K / ZN/ 01/10.10.07 and PS/K /IH/ 01/10.10.07.
Site of infection	:	Gill lamellae (mucous membrane)
Prevalence of infection	:	10% (3/30)
Etymology	:	The specific epithet *catli* has been given after the specific name of the host fish.

Discussion

The present species was compared with morphologically similar *Myxobolus* species i.e. *M. permagnus* Wegener, 1910 from gills, opercula and swim bladder of *Gobio gobio gobio* and *Scardinius erythrophthalmus*; *M. koi* Kudo, 1919 from gills of *Cyprinus carpio*; *M. catlae* Chakravarty, 1943 from gills of *Catla catla; M. bengalensis* Chakravarty and Basu, 1948 from gills of *Catla catla*; *M. gigi* (Fujita, 1927) Shulman, 1962 from kidney of *Fluvidraco nudiceps*; *M. aligarhensis* Bhatt and Siddiqui, 1964 from accessory respiratory organs, pharyngeal epithelium and fin of *Channa punctatus; M. chakravarty* Haldar et al 1983 from internal musculature of *Catla catla; M. amieti* Fomena et al 1985 from spleen, eye of *Ctenopoma nanum*; *M. pseudokoi* Li and Desser, 1985 from gills, skin of *Notropis cornutus; M. mahendrae* Sarkar, 1986 from gill rachii of *Catla catla; M. trichogasteri* (Sarkar, 1985) Gupta and Khera,1988 from gall bladder of *Trichogaster fasciatus*; *M. beninensis* Sakiti et al 1991 from gill arch connective tissue of *Sarotherodon melanotheron*; *M. hydrocyni* Kostoingue and Toguebaye, 1994 from gills of *Hydrocynus forskali; M. multivaderis* Mukhopadhyay and Haldar, 1998 from gills of *Catla catla* and *M. diamaensis* Diamanka et al 2007 from gill filaments of *S. melanotheron* but differ from all of the above species in morphometric characteristics (Table II).

In the present species the spores are oval to egg shape in outline. The anterior end of the spore is narrow, tapered, bluntly pointed but the posterior end is rounded. In this respect, it is comparable with *M. pseudokoi, M. trichogasteri, M. permagnus* and *M. diamaensis.* The polar capsules in the present species are elongated oval with anterior end bluntly pointed and posterior end rounded. They are situated posteriorly from the tip, placed parallel to each other in the spore body cavity in contrast to elongated, pyriform spores having pointed anterior ends with bulb-shaped polar capsules occupying nearly half of the spore body cavity in *M. pseudokoi.* Spores of *M. permagnus, M. diamaensis* and *M. trichogasteri* appears to be morphologically similar to spores of the present species but are exceptionally larger in *M. gigi, M. permagnus*, *M. trichogasteri* and also the presence of unequal polar capsules in *M. diamaensis* and *M. aligarhensis* differentiate them from the present species.

In view of the above differences, the present species under study is proposed as new to the science and named as *M. catli* sp. nov.

***M. kalmani* sp. nov.**

Plasmodia

Minute, present in the mucous membrane around gill lamellae. 11-12 spores are present per plasmodium.

Spore description (Figs. 3, 4, Table III)

(Measurements based on 8-10 spores in frontal view)

The spores are histozoic, measure 10.0x4.7µm and elongately oval to ellipsoidal in valvular view. Shell valves are thick, smooth, symmetrical and measure 0.5ìm in thickness. Shell valves of the spore appear thicker (stains dark blue with Heidenhains Iron-haematoxylin) along one sutural plane. Parietal folds are absent. Polar capsules are two, equal, measure 3.4x1.67µm, placed anteriorly and slightly converge toward the anterior end. Polar capsules are pyriform with pointed anterior end and rounded posterior end. Polar filaments form 6-7 coils and are arranged obliquely to the polar capsule axis. Polar filaments are thin, thread-like, equal and measure 45.7µm in length when extruded. A small intercapsular process is present. Capsulogenic nuclei are two, one beneath the polar capsule and another in between two polar capsules measuring 0.6-0.8 (0.7±0.14) ìm and 1.5-1.6 (1.5±0.70) ìm in diameter respectively. Sporoplasm is agranular and homogenous occupying whole of the extracapsular space behind polar capsules. Sporoplasmic nuclei are two, each measuring 1.5ìm in diameter. An iodinophilous vacuole is present measuring 1.3ìm in diameter.

Table II. Comparative description of *M. catli* sp. nov. with morphologically similar species (measurements are in micrometer)

Species	**Host**	**Site of infection**	**Locality**	**Spore**	**Polar capsule**
***M. catli* sp. nov.** (present study)	*Catla catla*	gill lamellae (mucous membrane)	Ropar wetland, Punjab (India)	8.3x4.7	3.3x1.67
M. permagnus Wegener, 1910	*Gobio gobio gobio, Scardinius erythrophthalmus*	gills, operculum, swim bladder	-	17.0-20.0x 10.0x11.5	7.0-11.2x 4.0-4.5
M. koi Kudo, 1919	*Cyprinus carpio*	gills	Japan	14.0-16.0x 8.0-9.0	8.0-9.0x 2.5-3.0
M. catlae Chakravarty, 1943	*Catla catla*	gills	India	14.5-16.5x6.18	10.3-12.36x 2.06-3.01
M. bengalensis Chakravarty and Basu, 1948	*Catla catla*	gills	India	8.9x6.6	4.8x2.8
M. gigi (Fujita, 1927) Shulman,1962	*Fluvidraco nudiceps*	kidney	Japan	12.0x6.0	7x0
M. aligarhensis Bhatt and Siddiqui, 1964	*Channa punctatus*	accessory respiratory organs, pharyngeal epithelium, fin	India	12.0-14.0x 6.0-7.5	7.2x2.2 and 6.0-7.0x2.5
M. chakravarty Haldar et al 1983	*Catla catla*	internal eye musculature	India	12.3x7.7-10.5	5.5-6.6x 3.3-5.0 and 4.4-5.0x2.2-4.4
M. amieti Fomena et al 1985	*Ctenopoma nanum*	spleen, eye	Africa	11.5-16.0x 5.5-8.5	6.0-10.0x 1.5-2.5
M. pseudokoi Li and Desser, 1985	*Notropis cornutus*	gills, skin	Canada	13.5x6.5	6.5x2.5
M. mahendrae Sarkar, 1986	*Catla catla*	gill rachii	India	12.7x9.2	6.98x3.73 and 5.44x3.42
M. trichogasteri (Sarkar, 1985) Gupta and Khera,1988	*Trichogaster fasciatus*	gall bladder	India	15.5x9.3	10.1x3.3
M. beninensis Sakiti et al 1991	*Saratherodon melanotheron*	gill arch connective tissue	Benin	10.5-14.0x 5.5-9.0	6.0-8.0x 1.5-3.0
M. hydrocyni Kostoingue and Toguebaye, 1994	*Hydrocynus forskali*	gills	Arica	13.0-14.0x 8.0-10.0	4.0-5.0x 2.0-3.0
M. multivaderis Mukhopadhyay and Haldar**,**1998	*Catla catla*	gills	India	5.5x3.44	3.0x1.5
M. diamaensis Diamanka et al 2007	*Sarotherodon melanotheron*	gill filaments	West Africa	11.8x7.36	6.1x3.03 and 5.0x2.46

Fig. 3. a Spore stained in Ziehl-Neelsen (valvular view)
b Spore stained in Iron-haematoxylin (extruded polar filaments) Scale bar = 0.005 mm

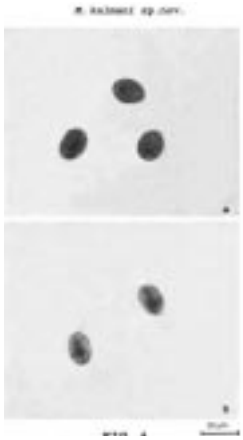

Fig. 4. a Spores stained in Ziehl-Neelsen
b spores stained in Iron-haematoxylin (extruded polar filaments)

Table III. Measurements (in µm) and ratio of *M. kalmani* sp. nov.

Characters	Range	Mean Values	SD
LS	9.5-10.5	10.0	0.7
WS	4.2-5.2	4.7	0.7
LPC	3.0-3.8	3.4	0.5
WPC	1.3-1.9	1.67	0.4
Ratio: LS/WS		2.1	
ICP		small	
NC		6-7	
Parietal Folds		absent	

Taxonomic summary of *M. kalmani* sp. nov.

Type host : *Cirrhina reba* (Ham.) vern chunni, mori, kursa

Type locality : Harike wetland, Punjab, India

Type specimen : Paratypes are spore stained in Ziehl-Neelsen and Iron-haematoxylin, deposited in the museum of department of Zoology, Punjabi University, Patiala, India. Slide no. ME/K/ZN/ 05/17/04/2010 and ME/K/IH/06/17/04/2010.

Site of infection : Gill lamellae (mucous membrane)

Prevalence of infection : 65.2% (15/23)

Etymology : The specific epithet *kalmani* has been given after the name of Dr. Kalman Molnar an eminent worker in the field of Protozoology, Veterinary Medical Research Institute, Hungarian Academy of Sciences, H-1581 Budapest, Hungry.

Discussion

The present species under study was compared with *M. gigi* (Fujita, 1927) Shulman, 1962 from kidney of *Fluvidraco nudiceps*; *M. curmucae* Seenappa and Manohar, 1980 from scales of *Puntius curmuca; M. hosadurgensis* Seenappa and Manohar, 1981 from gills, muscles of *Cirrhina mrigala*; *M. amieti* Fomena et al 1985 from spleen, eye of *Ctenopoma nanum; M. israelensis* Landsberg, 1985 from kidney, spleen of *Oreochromis niloticus*, *O. aureus*, *Sarotherodon galilaceus*; *M. utlouensis* Hemananda et al 2009 from cornea of *Clarias batrachus* and *M. harikensis* Kaur and Singh, 2010 from caudal fins (in between fin rays) but differ from all of the above in morphometric characterstics (**Table IV**).

Table IV. Comparative description of *M. kalmani* sp. nov. with morphologically similar species (measurements are in micrometer)

Species	Host	Site of infection	Locality	Spore	Polar capsule
***M. kalmani* sp. nov.** (present study)	*Cirrhina reba*	gill lamellae (mucous membrane)	Harike wetland, Punjab (India)	10.0x4.7	3.4x1.67
M. gigi (Fujita, 1927) Shulman,1962	*Fluvidraco nudiceps*	kidney	Japan	12.0x6.0	7.0
M. curmucae Seenappa and Manohar, 1980	*Puntius curmucae*	scales	India	9.8x7.6	4.9x2.5 and 3.9x2.4
M. mathuri Jayasri et al 1981	*Puntius saranae*	gills	India	8.7-23.5x 5.1-10.1	2.7-11.9x 1.8-4.6 and 2.7-7.8 and 1.8-4.6
M. hosadurgensis Seenappa and Manohar, 1981	*C. mrigala*	gills, muscles	India	10.5x6.25	5.37x2.3 and 3.3x1.43
M. amieti Fomena et al 1985	*Ctenopoma nanum*	spleen, eye	Africa	11.5-16.0 x 5.5-8.5	6.0-10.0x 1.5-2.5
M. israelensis Landsberg, 1985	*Oreochromis niloticus*, *O. aureus*, *Sarotherodon galilaceus*	kidney, spleen	Israel	11.5-14.0x 7.5-10.0	7.7x3.5
M. utlouensis Hemananda et al 2009	*Clarias batrachus*	cornea	India	12.28x 8.92	2.34
M. slendrii Kaur and Singh, 2009	*Cirrhina mrigala*	gill lamellae (mucous membrane)	India	14.87x3.4	5.74x1.48
M. harikensis Kaur and Singh, 2010	*Cirrhina mrigala*	caudal fin (in between fin rays)	India	10.1x8.5	5.0x3.1 and 1.7x1.4

The present species have spores elongately oval to ellipsoidal in shape. In this respect, the present species is comparable with *M. israelensis, M. amieti, M. curmucae, M. hosadurgensis* and *M. utlouensis.* Spores in the present species contain two polar capsules, equal, pyriform with anterior end pointed and rounded posterior end. They converge slightly towards the anterior end. A small intercapsular process is present. Although, spores of *M. curmucae* and *M. hosadurgensis* possess intercapsular process but differ in having unequal polar capsules. Spores of *M. israelensis* and *M. amieti* lack intercapsular process despite of having equal polar capsules. Spores of *M. utlouensis* have spherical polar capsules and a larger double crescent shaped intercapsular ridge.

The shell valves in the present species appear thicker at one sutural plane, similar thickened posterior shell valves have also been reported in *M. mathurii* Jayasri et al 1981 and in *M. slendrii* Kaur and Singh, 2009.

In view of the above differences, the present species under study is proposed as new to the science and named as *M. kalmani* sp. nov.

***M. moli* Fomena et al 1985 (Revised diagnosis and new nomenclature *M.* sp. 4 Fomena et al 1985)**

Plasmodia

Minute, present in the mucous membrane around gill lamellae. 12-13 spores are present per plasmodium.

Spore description (Figs. 5, 6, Table V)

(Measurements based on 10-12 spores in frontal view)

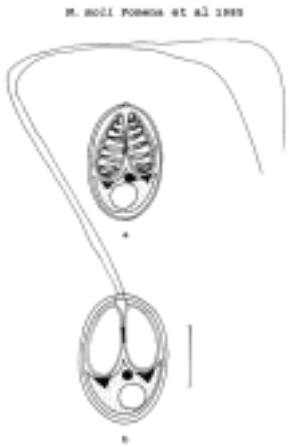

Fig. 5. a Spore stained in Ziehl-Neelsen (valvular view)
b Spore stained in Iron-haematoxylin (extruded polar filaments) Scale bar = 0.005 mm

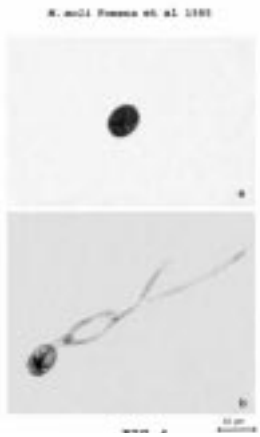

Fig. 6. a Spores stained in Ziehl-Neelsen
b spores stained in Iron-haematoxylin (extruded polar filaments)

Table V. Measurements and ratio of *M. moli* Fomena et al 1985

Characters	Range	Mean Values	SD
LS	9.1-9.3	9.2	0.14
WS	5.9-6.5	6.2	0.42
LPC	4.3-5.2	4.75	0.63
WPC	1.3-2.1	1.7	0.56
Ratio: LS/WS		1.4	
ICP		absent	
NC		6-7	
Parietal Folds		absent	

The spores are histozoic, measure 9.2x6.2µm, elliptical in valvular view having blunt anterior end with a conspicuous pore and rounded posterior end. Two shell valves are thin, smooth, symmetrical and measure 0.3ìm in thickness. Parietal folds are absent. Polar capsules are two, equal, measure 4.75x1.7 µm are anterior in position and converge slightly towards the anterior end of the spore. Polar capsules are broadly pyriform to elongately oval with bluntly pointed anterior end and rounded posterior end. They occupy more than half of the spore body cavity, contain 6-7 coils of polar filaments arranged perpendicular to the polar capsule axis and measure 32.9µm in length when extruded. An intercapsular process is absent. Capsulogenic nuclei are three, two beneath the polar capsule and one in between two polar capsules measuring 0.33 ìm and 0.67ìm in diameter respectively. Sporoplasm is agranular and homogenous occupying whole of the extracapsular space behind the polar capsules. Sporoplasmic nucleus is present measuring 1.3ìm in diameter. An iodinophilous vacuole is present measuring 3.33ìm in diameter.

Taxonomic summary of *M. moli* Fomena *et. al.* 1985

Host: *Amblypharyngodon mola* (Ham.) vern. molelia

Locality : Harike wetland, Punjab, India

Site of infection : Gill lamellae (mucous membrane)

Etymology : The species epithet is named after the vernacular name of the host fish *Amblypharyngodon mola*

Prevalence of infection : 10% (1/20)

Remarks

The present observations (LS/WS: 1.4) on *M. moli* Fomena et al 1985 are in conformity with the original description (LS/WS: 1.4) except for some variations in size of spore and polar capsules i.e. spores and polar capsules are smaller in the present species under study. Parietal folds and an intercapsular process are absent in the present material as in the original specimens. In the present species, spores are ellipsoidal with rounded anterior and posterior ends. A conspicuous pore is present at the anterior end and polar capsules occupy more than half of the spore body cavity. Earlier, the parasite was recorded in the muscles of *Raiamas* sp. from Cameroon. This species was of uncertain systematic status and incompletely described by Fomena *et. al.* 1985 as *M*. sp. 4. In the present study, it is described and named as *M. moli* after the vernacular name of the fish i.e. molelia. A new host- *Amblypharyngodon mola*, a new location- mucous membrane around gill lamellae and a new locality- Harike wetland are recorded for this parasite (Table **VI**).

Table VI. Comparative description of *M. moli* Fomena *et. al.* 1985 (Revised diagnosis and new nomenclature *M.* sp. 4 Fomena *et. al.* 1985) with original species (measurements are in micrometer)

Species	Host	Site of infection	Locality	Spore	Polar capsule
M. moli (present study)	*Amblypharyngodon mola*	gill lamellae (mucous membrane)	Harike wetland, Punjab (India)	9.2x6.2	4.75x1.7
***M.* sp. 4** Fomena et al 1985	*Raiamas* sp.	muscles (Western	Cameroon x7.0-10.5 Africa)	10.5-15.5 2.5-4.0	6.0-7.5x

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Parasitic infestation among the adolescent girls of Bangladesh

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Abstract : The present study was conducted in rural, urban and slum areas in and around Dhaka city, Bangladesh to investigate the prevalence of parasitic infestation among the adolescent girls (aged 10-19 years). Faecal samples were collected from 1570 adolescent girls. About one third (33.50%) of the adolescent girls were found to be infected with one or more protozoan (*Entamoeba histolytica, Giardia lamblia*) and helminth (*Ascaris lumbricoides, Trichuris trichura, Strongyloieds stercoralis,* hookworm) parasite infections. Among all detected parasites, *Ascaris lumbricoides* (14.20%) overwhelmingly infected the adolescent girls. The most common parasite combination infecting adolescent girls were found to be *Ascaris lumbricoides* + *Trichuris trichura* (1.84%) and *Ascaris lumbricoides* + hookworm (1.27%). The prevalence was higher in rural (49.62%) area compared to the slum (33.43%) and urban (18.22%) areas. The girls aged 12-13 years were more vulnerable for the parasitic infestation (48.75%, $p<0.08$). The findings of the parasitic infestation among the adolescent girls in this study indicate important public health problem. Measures to improve socioeconomic conditions, sanitary practice and rate of literacy in the slum and rural areas are recommended intervention approaches to control the extent of parasitic infestation.

Keywords **:** *Adolescent girls, parasitic infestation, rural, slum, urban.*

Introduction

WHO (1994) estimates suggested that approximately 1.4 billion, 1.2 billion and 1 billion persons are currently infected with various species of intestinal helminthes: *A. lumbricoides*, hookworm (*Ancylostoma duodenale/ Necator americanus*) *and T. trichura* respectively. The epidemiology and population biology of hookworms has been studied extensively and recently a mathematical models have been proposed to explain transmission and identify phases in the host-parasite relationship where available control measures are most likely to be effective (WHO, 1994).

Bangladesh is mostly a plain land and embedded with rivers and canals. The soil humidity and temperature contributes a lot towards parasitic infection. Several studies showed that intestinal parasitic infections are present all the time everywhere in this country (Nuruzzaman and Huda, 1974; Muttalib et al 1975; Shakur and Ehsan, 1993). Helminthiasis is a most common health problem in the tropics because the environment is favorable for the transmission of the helminthiasis in the community. In Bangladesh, among the helminth and protozoa parasites (*A. lumbricoides*, *A. duodenale*, *T. trichura*, *Enterobius vermicularis* and *E. histolytica*, *G. lamblia*) are common (Kuntz, 1960; Banu et al 2003; Khanum et al 2010).

Adolescent girls are generally more infected than their younger peers with intestinal parasites, like *A. lumbricoides*, *T. trichiura*, hookworm, *E. histolytica*, *G. lamblia* etc. In Bangladesh it was found that 58-90% of 4-13 years old children harboring *A. lumbricoides* and 60% of 1-40 years old people suffer from *E. vermicularis* infection (D'Silva et al 2003, Uddin et al 2005).

Adolescents are important segment of the whole population. It is the adolescent girls of today who will usher in the generation of the 21st century. There are very few studies on the prevalence of parasite among the adolescents' girls in comparison to other groups like women and children in the world and it is the case in Bangladesh too. The protozoan and helminth parasites are the oldest pathogens and remain an important part of infectious diseases among the world's poor who are the most susceptible population. Adolescent girls are particularly susceptible because of their more involvement with the domestic work. So the present study was undertaken to investigate the extent and the pattern of parasitic infestation in relation to age groups of the adolescent girls from rural, urban and slum settings of Bangladesh.

Materials and methods

The present investigation was a cross sectional study with a sample size of 1570 adolescent girls (aged 10-19 years) conducted during the period of June 2006 to May 2009. The six study areas are located in Dhaka district, Bangladesh

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representing rural (Kamrangirchar and Zinjira), urban (Savar and Lalbag) and slum areas (Mirpur and Mohammadpur). Faecal samples were collected from adolescent girls and carried out to the laboratory. The most recommended Formol Ether Concentration Method (Cheesbrough, 1987) was applied which was suitable for concentration of parasite eggs, cysts and larvae in fresh or preserved faeces. After concentration, the sediment was mixed up by using the stick. It was transferred to the slides by dropper and covered with cover glasses. The prepared slides were examined microscopically and the presence of parasite eggs, cysts and larvae were detected. The findings were confirmed by the help of experts and renowned books (Neva and Brown, 1994; Chatterjee, 2004).

Results

During the present investigation, total of 1570 stool samples were collected for the identification of different parasite species. Out of 1570 samples, 526 (33.50%) girls were found to be infected with one or more intestinal parasites.

The highest percentage of parasitic infection (49.62%) was found in rural Kamrangirchar and the lowest was in urban Savar (18.22%), comparatively higher rates of infection identified in Slum girls of Mohammadpur (33.43%) and Mirpur (34.41%) (Table I).

Table I. Prevalence of infestation of intestinal parasites among the adolescent girls in different study areas.

Study areas		Total no. of stool samples of the adolescent girls examined	No. of parasite positive cases	Prevalence (%)
Rural areas	Kamrangirchar	270	134	49.62
	Zinjira	230	105	45.65
Urban areas	Lalbag	310	65	20.96
	Savar	225	41	18.22
Slum areas	Mohammadpur	320	107	33.43
	Mirpur	215	74	34.41
Total		1570	526	33.50

Age of the adolescent girls observed as an important factor in parasitic infection. The highest prevalence (48.74%) of intestinal parasite was found at the age group of 12-13 years and the lowest (14.09%) at 18-19 years among all five age groups (Fig.1). So, the prevalence was negatively correlated with age groups ($r = -0.83$, $p<0.08$) which implied that as the age increased, rates of infestation tends to decrease (Fig.1).

Two protozoan parasites (*E. histolytica* and *G. lamblia*) and four helminthes parasites (*A. lumbricoides, T. trichura*, Hookworm and *S. stercoralis*,) were identified during the present investigation (Table II).

In case of protozoan single parasite species, prevalence of *E. histolytica* (3.62%) was comparatively higher than *G. lamblia* (2.67%). Regarding helminth single parasite species, *A. lumbricoides* was found to be overwhelmingly dominant parasites in all age groups and highest prevalence 19.65% was found in 12-13 years age category. The next prevalent helminth was *T. trichura* followed by hookworm and *S. stercoralis*. Importantly, all of the helminth parasites infected particular age group of 12-13 years in higher percentage. Chi-square (χ^2) test showed that age group was not significantly associated ($p>0.05$) with infected by protozoan parasites on the other hand, infected by helminth parasites was found to be significantly associated ($p<0.000$) with age groups (Table II).

Age of the adolescent girls, appears to be an important variable for the infestation of intestinal parasites; nevertheless, the infestation of double parasite (considered presence of two intestinal parasites at a time in a single host) was not highly prevalent in this study. Considering all of the five age groups, adolescent girls of 12-13 years were more infected (7.21%) as compared with other age groups and least infection (1.34%) was found in 18-19 years (Table III).

Among all types of parasite pairs, *A. lumbricoides* and *T. trichura* parasite combination (2.48%) mostly infected the 12-13 years aged adolescent girls. Another parasite combination (2.33%), *A. lumbricoides* and Hookworm was found in the same

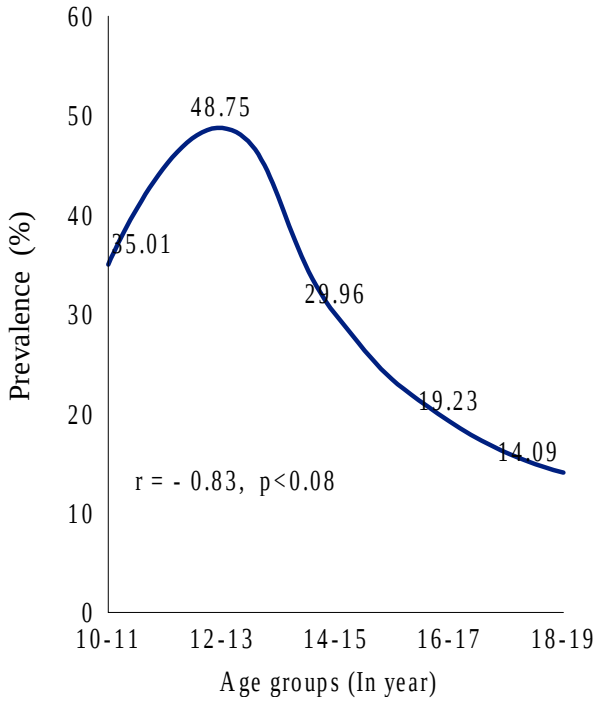

Fig. 1. Prevalence of intestinal parasites in different age groups among the adolescent girls.

Table II. Prevalence of single infection of protozoan and helminth parasites in different age groups of the adolescent girls.

Age groups (In year)	Total no. of stool samples of the adolescent girls examined	Infected by Protozoan Parasites			Infected by Helminth Parasites				
		Infected by EH	Infected by GL	Total	Infected by AL	Infected by TT	Infected by HW	Infected by SS	Total
		n	n	n	n	n	n	n	n
		P	P	P	P	P	P	P	P
		(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)
10-11	514	19	17	36	80	24	7	0	111
		3.69	3.30	7.00	15.56	4.66	1.36	0.00	21.59
12-13	402	21	12	33	79	23	16	4	122
		5.22	2.98	8.20	19.65	5.72	3.98	0.99	30.34
14-15	297	9	7	16	36	10	6	3	55
		3.03	2.35	5.38	12.12	3.36	2.02	1.01	18.51
16-17	208	4	5	9	18	5	3	0	26
		1.92	2.40	4.32	8.65	2.40	1.44	0.00	12.5
18-19	149	4	1	5	10	4	0	0	14
		2.68	0.67	3.35	6.71	2.68	0.00	0.00	9.39
Total	1570	57	42	99	223	66	32	7	328
		3.63	2.67	6.30	14.20	4.20	2.03	0.44	20.89

AL = *Ascaris lumbricoides*, TT = *Trichuris trichura*, HW = Hookworm, *SS* = *Strongyloides stercoralis*, EH = *Entamoeba histolytica*, GL = *Giardia lamblia*, P = Prevalence, n = number

For protozoan parasites :

Chi-square (χ^2) = 6.89, p<0.142

For helminth parasites :

Chi-square (χ^2) = 43.69, p<0.000

age category. So it was evident from this study that girls aged 12-13 years were identified as a remakably risk group for parasitic infestation (Table III).

Table III. Prevalence of double infection of protozoan and helminth parasites in different age groups of the adolescent girls.

Age groups (In year)	No. of stool samples of the of the adolescent girls examined	Infected by EH+ GL	Infected by AL+ GL	Infected by AL+ EH	Infected by TT+ GL	Infected by TT+ EH	Infected by AL+ TT	Infected by AL+ HW	Infected by AL+ SS	Infected by TT+ HW	Overall Prevalence (%)
		n	n	n	n	n	n	n	n	n	n
		P	P	P	P	P	P	P	P	P	P
		(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)
10-11	514	1	1	1	1	2	9	7	0	3	25
		0.19	0.19	0.19	0.19	0.38	1.75	1.36	0.00	0.58	4.86
12-13	402	3	2	3	0	0	10	9	1	1	29
		0.74	0.49	0.74	0.00	0.00	2.48	2.23	0.24	0.24	7.21
14-15	297	0	1	1	2	0	6	2	2	0	14
		0.00	0.33	0.33	0.67	0.00	2.02	0.67	0.67	0.00	4.71
16-17	208	0	0	0	0	0	3	1	0	0	4
		0.00	0.00	0.00	0.00	0.00	1.44	0.48	0.00	0.00	1.92
18-19	149	0	0	0	0	0	1	1	0	0	2
		0.00	0.00	0.00	0.00	0.00	0.67	0.67	0.00	0.00	1.34
Total	1570	4	4	5	3	2	29	20	3	4	74
		0.25	0.25	0.31	0.19	0.12	1.84	1.27	0.19	0.25	4.71

AL = *Ascaris lumbricoides*, TT = *Trichuris trichura*, HW = Hookworm, SS = *Strongyloides stercoralis*, EH = *Entamoeba histolytica*, GL = *Giardia lamblia*, P = Prevalence, n = Number.

E. histolytica + *A. lumbricoides*+ *T. trichura* triplet parasite combination (considered presence of three parasites at a time in a single host) was recorded as highest (0.70%) among all triplet categories. Similar prevalences (0.38%) were found in two other triplet combinations. The combination of *A. lumbricoides* + *T. trichura* + *S. stercoralis* was identified as the least prevalent (0.12%) combination (Fig. 2).

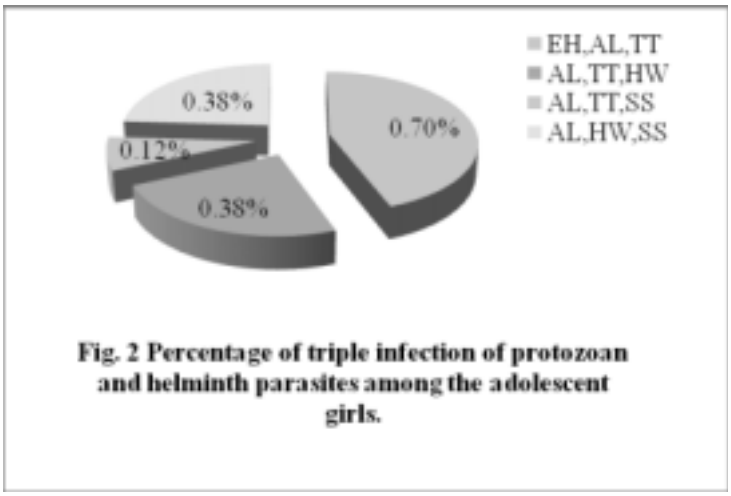

Fig. 2 Percentage of triple infection of protozoan and helminth parasites among the adolescent girls.

AL = *Ascaris lumbricoides*, TT = *Trichuris trichura*, HW = Hookworm, SS = *Strongyloides stercoralis*, EH = *Entamoeba* histolytica, GL = *Giardia lamblia*

Discussion

Out of 1570 samples, the overall parasitic infestation was recorded as 33.50% with the highest prevalence (49.62%) in rural area (Kamrangirchar) and the lowest (18.22%) in urban area (Savar). The prevalence in slum areas was more than 30% which was comparatively lower than rural but higher than the urban area. Rahman (2009) reported overall infection as 33% in his study among the people of Chittagang and Chittagang Hill Tracts which was almost similar with the present study. Hyder et al (1998) reported gross prevalence (33.3%) of parasites among the adolescents and adult population in different rural areas of Mymenshing district of Bangladesh, which was also closely related with our findings. Uddin et al (2005) studied the prevalence of parasitic infestation among the adolescent girls in two rural areas, Kutombopur and Savar and reported overall prevalence as 71.01% with 62.5% in Kutombopur, which was much higher than the rural area of the present study. Rao et al (2003) reported overall prevalence 57% among tribal adolescent girls of Nepal. The findings were higher than the present work due to differences in study population study and prevailing socioeconomic condition.

Among all age groups the highest prevalence (48.74%) was observed among the adolescent girls in the present study who were in 12-13 years of age category. In another study by Uddin et al (2005)**,** highest prevalence (87.50%) was also recorded among female adolescents aged 12-14 years. So it was evident that adolescents of this particular age group were more vulnerable for parasitic infestation than other age groups.

Regarding single parasitic infestation, *A. lumbricoides* overwhelmingly dominated the others protozoans and helminthes. Out of 1570 samples, 223 (14.20%) was found to be infected with *A. lumbricoides*. The next prevalence of single parasitic infection was *T. trichura* (4.20%) followed by *E. histolytica* (3.63%), *G. lamblia* (2.67%), Hookworm (2.03%) and *S. stercoralis* (0.44%). Considering the age groups, adolescents' girls of the 12-13 years age category was found to be more susceptible for parasitic infestation. About 20% of the girls of this age group were infected by *A. lumbricoides* which was much higher than the average 14.2% of all age groups for this particular parasite.

Prevalence of single parasitic infestation reported by Sharma et al (2004) in school children (4-19 years) in the northeastern part of Kathmandu valley, Nepal were *T. trichura* (43.6%), *A. lumbricoides* (13.8%) and Khanum et al (2001) observed 20.39% of *A. lumbricoides* and 15.30% *T. trichura* among the 2-16 years of subjects from lower income families of Dhaka city. The prevalence of *A. lumbricoides* (14.20%) in the present study was almost similar with the findings of Sharma et al (2004) but it was different from the findings of Khanum et al (2001) and the prevalence of *T. trichura* (4.20%) was much lower than their studies. A Brazilian study by Ferreira (2001) reported overall prevalence of *T. trichura* as 5% which was almost similar (4.20%) with the present study. Osazuwa et al (2011) determined the overall prevalence of intestinal helminths in children and adolescents of three rural communities in Nigeria were *A. lumbricoides* (75.6%), hookworm (16.19%) and *T. trichiura* (7.3%) which was quite different from the findings of the present work, this difference might be due to the study population, location, prevailing environment and socioeconomic status.

Ahmed and Talukder (2002) found the prevalence (69%) of *A. lumbricoides*, (39%) of *T. trichura* and Hookworm 8% among the school children (aged 9-16 years) from rural and urban areas of Bangladesh and further proved that the *A. lumbricoides* as the dominant parasite but the prevalence of this parasite was much higher than the present investigation and this difference might be due to the study population, location, prevailing environment and socioeconomic status.

According to WHO (1987), as much as 60% of the world's population were infected with gut parasites. Hamimah et al (1982) found that prevalence of intestinal protozoas like *E. histolytica* was 2.3% and of *G. lamblia* was 2.6% in a study in Kualalumpur, Malaysia. These values were almost similar with our study. On the other hand, in another work in Malaysia, Aza et al (2003) recorded higher prevalence of *E. histolytica* 21% and of *G. lamblia* 8.6%. Azian et al (2007) in a study on aborigin community in Pehang, Malysia, noted that the prevalence of *G. lamblia*, and *E. histolytica* were 29.2% and 26.2% respectively.

The extent of double infection was lower than that of single infection in the present study. The parasite combination (*A. lumbricoides* and *T. trichura*) was 1.84% and another combination (*A. lumbricoides* and hookworm) was 1.27% in the observed samples. Opara et al (2007) were found 1.6% prevalence of *A. lumbricoides* and *T. trichura* combination among the school children which was closely related with the present investigation. But their other finding, 3.3% of *A. lumbricoides* and hookworm combination was higher than that of the findings of our study. Kaur and Sween (2007) reported 17.64% of *A. lumbricoides* and hookworm combination which was much higher than that of the findings of the present study.

Rahman (2009) reported 0.28% of *E. histolytica* + *A. lumbricoides*+ *T. trichura* and 0.03% of *A. lumbricoides* + *T. trichura* + *S. stercoralis* triplet parasite combinations only from the female subjects which were much lower prevalent from our study

findings (0.70% and 0.12% respectively). Opara et al (2007) stated the prevalence (3.3%) of *A. lumbricoides* + *T. trichura* + hookworm triple parasite combination among the school children while it was comparatively higher than our findings (0.38%). The difference may be due to the study population and environmental obligation.

The higher prevalence of intestinal parasites among the adolescent girls in Bangladesh may be due to its climate, temperature and environment, all of which favour the growth of different parasites. The adolescent girls of the rural and slum areas were more vulnerable to parasitic infestation than urban adolescent girls due to the differences in hygienic conditions, rate of literacy and prevailing socioeconomic conditions.

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A Minireview : matrix metalloproteases-an invasive arm of nematode parasites

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Abstract : Matrix metalloproteases (MMPs) are a family of Zinc-dependent endopeptidases responsible for degrading extracellular matrix (ECM) components and has been responsible for diverse set of developmental processes. However, expressions of many MMPs have been associated with several pathological conditions. It has been established that the MMPs are conserved throughout the animal kingdom and studies of invertebrate MMPs have demonstrated that primarily they are involved in various developing functions in hydra, *Drosophila*, sea urchin and nematodes. The synthesis of these proteolytic enzymes and their release as excretory and secretory (ES) products have been reported in various parasitic nematodes. Host invasion and tissue migration of several nematodes have been linked to the expression and release of parasite-derived proteases. Studies with enzyme inhibitors suggest that the enzyme may be a metalloprotease. Moreover, substrate impregnated zymographic analysis of extracts and ES products of different nematode parasites have revealed the multiple enzyme activities of MMPs with various molecular weights. Molecular cloning of a gene encoding matrix metalloproteinase-like protein from *Gnathostoma spinigerum* has also been done. This MMP-like protein of *G. spinigerum* possesses the catalytic domain, but lacks the propeptide and hemopexin-like domains found in other MMPs. More research on MMP degradome in nematode parasites can provide valuable information for intense evaluation of pathogenesis caused by these parasites.

Key words **:** *Endopeptidases, Matrix metalloprotease, MMP, Nematode parasites.*

Introduction

It has been established that various biological processes including cell migration, wound healing, tissue differentiation, immune system have been accomplished by morphological changes in extracellular matrix (Stocker et al 1995). Matrix metalloproteases (MMPs) are the major group of enzymes with ability for degrading extra cellular matrix proteins and this process of ECM remodelling is responsible for all these necessary processes (Shapiro,1998). The MMPs constitute a part of large family of zinc-dependent endopeptidases. These proteins may be found from bacteria to advanced forms of life even in several viruses (Ugalde et al 2010) and have been considered as a group of highly conserved proteins throughout the course of evolution. These enzymes seem to play various functional roles and touch many aspects of physiological and pathological processes not only in vertebrates but also in invertebrates (Masova et al 1998). The parasitic nematodes are the major group of invertebrates, which dwell in different organs of vertebrate hosts (Anderson,1992). Many of these parasites to complete their life cycle have to migrate or invade through various organs of their hosts. During this journey, expression and release of MMPs by various parasitic nematodes have been associated with the pathology resulting from histolysis (Hotez et al 1990; McKerrow et al 1990; Lai et al 2005; Williamson et al 2006). Genetic analysis and cDNA cloning of MMPs have been evaluated in few parasitic nematodes (Newport et al 1988; Uparanukraw et al 2001; Salter et al 2002).

MMPs and nematode parasites

Metalloproteases comprise a heterogeneous group of proteolytic enzymes whose main characteristic is the utilization of a metal ion to polarize a water molecule and perform hydrolytic reactions. MMPs are a major group of zinc-dependent endopepdidases with a ability to cleave one or more extracellular matrix constituents as well as nonmatrix proteins (Hotez et al 1990; Williamson et al 2006). These enzymes include a wide range of proteases in many organisms and play essential roles in multiple biological processes. By virtue of these proteolytic property proteases regulate a variety of cellular processes such as cell proliferation, cell-cycle progression, tissue differentiation and migration, apoptosis, senescence, DNA replication and autophagy. In metazoans, proteolytic activities are also involved in the maintenance of tissue homeostasis and in the regulation of different physiological processes such as fertilization and fecundation, embryonic development, wound healing, tissue remodeling, immune response and angiogenesis (Ugalde et al 2010). However, expressions of some of the MMPs have been associated with different pathological manifestations, which are predominantly associated with inflammation, arthritis, cancer

metastasis and parasitic invasion through host's tissues (McKerrow et al 1990; Hawdon et al 1995; Ugalde et al 2010). Life cycles of many nematode parasites consist of different migratory as well as invasive larval stages in host environment, even sometimes young and adult may be invasive in their nature (McKerrow et al 1990; Lai et al 2005). Host invasion and tissue migration of several nematodes have been linked to the expression and release of parasite-derived proteases. In nematodes, MMPs are the proteases which are thought to play an important and essential role in these migratory and invasive phenomena (McKerrow et al 1990). According to their substrate specificity MMPs can be categorized as collagenases, gelatinases, elastases, Stromelysins and membrane-type MMPs. Nematode MMPs generally include collagenases, gelatinases and elastases (Petralanda et al 1986; McKerrow et al 1990; Healer et al 1991).

Research on helminth derived MMPs got momentum in eighties. Most of these works evaluated MMPs from excretory-secretory (ES) products as well as the extracts of parasites (Lai et al 2005). Initial period of MMP research in nematodes included detection and measurement of activity in different life stages of nematode parasites.

Petralanda et al.1986 determined collagenilytic activity in extracts of adult worms, in living microfilariae of *Onchocerca volvulus* and in live infective larvae and adult female worms of *Brugi malayi*. In culture, infective larvae of *B malayi* also secreted large amounts of collagenase. Studies with enzyme inhibitors, antigen-antibody reactions and immunoprecipitation indicated that this protease might be a metalloproptease. In another experiment scientists (Robertson et al.1989) did characterize this metalloprotease in *Toxocara canis* by using different techniques. Various forms of enzyme have been determined by zymography where they found two bands with molecular weights of 120 and 32 kDs. This group also determined the optimum pH at 9 with less activity at pH 5 and 7. As a potent inhibitor Phenylmethylsulfonyl fluoride was used to characterize the metalloprotease nature of these proteins.L3 Larvae of a nematode parasite *Strongyloides stercoralis* have an ability to migrate through tissue at a speed of 10 cm per hour (McKerrow et al 1990). Their studies indicated that this process of migration was facilitated by a metalloprotease with elastase activity. This invasive property of elastase has also been determined by inhibition study and author indicated that the parasitic virulence factor may be guided by metalloprotease and could be a molecule for therapeutic purpose. In another study inhibitor as zinc-chelator like 1,10-phenanthroline has been used to prove that proteolytic activity of MMP is zinc-dependent(Hawdon et al 1995). However this study concentrated on the role of MMP in transition from free-living to parasitic mode of life. In 1990, Hotez and colleagues published a work including the study of metalloprotese from a human hookworm species *Ancylostoma duodenale and* a zoonotic species *A. caninum* and they found that these two species could synthesis MMPs of similar moleculat weights(68 and 38 kDs)and properties that might cause tissue degradation, exsheathment and ecdysis(Hotez et al 1990; Richer et al 1992).In their study, radiolabelled fibronectin has been degraded by both species of *Ancylostoma* larvae,which could be corroborated with the activity of MMPs found in *Strongyloides stercoralis* larvae and *Serratia marcescens* (Hotez et al 1990). Along with the determination of molecular weight of metalloprotease,found in larval ES product and adult stages of *Nippostrongylus brasiliensis* Healer et al.1991 also characterized the zymogen form of MMP in larval somatic extract. However, it has been found that uninfected rat intestinal tissue has similar kind of protein with identical molecular weight, suggesting the uptake of this metalloprotease by the parasite. A high pressure liquid chromatography technique has been used to purify a zinc metalloprotease ,isolated from in vitro culture fluids of *Trichuris suis* adults. (Hill et al 1993). Determination of isoelectric point and immunohistochemistry of MMP has also been done in this experiment.

Analysis of tissue extracts and ES products of *Angiostrongylus cantonensis* showed the activity of different form of MMPs (Lai et al 2005). They examined gelatinase activities from extracts of L1, L3, young and adult stages of this parasite. Five gelatinase bands (94,86,66,42 and 30 kDs) were observed in L3 larval stages, and minimum number of bands (94 and 72 kDs) were evident in young and adults. All these bands of various stages showed common characteristics with MMP, cystein and serine proteinase. Zymographic bands of L1 (105,94,42 kDs) and L3((105,66,50, and 30 kDs) ES products were proved to have proteolytic activity whereas adult and young ES products had no gelatinase activity. Of these bands obtained from larval stages, 94 and 105 kDs were gelatinase and others were metalloproteinases. In this experiment about 2000 larvae were used to detect enzyme activity in contrast to another experiments (Lai et al 2004 and Lee et al 2004) when 60 larvae were used to infect mice and MMP activity was determined. This differentiation led the author to suggest that in later experiments MMPs were secreted by the host not by parasite. Simultaneously, several authors provided information related with the activities of MMPs in different helminthes and MMP mediated histolysis of skin and intestinal walls (Tort et al 1999) and degradation of ECM proteins (Petralanda et al.1986). It has been evident that after ingestion, *A. cantonensis* larvae could invade and penetrate host stomach or intestinal wall (Alicata,1965). Considering all these experimental evidences, in their publication Lai

and group ultimately suggested that MMPs secreted by larvae could be associated with parasite spreading and pathogenesis in host.

In developing countries 740 million people are infected with hookworms (deSilva et al 2003). In most of the cases hookworm infect took place by penetrating host's skin and after getting inside of host, larvae migrate by invading tissues of various organs(Hotez et al 1990).Proteases have been considered as invasive arm for tissue penetration by parasitic helminthes and many experiments revealed that characterization of protease enzyme activity has been evaluated from larval ,young and adult crude extract as well as excretory and secretory (ES) products of many parasites(knox et al 1990; Gamble et al 1996; Halfner et al 1998 and Lai et al 2005).

Williamson et al. 2006 did an experiment where they found an astacin-like metalloprotease (*A*c-MTP-1) activity in ES product of *Ancylostoma caninum* L3 larvae.This *A*c-MTP-1 has a sequence similarity with zinc-metalloprotease . L3 larval stage of *A caninum* can exclusively express Ac-MTP-1and it's activity in culture medium indicates its role in host tissue invasion. Activity of metalloprotease in this study has been proved both by native and recombinant emzymes and using immunoelctron microscopy, site of synthesis in the secretory granules of the glandular esophagus of L3 and its course of transportation to cuticle and followed by secretion from cuticle have been evaluated. More recently an orthologue of Ac-MTP-1 known as Ay-MTP-1 has also been detected in *Ancylostoma ceylanicum.* This group of scientists suggested a probability of using MTP-1 as the target molecule for developing vaccine to prevent larval migration through tissues.

Similar kind of metalloprotease has been characterized from sheep barber's pole worm *Hemonchus contortus* (Halfner et al 1998). However, no recombinant enzymes have been used in this study. Uparanukraw et al 2001 has used the technique immunoscreening with the monoclonal antibody to synthesise a cDNA clone from L3 larvae of *Gnathostoma spinigerum*. From this clone, they have identified a gene of 732 bp encoding a metalloprotease having 33-39% similarity with MMP of *Caenorhabditis elegans.*

From above discussion it has been assumed that parasitic nematodes have the ability to modify or degrade host's extracellular matrix by secreting metalloproteases. This process of invasive nature assists these parasites to migrate through different host tissues causing pathogenesis. Simultaneously our knowledge related with characterization as well as pattern of expression of genes of these proteases in nematode parasites is not satisfactory.

In conclusion,it has been found that characterization of MMPs in parasitic nematode have been evaluated but not with specific and sufficient information. Still we don't have that much of knowledge about different MMPs in many other nematode parasites. Present study needs much attention towards more intense morphological and functional characterizations of gene or gene family of MMPs and all these studies related with parasitic helminth secreting metalloproteases and relevant gene family can help to investigate more intensely the pathogenesity as well as to develop antimetalloprotease drug to combat against helminth parasites.

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Evaluation of sarcocyst infested bovine carcassess

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Abstract : The study was conducted to evaluate the Prevalence of sarcocyst in bovine carcasses, total 120 buffaloes (60male 60female), 120 beef (60male,60 female) of age group 5–15 yrs were randomly taken from the Tanagra Slaughter House, Kolkata. In addition, age and gender of the investigated animals were assessed by visual inspection of teeth, horns, and sexual organs. Oval shaped macrocyst were observed in oesophagus,heart, skeletal muscle, intercostal,tongue, diagphram. Sections of cardiac muscles revealed presence of sarcocyst. Out of 120 buffaloes examined, 29.2% and 120 beef examine, 23.3% animals showed sarcocysts infection. The prevalence of sarcocysts noted was highest in oesophagus (65%) in buffaloes, and lowest in Diaphragm (10%). In beef,also highst in oesophagus(55%) and lowest in tongue (20.1%).But no infection was recorded in intercostals in buffaloes and intercostal, diaphragm in beef carcasses. animals when slaughtered during pre monsoon and post monsoon period showed higher 20% of infection in male,18.3% of infection in female in buffaloes and 18.3% of infection in male,15% of infection female in beef in their respective carcasses than that of the other animal slaughter during pre monsoon and post monsoon period. Since the period of the study is limited, the work was only confined to organ wise prevalence, clinico-pathological, economic loss of carcasses and some patho morphological changes and to establish the pathologic effects of sercocystosis in bovine.

Key words **:** *sarcocystosis, buffalo, organ, economic impact.*

Introduction

India is endowed with the largest livestock population in the world. It accounts for 57 per cent of the world's buffalo population and 15 per cent of the cattle population. According to Livestock Census (2003), the country has about 18.5 crore cattle and 9.8 crore buffaloes. The contribution of livestock to Indian GDP accounts for more than of total 32% share from Agriculture. as per current estimate,animal sector contributes 180,000 crores and more than 50 crores daily. Livestock sector not only provides essential protein and nutritious human diet through milk, eggs, meat, etc., but also plays an important role in utilisation of non-edible agricultural by-products. Livestock also provides raw material/by products such as hides and skins, blood, bone, fat, etc.

The prevalence of the disease poses a threat due to mortality and morbidity losses in buffaloes and beef.Infectious diseases cause significance losses affecting the economy of the owner as well as the country. Besides losses, there are export restrictions and increasing danger of public health hazards. In case of outbreak of epizootics, the losses are much more colossal. Parasitic diseases are primarily concerned with the severe production losses, both in quality and quantity of meat and milk. Besides, some of them are of zoonotic importance. Sarcocystosis is one of such serious protozoan parasitic disease as its causes high rate of morbidity losses in young animal (Dubey et al, 1989) along with the symptoms of reduce milk production.Besides it, has a zoonotic significance. Thus, it has aroused the interest of various scientists. Its sites of location are in the muscular system mainly besides the other organs, affecting the quality and quantum of meat production.

In sarcocystosis, the endothelium and the muscles of other soft tissues are invaded by the protozoan of the genus sarcocystis. Heart, diaphragm and the skeletal muscles are the preferred organs of predilection of sercocystosis sp in the intermediate hosts and can persist throughout the life in the host but many start to disappear after 3 month of inoculation (Mitchell et al. 1988). Meat is heavily infected and may be condemned as unit of human consumption (Olsen, 1974). The definitive hosts are infected when it ingests tissues containing sarcocysts. All these factors are responsible for poor diagnosis of the disease and thus increase the importance of more extensive research on this disease.

Material and methods

During the period from March to November-2010, sample was collected on the basis of initial clinical symptoms and gross pathological lesions observed during examination of carcass afterwards. Animal showing initial clinical resembling sarcocystis

infection were primarily suspected for the infection. The period of collection was categorized by premonsoon (March to May), monsoon (June to August), and post monsoon (September to November).

In total 120 buffaloes (60male 60female),120 beef (60male,60 female) of age group 5–15 yrs were randomly taken from the Tanagra Slaughter House, Kolkata.

In addition, age and gender of the investigated animals were assessed by visual inspection of teeth, horns, and sexual organs. The oesophagus, heart, tongue, cervical and abdominal muscle, diaphragm as noted by Biswas et al.(1992) were chosen for inspection and collection of samples. Immediately after the animals were slaughtered, ocular inspection was made in order to detect macroscopic Sarcocystis cysts.

The sample are collected in the polythene bags containing ice and brought to the pathological laboratory. Each morbid tissue was then divided into 2 parts, one part is kept as room 4 degree c temperature meticulous research and recovery of sarcocysts and the other part of the same tissue was preserved in 10%formal saline.

Esophageal muscles were examined for the macroscopic cysts against lights after recovering the superficial fascia, fat and connective tissues where as to detect the minute size sarcocysts and oesophagus were ballooned by filling their lumina with water (jain and shah,1985).The macroscopic cysts were teared out with and forceps while the microscopic cysts required treatment with 1% pepsin or 1% trypsin at room temperature for 1 hour which is known as pepsin digestion technique or trypsin digestion technique (Jaquobs et al,1960).

Histo-Pathological study

Muscular samples sized approximately 15 mm × 10 mm with or without macroscopically visible sarcocysts were cut from each of the muscular tissues. The samples were fixed in neutral buffered 10% formalin, processed by standard histological techniques, sectioned at 5 ìm, stained by Haematoxylin and Eosin as per the method describe by Lillie(1965), finally,sections were mounted with DPX and examined by light microscopy for the presence of sarcocysts or for histopathological lesion.

Results and discussion

1. Clinical diagnosis

Antemortem examination

A preliminary diagnosis of sarcocystosis in buffaloes and beef were made based on manifestation of common clinical signs during ante mortem examination prior to slaughter of the animals.

Apparent symptoms of the diseases viz fever ranging between 103-108° F, pale mucous membrane, reduced body weight,as reported earlier by Johnson et al. (1974),Jungmman et at,(1977); Seteu (1981); Chowdhury (1999);Gharagozlou et al. (2001) were taken into consideration for clinical diagnosis. Haydorn(1977); Foreyt et al.(1995); Dubey (2001).

Post mortem examination

After opening the carcass, the individual organs were critically examined oesophagus,heart, skeletal muscle, intercostal,tongue, diagphram were thoroughly dissected.Oval shaped macrocyst were observed in the oesophagus and ther were haemorrhage in heart which confirmed the reports of Paikne et al.(1980).

2. Pathological observation

Gross lesion : Oesophagus revealed oval shaped macro cyst (fig.no.1) Haemorrhages were observed in cardiac muscle which confirmed the reports of Paikne et al.(1980). Other organs revealed no macroscopical changes.

Oesophagus

Histopathological sections showed the presence of sarcocysts within the muscle fibre.(fig no.2) occasionally,some sections had more than one cyst embedded in the muscle fibre (fig no.2) similar observations were reported by Gajadhar et al.(1986) who has recorded massive cellular infiltration in all the muscles lodging the cyst.loss of striation of the muscle were quite clear and evident in some sections (fig no.3) which is confirned the reports of Szarek (1982); Mohanto et al.(1995) who also disintegration of muscle fibre along with the loss of striations.

3. Histopathological observation

Cardiac muscle

Sections of cardiac muscles revealed presence of sarcocyst (fig no.4). Fibrosis in the area of degenerated muscles also observed in some cases. These finding were similar to these reported earlier by Szarek (1982); Mohanty et al.(1995); Raphael

(2000). Rupture of the capillaries might be brought about by the rupturing second generation schizonts. This might be initiated an inflammatory responses and migration of the cellular components of the blood resulting in the disorganization of the myofibrils.

Skeletal muscle

Histopathological section revealed loss of striation and degeneration of the muscle fiber (fig no.5) along with fibrosis in some region which corroborated the findings of Mohanty et at. (1995) and Raphael (2004), where they are observed similer type of result in muscle of sarcocysts infected carcasses.

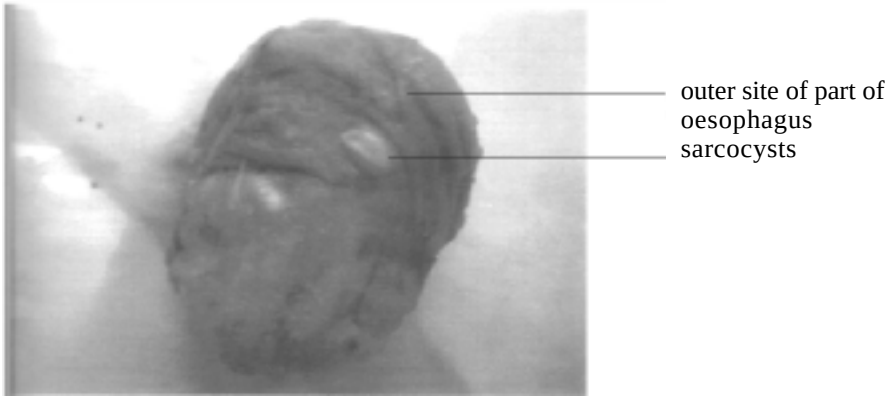

Figure 4. cooked rice type sarcocysts in oesophageal muscle.

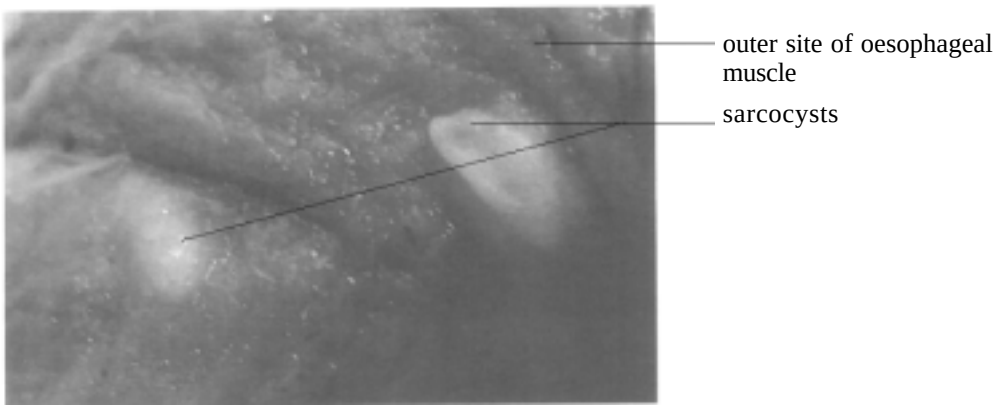

Figure 5. Higher magnification of figure no 4. 100x

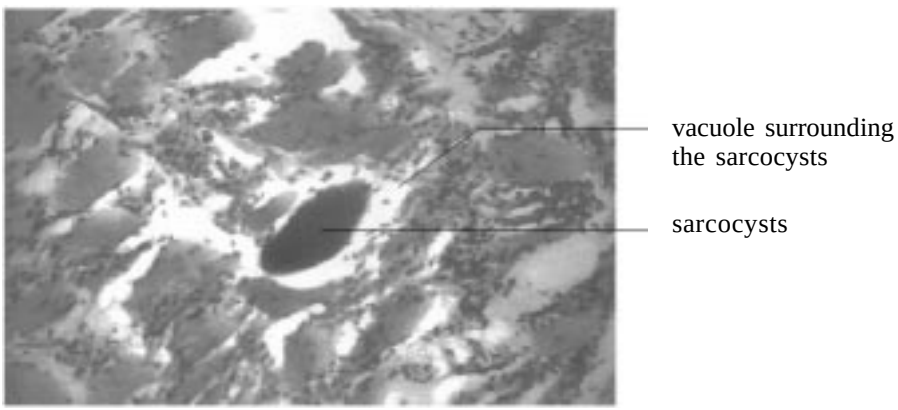

Figure 6. Loss of striation of muscle and cellular infiltration surrounding the sarcocysts in oesophagus. (magnification)

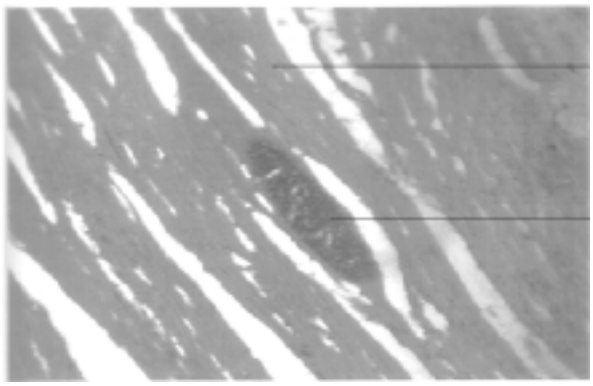

Figure 7. Sarcocysts located in cardiac muscle.

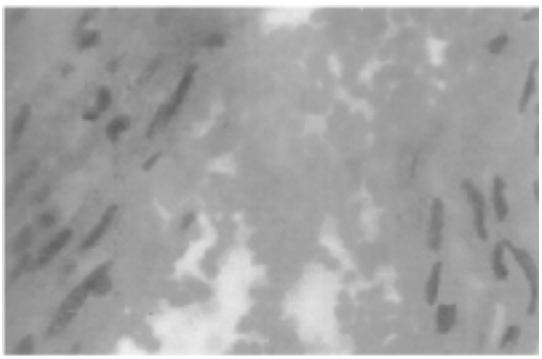

Figure 8. Degeneration of myofibrils with complete loss of striations.

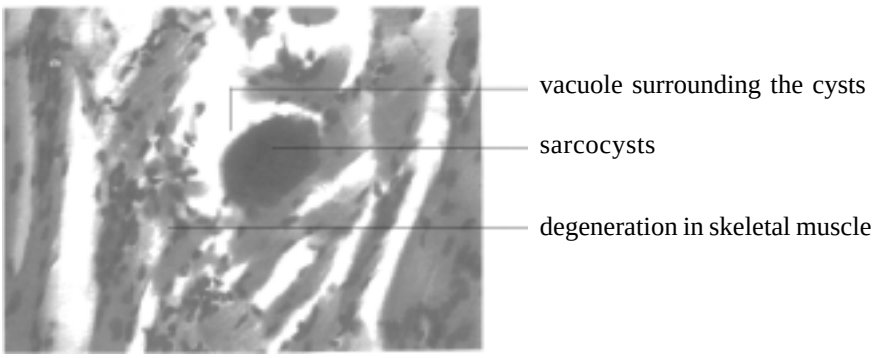

Figure 9. Loss of striations and degeneration in skeletal muscle with cellular infiltration.

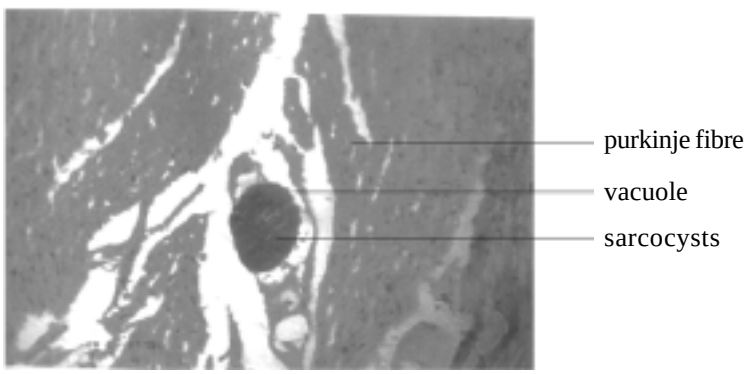

Figure 10. Presence of sarcocysts in purkinje fibre in heart.

4. Prevalence

For the present study of the heart, oesophagus, diaphragm, tongue and skeletal muscle were collected from 120 beef (60 male,60 female),120 buffaloes (60 male,60 female) during slaughter.Out of 120 buffaloes examined, 29.2% and 120 beef examined, 23.3% animals showed sarcocysts infection. Earlier study conducted in West Bengal,Biswas et al.(1992) reported 60.93% prevalence rate of sarcocysts infection in beef cattle.the distribution of sarcocysts in different muscle and tissues was presented in table no....

Table 1. Tissue wise Prevalence of Sarcocystosis in Buffaloes

Musculature Site	Number Examine	Number positive	Percentage
Oesophagous	20	13	65
Diaphragm	20	02	10
Tongue	20	07	35
Heart	20	09	45
Skeletal Muscle	20	04	20
Intercostal	20	–	–
total	120	35	29.2

Table 2. Tissue wise Prevalence of Sarcocystosis in Beef

Musculature Site	Number Examine	Number positive	Percentage
Oesophagus	20	11	55
Diaphragm	20	–	–
Tongue	20	4	20.1
Heart	20	8	40
Skeletal Muscle	20	5	25
Intercostal	20	–	–
total	120	28	23.3

The prevalence of sarcocysts noted was highest in oesophagus (65%) in buffaloes, and lowest in Diaphragm (10%). In beef,also highst in oesophagus(55%) and lowest in tongue (20.1%).But no infection was recorded in intercostals in buffaloes and intercostal, diaphragm in beef carcasses.

It was observed that bovine oesophagus muscle was most prevalent site for sarcocystis infection which confirmated the reports of Biswas et al,(1990); camisasca et at.(1990); Gharagozlou et at.(2001).

And second position of sarcocystis infection in heart but Santana (1982); Desh pande (1983); O'-toole et al (1986); Carvalho (1993); Mohanto et al.(1995), while Biswas et al, reported that 47.83% infection in heart in carabeef in wast Bengal.

In this study, no infection was recorded in intercostal and diaphragm both in carabeef and buffaloes might be due to the fact that the portion of these collected for the study was devoid of sarcocysts or parasites remain in assested in larval stage due to environmental condition.

Table 3. Prevalence of Sarcocystis spp. according to age and gender in water buffaloes

N=120 (N = Total no. Of Organism)

Age Range (years)	Gender	No. examined	No.positive	
			No.	%
2-3	Male	20	6	15
	Female	20	2	10
4-5	Male	20	8	41
	Female	20	7	36
6-7	Male	20	13	65
	Female	20	10	50

Table 4. Prevalence of Sarcocystis spp. according to age and gender in beef
N=120 (N = Total no. Of Organism)

Age Range (years)	Gender	No. examined	No.positive	
			No.	%
2-3	Male	20	2	10
	Female	20	0	00
	Total			
4-5	Male	20	9	
	Female	20	45	7
	Total		36	
6-7	Male	20		
	Female	20	11	
			54	
			7	
			36	

In the both male and females the prevalence of sarcocystis infection increased with age. Overall, the prevalence of infection was lowest in 2-3 years old animal (10% in male, 15% in female in case of buffalo and 10% of male only in case of beef) and highest in 6-7 years old animals (65% in male, 50% in female in case of buffalo but 54% in male, 34% in female in case of beef).There was no difference between the prevalence in the age groups 4-5, 6-7.

Observation of infection of higher age group (6-7) in both the species of animals might be due to the fact that, older animal's finds longer duration towards organism and there by exhibit the symptom in the form of macro and micro cysts in different vital organ. Where as in lower age group animals such exposure are often limited. The statement is in agreement with the observation of Huong,(1999) reported prevalence increased with age from a 57% infection rate among 2-3 yr old animals to 93% among 6-7 yrs olds.

The month wise prevalence revealed highest infection in post monsoon season (September to November) i.e. 20% in male, 18.3% in female in case of buffalo but 18.3% in male, 15% in female in case of beef and lowest in pre monsoon season (March to May) i.e. 8.3% in male, 3.3% in female but only 3.3% in male, which is in partial agreement with Singh et al (1992).,Pathkar and shah (1991) and Wadajkar et.al (1992) where they also reported the similar observation.

Reason behind such observation might be due to acquire such infection during monsoon period when chances of getting infection where more from the pasture and such infection got settle with the system/organ differently of the animal carcass. Thus such animals when slaughtered during pre monsoon and post monsoon period showed higher 20% of infection in male,18.3% of infection in female in buffaloes and 18.3% of infection in male,15% of infection female in beef in their respective carcasses than that of the other animal slaughter during pre monsoon and post monsoon period.

Economic impact

There has been very critical study of the economic loss caused by sarcocystis sp infection, but circumstantial evidence indicates that they are responsible for a market loss in production. This infection also changes the quality and quantity of carcasses of buffalo and beef and reduces the market value than that of its original value.

Table 5. Approximate economic loss due to sarcocyst infection buffalo carcasses
≈ Number of slaughtered animals 500 per day in Tangra Slaughter House, Kolkata

Infected organs	% of damage	≈Weight of each organs (kg)(up to 6-7 age)	≈Market value of each organ (Rs)	% of damage in total slaughtered animals	Market value of normal carcasses (Rs)	**Carcasses value of % of damage (Rs)
Oesophagus	65	1.5	50/piece	325	25000	16250
Diaphragm	10	1	10/piece	50	5000	500
Tongue	35	500 gm	15 (500 gm)	175	7500	2625
Heart	45	2.5	20/piece	225	10000	4500
Skeletal Muscle	20	5*	50/Kg	100	25000	5000

*In 20%of the carcasses, the extent of the damage caused by the Sarcocyst infection was found to be high,resulting a partial condemnation of skeletal muscles, totalling the loss of the muscle about 5 kg on average /carcasses.

**These amount is the approximate direct loss for the infection but the intensity of infection has a direct effect on the health and growth of the animal.This part of loss is not covered with in area of the present study

Above table no. Showing the great economic loss due to cause of sarcocystosis infection of buffaloes carcasses and also indicates the reduction of market against normal due to this disease.

Table 6. Approximate economic loss due to sarcocyst infection beef carcasses

≈Number of slaughtered animals 500 per day in Tangra Slaughter House, Kolkata

Infected organs	% of damage	≈Weight of each organs (kg)(up to 6-7 age)	≈Market value of each organ (Rs)	% of damage in total slaughtered animals	Market value of normal carcasses (Rs)	**Carcasses value of % of damage (Rs)
Oesophagous	55	1.5	54/Piece	275	27000	14850
Tongue	20.1	500 gm	12 (500 gm)	100.5	6000	1206
Heart	40	2.5	20/piece	200	10000	4000
Skeletal Muscle	25	5*	70/Kg	125	35000	8750

*In 20%of the carcasses, the extent of the damage caused by the Sarcocyst infection was found to be high,resulting a partial condemnation of skeletal muscles, totalling the loss of the muscle about 5 kg on average /carcasses.

**These amount is the approximate direct loss for the infection but the intensity of infection has a direct effect on the health and growth of the animal.This part of loss is not covered with in area of the present study

Above table no. Showing the great economic loss due to cause of sarcocystosis infection of beef carcasses and also indicates the reduction of market against normal due to this disease

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Suppression of *Fusarium oxysporum* affecting cucumber host by treatment with plant growth-promoting rhizobacteria

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Abstract : *Fusarium oxysporum* var *cucumerinum* is one of the most predominant pathogen affecting cucumber hosts. The disease is mostly prevalent during warm summer to monsoon season when the temperature ranges between 28^0C-34^0C and the relative humidity ranges between 90-98 %. Several studies have been conducted since the past few decades related to the suppression of various pathogens affecting cucumber plants by application of plant growth-promoting rhizobacteria (PGPR) and subsequent induction of systemic resistance in the cucumber hosts. Our present study was aimed at testing the efficiency of the PGPR isolated from the rhizosphere of some members of cucurbitaceae in retarding Fusarium wilt pathogen in cucumber hosts in Indian agro climatic background with special emphasis to Bengal. This experiment was conducted in the month of August to October '2010. Roots of cucumber (Cucumis sativus) seedlings of two weeks old were treated with PGPR suspension for an hour and transplanted to pots of 10 cm diameter. Fungal inoculums were added to the sterile soil seven days after transplantation. The number of infected leaves, diseased plants and the plant growth parameters were observed twice weekly for five weeks. The disease severity was found to be least in plants treated with bacterial isolates which was evident from the delayed disease symptom development and reduced number of dead plants. In contrast to this the pathogen treated plants showed disastrous results.

Keywords **:** *Plant Growth Promoting rhizobacteria, suppression, pathogen.*

Introduction

Fusarium oxysporum is a widely distributed soil-borne plant pathogen. (Fravel et al, 2003). It affects a wide range of hosts, mainly flowers, fruits vegetable yielding crops viz. carnation, cucumber, water melon, muskmelon, tomato, legumes like pea, beans; some field crops such as cotton, jute, tobacco, plantation crops like banana, sugarcane, coffee etc. (Agrios, 2005)The pathogen thrives well in warm humid climate specially in sandy soil chiefly causing Fusarium wilts. In Indian agro- climatic background, the pathogen is more prevalent in temperate areas i. e. in the northern states during summer when temperature ranges lie between 15°C to 25°C and relative humidity is moderately high ;in the states lying in the eastern and southern part of the country the disease incidence related to the pathogen is more frequent under warm-humid climatic conditions during the rainy season. In India, banana, tomato, gladiolus and vanilla are the chief cash crops affected by the attack of this organism. In Fusarium wilts infected plants become stunted and soon wilt and finally die, occasionally entire fields are affected before the crop is harvested. Serious losses are caused by rather high soil and air temperatures. (Nelson, 1981). Several cucurbits are cultivated in India all the year round. Of them, cucumber (Cucumis sativus) is a warm loving, second most largely grown cucurbit after water melon; cultivated throughout the year in garden lands and riverbeds in tropical, sub-tropical and temperate regions of Indian territory. Cucumber is popularly known in India as 'khira' and gherkins. A well drained loamy soil is most preferred by this crop. In sandy river beds, alluvial substrata and subterranean moisture of river streams support cucurbits. The optimum temperature for growth is 26. 4°C. The crop is susceptible to frost and excess humidity makes the crop vulnerable to diseases to powdery mildew, downy mildew, Fusarium wilts, pests such as fruit fly. The immature fruits owing to their juicy and refreshing taste are commonly taken as salad or as vegetables in curries and pickles, (Singh, 2008). Tender leaves are also used as vegetables. The fruits are used as astringent and seeds are valued as spices and condiments. (More, 2001) The annual production of the crop in recent years is 50. 667 mt in 2000-2001 of which a significant portion is exported, the quantity being 10, 766mt in 1997-98, thus earning an enormous amount of money. The main importers of fresh or chilled gherkins from India

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are Belgium, Spain and USA; preserved gherkins Belgium, France, Spain and USA; and prepared/preserved gherkins USA, Belgium, Canada, France, the Netherlands, Spain and UK. The total amount estimated to be imported by USA is about 1.20 lakh tones in 2008-2009. (PNB report).. A number of varieties like Poona khira (a small-size pale-green fruit cultivated in western Maharashtra), Balam khira (cultivated in Saharanpur in Uttar Pradesh); and the Darjeeling and Sikkim varieties (grown in hills of North Bengal) have become very popular. Sheetal, Pusa Sanjog and Priya are some of the varieties developed by Indian researchers (ICAR bulletin). Powdery mildew, downy mildew, anthracnose, Alternaria blight and Fusarium wilt are reported to be serious fungal diseases of cucumbers causing severe economic loss. Aphid-transmitted cucumber mosaic virus damages the crop to a great extent. Fusarium wilt of cucumber is caused by Fusarium oxysporum f. sp cucumerinum. Susceptible plants are infected through the roots during all stages of growth and eventually resulting in plant wilting and yield losses as high as 100%(Sherf and MacNab, 1986). Powdery mildew and anthracnose can be effectively controlled by Bavistin (0.1%) or Calixin (0.05%), whereas downy mildew can be controlled effectively with DithaneZ-78 (0.3%) and Ridomil (0.2%). Seed treatment with Bavistin (0.2%) checks Fusarium sp infection in cucurbit seeds (FAO). The use of fungicides for curbing fungal diseases has been a conventional practice, but such chemicals are leached off from the soil to the neighbouring water bodies along with the irrigation water. Thus causing eutrophication of such water bodies and hence affecting aquatic life even leading to increased mortality of fishes. Such chemicals viz carbamates consisting of dithiocarbamates, e. g. Dithane, organomercury compounds like methyl ethyl mercuric chlorides are biomagnified as it passes through different levels of consumers in the human food chain leading to detrimental and toxic effects on human health. (Roy, 2003). Prolong use of such fungicides have enabled the pathogen to develop resistance against them (Baysal et al. 2009). In this context WHO Directive related to water intended for human consumption has outlined the maximum admissible limit for pesticide concentration in water is 2µg/l. With the increase in concern to reduce environmental pollution levels both at the global as well as at the local level, scientists are emphasizing and advocating organic farming and integrated pest management practices in an endeavor to accomplish sustainable agricultural system and to protect the fragile ecosystem from further damage. (UNDP, UNEP).

Several bacteria have been reported to be effective in biocontrol of diseases of cucumber viz, *Pseudomonas putida* and *Serratia mercescens* in control of Fusarium wilt (Liu et al. l995), *Bacillus subtilis, Bacillus pumilis, Curtobacterium fluccumfaciens* in combating angular leaf spot caused by *Psudomonas syringae* pv lachrymans and anthracnose caused by *Colletotrichum orbiculare*. (Raupach et al., 2000), root and crown rot of cucumber caused by *Pythium aphanidermatum* can be suppressed by *Pseudomonas corrugata* and *Pseudomonas aureofaciens* (Chen et al. 2000);*Bacillus amyloliquefaciens, B. pumilis, B. sphaericus* against *Rhizoctonia solani* causing damping off, *Ralstonia solanaraceum* and wilt disease respectively(Jetiyanon, 2002). From previous studies it has been noted that antagonistic bacteria not only produce β1, 3-glucanase but also trigger defence related enzymes(Jayaraj, 2004).

The objective of the present study is to investigate the capability of *Bacillus* spp isolated from rhizospheric soil in curbing Fusarium wilt disease of cucumber in Indian agro -climatic background.

Materials and methods

Isolation of bacteria, screening of isolates based on antagonistic ability and its identification. Bacteria were isolated from the rhizosheric, rhizoplane soils obtained from research plots as well as agricultural fields of Nadia and Hooghly districts during March –June, 2009, collected in sterile plastic bags and were suitably labelled. The soil samples were then plated to nutrient agar plates by serial dilutions and adding inoculums at 10^{-5}-10^{-8}. Pure cultures of morphologically different isolates were obtained by repeated serial dilutions and sub culturing. The isolates were tested for their antagonistic abilities against fungal pathogens viz. *Fusarium oxysporum* (obtained from NCIM, National Chemical Laboratory, Pune, India), by dual culture technique on Potato Dextrose Agar (PDA) plates supplemented with yeast extract and peptone. Bacterial streaks were drawn at a distance of 1cm from the periphery of the plates and fungal discs 5mm in diameter were placed at a distance of 7 cm from the bacterial inoculums. The two organisms were placed on the plates at the same time and incubated at a temperature of 28±2°C. The inhibition zone was measured at every 24 hours interval for 7 days. Three replica of each were maintained and the zone of inhibition was compared against control. The isolates showing the greatest antagonistic activity were selected for further experiments.

The isolates were then tested for their preliminary biochemical characteristics such as-amylase production, urease production, catalase production, oxidase production, H_2S production, citrate utilization, pH tolerance(3-12), carbohydrate

utilization(3-30%), etc. They were then morphologically characterized by Gram's staining, flagellar staining (West,) and capsule staining (Graham and Evans,). The test results were utilized for bacterial identification following Bergey's manual of determinative bacteriology(8th edn.) and run in biolog machine to get accurate results.

In vitro screening of isolates for their plant growth promoting activities.

The biocontrol and plant growth promoting attributes of selected isolates were tested by standard procedures, viz. chitinase, β-1, 3 glucanase production were determined following the method of Cattelan et al. (1999). IAA assay was performed by culturing the cells in Luria Bertani broth supplemented with 0.2% tryptophan (Gordon and Weber, 1951), Phosphate solubilization ability of the strains were detected by spotting them on Pikovskaya's agar plates and observing for halo zones around the colony. Siderophore production was detected on CAS blue agar medium following the method of Schwyn and Neilands (1987). HCN production was determined by growing the isolates in King's B medium (1954) plates amended with 4.4g l^{-1} of glycine and placing filter paper strips soaked in picric acid solution (2.5 g l^{-1} of picric acid and 12.5 g l^{-1} of Na_2CO_3) in the lid of each petridish (Lee et al. 2008).

Study period and area.

Disease assessment and plant vigor test experiments were carried out during the period November to January, 2009 in the net house of our department located adjacent to the department, enjoying same meteorological parameters as in the Kalyani area of Nadia district and glass house respectively having suitable temperature and humidity regulation arrangement.

Raising of seedlings under green house conditions.

Plant material : Cucumber (*Cucumis sativus* L.) cultivars belonging to the local variety baro pata were selected for our experiment.

Seeds were surface sterilized with 0.01% $HgCl_2$ by dipping in the solution for 45 seconds and then washed with sterile water consecutively for three times. The seeds were then allowed to imbibe water overnight and then wrapped in moist piece of sterile cloth for germination in a petridish. The germinated seeds were then spread on 10 cm diameter pots containing sterilized mixture of field soil, leaf compost, sand and organic manure in the proportion of 4:2:2:2 w/w. Two sets were maintained in green house and net house respectively. Green house sets were exposed to 29-30/-20-25°C (day/night) temperature (provided by natural as well as artificial light) and 84-89% relative humidity. Plants were watered when necessary.

Confirmation of the virulence of fungal pathogen.

The culture of fungal pathogen obtained were utilized to infect cucumber host seedlings of fifteen days old by adding fungal inoculum of intensities (2/100) g of inoculums/soil grown in sand maize meal in pots of 5cm diameter containing sterile sand. Water was sprayed on the seedlings at critical intervals prior to the drooping of the plants. Fungi was isolated from seven day old infected plant that showed wilting and drooping of leaves, on water agar, after surface sterilization. The isolated pathogen was then sub cultured on PDA plates and incubated at 28°C for 7-8 days till the appearance of spores.

PGPR mediated ISR against fungal pathogen.

Cucumber seedlings with one fully expanded true leaf were used for the experiment. The experiment was designed following completely randomized block design model. Eight treatments and three replica of each were maintained in our experiment. The treatments were: treatment with *B. subtilis* (B101) of the seedlings followed by challenging with *F. oxysporum* at 15 day old stage, similar treatment with *B. pumilis* (B102), and a combination treatment of both challenged with fungal inoculum, acontrol treatment of each, pathogen control and water control.

Bacterial treatment : Bacterial treatment was done by seed surface sterilization, followed by dipping of 1 gm of seeds in bacterial suspension (10 ml having a cfu of 108 per ml) for 4hours. 10 ml of inoculum was placed in a petridish and about 1gm of carboxy methyl cellulose were added for the bacterial suspension to adhere to the seeds and seeds allowed to air dry overnight before sowing.

Inoculation of cucumber host with fungal pathogen.

Fusarium oxysporum PDA disc of 5mm diameter, three in number were placed in 12 g of sand maize meal and incubated at 28°C for 5days. The inoculums was then thoroughly nixed with the soil along with sterile distil water. CFU was found to be of the order 108 per ml.

Assessment of disease progress and plant growth promotion under glass house condition.

Disease severity was determined following the 0-4 visual scale of the rhizome and root according to Rothrock (1987) in which the different numerical values indicates the following: 0=rhizome and root with no symptom, 1=lesions less than 25%, 2=lesions ranging between 25-50%, 3=lesions 50-75%, 4=lesions 75-100%.

The disease ratio, disease index and biocontrol effect were calculated using the formula proposed by Li et al. (2008).

Disease ratio (%) = diseased plants X100/ diseased plants + healthy plants

Disease index (%) = Σ (grade of disease severity X diseased plant of this grade) X 100/total plants assessed X highest grade of disease severity.

Biocontrol effect (%) = disease index of pathogen control-disease index of bacteria treatmentX100/disease index of pathogen control.

The plant growth promoting activities of the biocontrol bacteria was evaluated based on the length of root and shoot, biomass of root and shoot and the seedling vigor index (ISTA, 1966). The seedling vigor index was calculated using the formula: Vigor index= percentage of germination X (mean root length + mean shoot length) (Abdul Bake and Anderson, 1973). Percentage of seed germination was also calculated.

Statistical analysis.

Each experiment was repeated thrice Results were expressed as mean ± standard error. The analysis of variance of the treatment effects on the plant growth parameters as well as biocontrol were performed using the statistical analysis software of SPSS 10 by means of one way ANOVA with factorial devices of treatments and interactions. Analysis of difference between the categories was calculated using LSD and the results at the confidence level of .001-.05 was considered statistically significant.

Results and discussion

Selection, screening and identification of antagonistic bacteria.

90 bacterial isolates were isolated from the rhizospheric, rhizoplane and root invaded regions of cucurbits growing in the Nadia and Hooghly districts and screened against *F. oxysporum* by dual plate assay Six isolates were selected with strong antagonistic potential and isolate B101 was found to be the best among all. The plant growth promoting ability of the six isolates were screened by invitro assay as shown in table 1. B101 and B102 showed the best chitinase and β-1, 3 glucanase activity. With respect to IAA and HCN production, siderophore production and phosphate solubilization, these two isolates proved to be superior than the other isolates in consideration. From the above results it is evident that isolate B101 and B102 does not only possess good biocontrol ability but also exhibited maximum plant growth promoting potential. Some of the attributes of the two selected isolates showed distinct β1,3 glucanase and chitinase activity HCN production that are indicative of their biocontrol ability (Lee, 2008). Such correlation with respect to biocontrol ability and plant growth promotion is well established for *Bacillus* species (Singh, et al. 2008)

Table 1. Plant growth promoting and antagonistic characteristics of bacterial isolates

Isolates Antagonism against	IAA[a]	Phosphate solubilization[a]	Siderophore[a]	HCN[a]	Chitinase[a]	β1, 3 glucanase[a] *F.oxysporum*[b]
B101	+++	+++	++	++	+++	+++ 20.5 a
B102 ++	++	++ ++ ++	++ 19.6b			
B103	+	++	-	-	++	- 11.5c
B104	-	+	-	-	+	- 9.5c
B105	-	+	-	-	-	- 8.7c
B106	-	+	-	-	-	- 5.3d

Values bearing as exponents different lowercase letters in the same row are significantly different ($p<0.05$) in accordance with LSD test.

[a]- = negative; + = positive, ++ = good; +++ = excellent.

[b]Results are the means of three replications.

Carbon substrate utilization of absolutely pure strains were analyzed by Biolog and the database comparison revealed 99% similarity with *Bacillus subtilis* and *B. pumilis*. Based on the physiological and biochemical test, isolate B 101was identified and confirmed as *B. subtilis* and B 102 as *B. pumilis respectively*.

Biocontrol of Fusarium wilt by B101, 102 under glass house condition.

The effect of B101, 102 and their combination on *F. oxysporum* infection of cucumber was evaluated under green house conditions (Table 2). Pathogen controls exhibited initial appearance of disease symptoms on stem that spread rapidly to the aerial parts of the plant accompanied by wilt of the entire plants, whereas the pgpr treated plants showed little disease symptoms of which single treatment withB101 showed slower progress of the disease compared to 102 and the combination treatment showed still less. Thus a significant (P<.05) decrease in disease severity, disease ratio and disease index was recorded in the order B101+102>b101> B102. The identification of antifungal compounds produced by *Bacillus subtilis* and *B. pumilis* needs to be further studied. However the role of lytic enzymes cannot be ruled out and this possibility seems to be plausible as chitin and glucan are prominent cell wall components of fungi. The role of such enzymes in biocontrol has been demonstrated in other B. *subtilis* strain and B. *pumilis* by coworkers (Leelasuphakul et al. 2006, Raupach 1998)

Plant growth promoting effect of the isolates under glass house condition.

From table 3, it is clear that the root and stem length, fresh weights significantly increased (P<. 01) in plants treated with PGPR, the maximum being in case of the combination treatment. Seed germination rate, viability, seedling emergence and vigor

Table 2. Effect of isolate B101(*B. subtilis*) and B102(*B. pumilis*) on biological control of *Fusarium* wilt of cucumber.

Treatments	Disease ratio(%)	Disease index(%)	Biocontrol effect(%)
Seed soaking with	B101		12.16b
	10.12b		73.60b
Seed soaking with	B102 17.	23c	18.43c 50.88c
Seed soaking with	B101+B102	11.12a 9.11a	79.24a
Pathogen control	42.77d	38.42d	-
B101 control	-	-	-
B102 control	-	-	-
B101+B102 control	-	-	-
Pathogen control	-	-	-
Untreated control	-	-	-

Results are the means of three replications. Means followed by lowercase letters are significantly different by LSD test at p<. 05 – indicates no diseased plants are recorded in the treatments.

Table 3. Effect of isolate B101 (B.subtilis), B102 (B.pumilis) on plant growth promotion of cucumber.

Treatments Vigor Index	Percentage of germination (%)	Shoot length (cm)	Root length (cm)	Shoot fresh weight (g seedling^{-1})	Root fresh weight (g seedling^{-1})	
B101	95.0a	18.70b	25.27B	3.45B	1.60B	4117.53b
B102	93.0a	17.20b	21.67C	3.42B	1.57B	4110.12b
B101+B102	98.0a	19.12b	25.34B	3.49B	1.66B	4119.34b
B101 control	94.0a	22.40a	28.65A	3.67A	1.83A	4747.65a
B102 control	93.66a	21.43a	27.19A	3.12A	1.80A	3575.06c
B101+B102 control	92.0a	22.50a	28.99A	3.71A	1.89A	4756.00a
Untreated control	82.0b	15.53c	20.87C	3.04D	1.21D	2983.67d
Pathogen control	78.0c	14.13d	18.43D	2.85D	1.11E	2541.47e

Values are the means of the three replications.Mans followed by different lowercase letters are significantly different by LSD test at P<0.05 and different uppercase letters are significantly different by LSD at P<.01

index of plants were also significantly increased at P<. 05 upon PGPR inoculation. Further the plants of the PGPR controls without the inoculation of *F. oxysporum* exhibited the best plant growth promotion. Enhanced plant growth parameters may be attributed to the enhanced production of IAA by both the strains. (Asghar et al. 2002). Application of *B. subtilis* GB03 and *B. amyloliquefaciens* IN937, a strain mixture has been reported to have enhanced the plant growth of *Arabidopsis* hormonal mutant(Ryu et al. 2007). Ashrafuzzaman et al. (2009) had revealed the increased rice seed germination and seedling growth by application of PGPR was probably due to the induction of IAA production and phosphorous solubilization. The production of siderophore and the phosphate solubilization ability could combat low iron stress, enhance the uptake of minerals, increase the root proliferation and thereby promote plant growth (Grossman et al. 1993; Gupta 2002).

From the above study it may be concluded that *Bacillus subtilis* and *B. pumilis* having a potential use in commercial agriculture for their yield enhancing potentiality, are equally competent to combat Fusarium wilt disease in cucumber. Efforts are to be made to design a suitable commercial formulation comprising of both the strains effective in combating widespread disease incidence of *Fusarium* wilt incidence in Indian agro climatic background. Previously some isolates of *Bacillus* have been successfully commercialized and marketed by Gustafson under the brand name Kodiak is widely used for suppression of cotton diseases in US (Brannen and Kenney, 1997). With the growing interest and concern of the scientific world for organic farming and sustainable agricultural practices, biocontrol agents for suppressing soil borne pathogen are gaining popularity since the last few decades that suggests that Bacillus will be increasingly used as biofungicide in a broad spectrum of horticultural crops in the near future.

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Report on a fungal parasite of plant parasitic nematodes of Manipur, India

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Abstract : The ectomycorrhizal (ECM) root of pine wood possesses a distinct mantle of fungal mycelium. The root growth patterns of the host plant are often altered by ECM development on the root system. In *Pinus* spp., it is often observed that proliferation of short root is stimulated by fungal colonisation. A very large number of fungal genera have been identified in the ECM association e.g. *Cenococcum*, *Hymenoscyphus*, *Laccaria* etc. (Ruess *et al*., 2000). These mycorrhizae are found to be pathogenic to nematodes. They may be either endoparasitic or predaceous. During a survey for plant parasitic nematodes of pine woods in Manipur, several fungi and bacteria were found to parasitize the nematodes. One of fungi is *Cladosporium cladosporioides*. The presence of the fungus is the first report of the occurrence of nematophagous fungi from Manipur. It has been isolated, characterised and tested for pathogenecity on nematodes. The results of the study are presented elaborately.

Keywords **:** *Cladosporium cladosporioides, ectomycorrhizal, nematodes, Pinus*

Introduction

The pine root is ectomycorrhizal (ECM) root, also known as “sheathing” mycorrhizae because of the presence of a mantle of fungal mycelium or distinct sheath that covers the growing roots. Plants have a fungal association in intimate mutualistic symbiosis, where both partners are living and exchanging substances from both directions *i.e*., a biotrophic association. Root growth patterns of the host plant are often altered by ECM development on the root system. In *Pinus*, the proliferation of short roots is stimulated by fungal colonisation and dichotomy of short roots is very prominent in pine ECM. A very large number of fungal genera have been identified in the ECM association e.g. *Cenococcum*, *Hymenoscyphus*, *Laccaria* etc. (Ruess *et al*., 2000).

Ectomycorrhizal roots of pine are associated with several floras of predaceous fungi. These mycorrhizal fungi were found to parasitize nematodes. Some of the fungal parasites are *Dactylella oviparasitica* (Sterling and Mankan, 1978), *Fusarium oxysporum*, *Paeceilomyces liliacinus*, *Pseudopapulospora* sp., *Kendrickii* sp. and *Verticillium chlamydosporium* (Godey *et al*., 1983), *Aureobasidium pululans* (Morgan-Jones *et al.*, 1984), *Cladosporium herbarum* (Pers.) Link ex-Gray (Kannam & Lingaraju, 1999) etc. Considering the importance of nematophagous fungi for the control of plant parasitic nematodes in integrated approach, a survey was conducted for the isolation of nematophagous fungi of nematodes associated with pine woods in Manipur. The results are illustrated here.

Materials and methods

One hundred and thirty seven soil samples were collected from around the roots of pine trees from different pine growing areas of Manipur including Waikhom Pine Reserve Forest of Thoubal district. The nematodes were extracted using Cobb’s (1918) sieving and decanting method followed by modified Baermann’s funnel technique (Thorne, 1961). The nematodes, thus collected were observed under stereoscopic binocular microscope.

For isolation of the fungus, the infected nematodes were transferred to the Potato Dextrose Agar, amended with 0.05% Bistrepen and incubated at 25ºC for five days. The fungal colony developed from the infected nematode was transferred to PDA slants in pure culture for further studies.

Inoculation experiments of this fungus on healthy plant nematodes were also performed. The healthy nematodes were transferred on sterile agar blocks (1.7% water agar), placed on sterilised glass cavity slides. The agar blocks containing healthy nematodes were inoculated with 0.1 ml spore suspension of *Cladosporium cladosporioides.* These cavity slides were incubated in sterilised petridishes at 25ºC. Uninoculated nematodes on agar blocks were also kept as control sets. Observation of the nematodes under stereoscopic binocular microscope for fungal infection was done at 24 hours interval.

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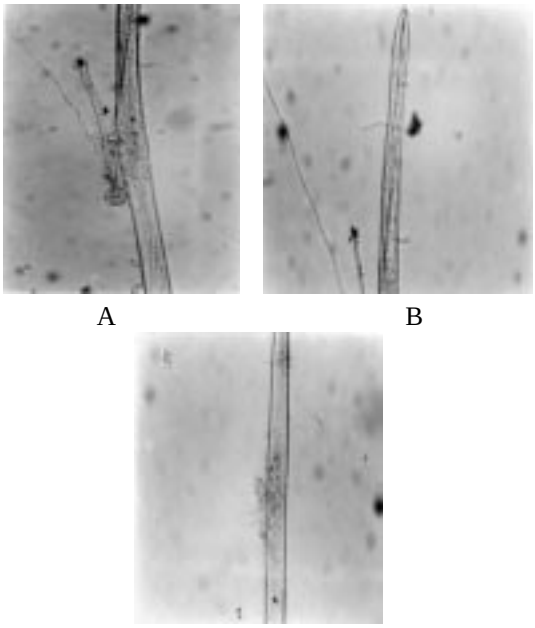

Fig. 1 : *Xiphinema* spp. infested with bacteria and fungi
A - *Xiphinema* spp. infested with bacteria and fungi at reproductive area
B - *Xiphinema* spp. infested with bacteria and fungi at anterior body region
C - *Xiphinema* spp. infested with bacteria and fungi at mid-body region

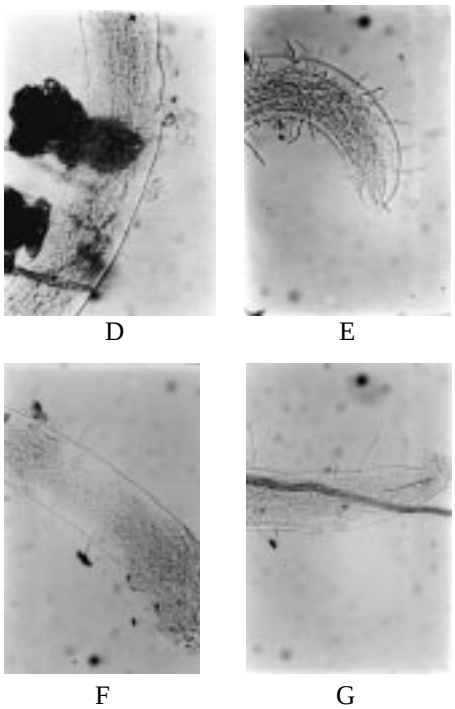

Fig. 2 : *Aporcelaimus* sp. infested with bacteria and fungi
D - *Aporcelaimus* sp. infested with bacteria and fungi at reproductive area
E - *Aporcelaimus* sp. infested with bacteria and fungi at tail region
F - *Aporcelaimus* sp. infested with bacteria and fungi at mid body
G - *Aporcelaimus* sp. infested with bacteria and fungi at cephalic region

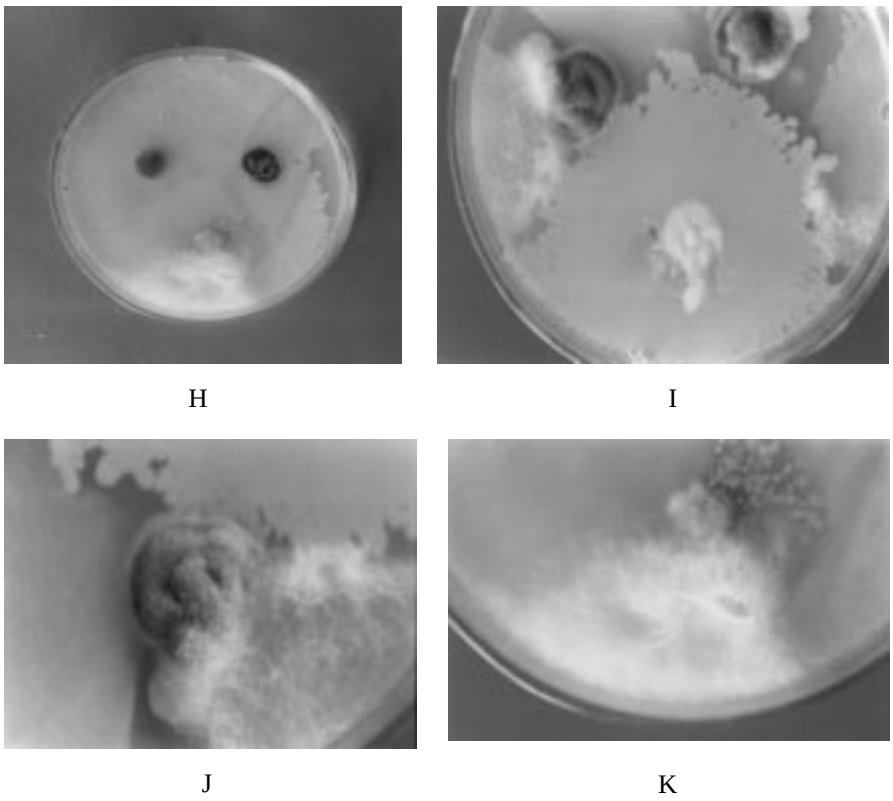

Fig. 3 : Crude cultures of fungus and bacteria
H - Crude cultures of fungus and bacteria.
I - Crude cultures of fungus and bacteria (Magnified)
J - Crude cultures of fungus and bacteria (Magnified)
K - Crude cultures of fungus and bacteria (Magnified)

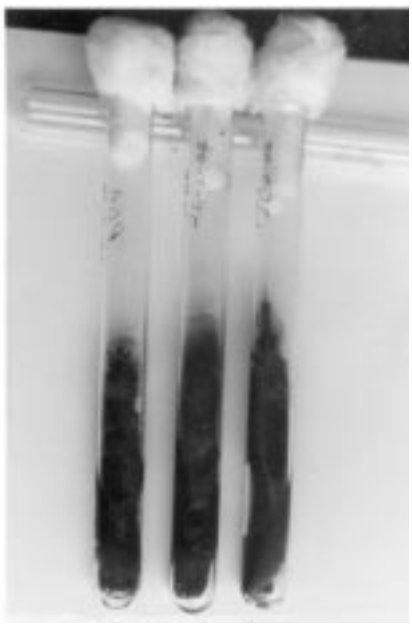

Fig. 4 : Slants of pure culture of *Cladosporium Cladosporioides.*

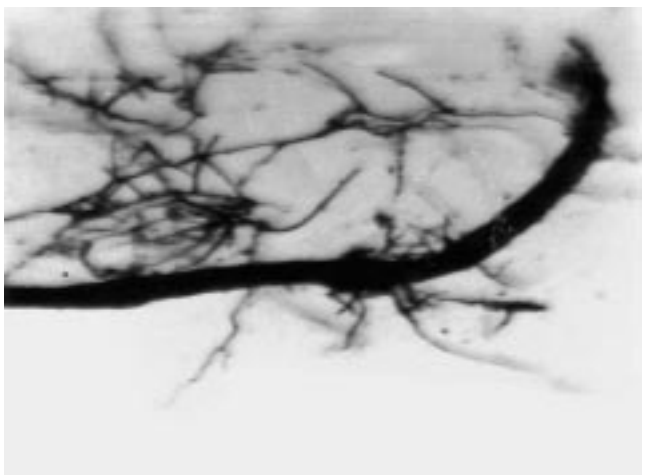

Fig. 5 : *Scutellonema* sp. inoculated with *Cladosporium cladosporioides.*

Results

Several species of fungi and bacteria were found parasitizing the nematodes. *Xiphinema* spp., considered as nematode vector of plants which can transmit viruses to plants during their feeding of soft rhizospheric parts and *Aporcelaimus* spp. were found to be highly parasitized by *Cladosporium cladosporioides.* Parasitism of *Scutellonema* spp. by *Cladosporium cladosporioides* was seen during the study.

Among two species of fungi and one species of bacterium, associated with *Xiphinema* spp. and *Aporcelaimus* spp., the fungus *Cladosporium cladosporioides* was found frequently inteteracting with the nematodes. The present finding is the first report of a nematophagous fungus of plant parasitic nematodes from Manipur. We again examined parasitism of *Cladosporium cladosporioides* against *Scutellonema* sp. for its possible control by inoculation. The fungi attack the nematodes through the production of sticky spores and mycelia that on contact adhere to the cuticle and germinate forming germ tubes through which penetrate nematodes. Then they extend their hyphae inside the nematodes after penetration of the cuticle by conidia formation. These hyphae multiply profusely within 15 days at room temperature. The infestation of nematodes starts within 48 hours of exposure at the same room temperature. The fungus was found to completely infest a healthy nematode within 15 days of exposure to the fungus. Some nematodes had been found to be totally parasitize within 72 hours of exposure.

Chowdhury and Kaushal (1984) reported *Cylindrocarpon uniseptatum* inhabiting unhatched eggs of *Heterodera avenae*. Sikora *et al.*, (1993) worked on *Verticillium chlamydosporium,* Ashraf and Khan (2008) on *Paeceilomyces lilacinus* infesting *Rotylenchulus reniformis*, Khan and Goswami (1995) on *Aspergillus niger* and Khan (2002) on *Fusarium solani*. Nematodes in soil are subject to parasitism by bacteria and fungi like *Pochonia chlamydosporia* (Kerry, 2002). Various aspects of biological control of nematodes using fungi have been reviewed by Jaffee (1992), Siddiqui & Mahmood (1998) and Kerry (2001). The fungus immobilises the nematode by secreting extracellular enzymes at the point of contact to overcome the cuticle to allow further parasitism (Tunlid *et al*., 1994). The endoparasitic fungi with adhesive spores attach to the anterior and posterior part of the juvenile nematodes and infect directly from the spore. The mechanism of parasitism is suspected to be due to nematotoxic substances secreted by the fungus (Kamra and Ganguly, 2002). Sankaranarayan *et al*., (2002) studied on the detail parasitism of *Meloidogyne incognita* eggs by *Fusarium oxysporum* and some other fungi. Goswami and Singh (2002) dealt on the effect of *Aspergillus niger* and *Cladosporium oxysporum* on root-knot nematode multiplication on eggplant. Kannam and Lingaraju (2002) worked on interaction of *Cladosporium herbarum* and *Heterodera cajani* in the presence of organic amendments.

Discussion

Species of *Xiphinema* are polyphagous and parasitize a wide variety of cultivated and wild floras. Their feeding in root tip results in formation of cork due to impregnation of cell walls with suberin and phelloderm and induction of terminal galls or curly tip produced by hypertrophy or hyperplasia of cortical cells of one side. The galls become attractive feeding sites for other worms of the same species. *Xiphinema* spp. have long stylets and capable of feeding even up to cells lying deeper in the vascular bundles particularly in case of young roots. They are vectors of soil borne viruses due to their sub-terranean mode of life and root parasitism. Hewitt *et al*., (1958) proved that *Xiphinema index* transmits Grapevine Fanleaf virus (GFLV). The plant parasitic nematode, *Scutellonema* spp. feed on the peripheral tissues of roots, cause necrosis on tubers even during storage, thus causing huge qualitative losses.Necrosis also leads to secondary attack by fungi and bacteria. One of the species, *Scutellonema bradys* (yam nematode) is economically the most important and causes dry rot of yam (*Dioscora* sp.). *S. cavenessi* is important on groundnut in Senegal.

Most of the *Cladosporium cladosporioides* isolated during the present study was cultured from infected *Aporcelaimus* spp. The fungi were also found to parasitize *Xiphinema* spp., one of the economically important nematode vectors in natural conditions of soil. They immobilise the nematodes by trapping with their hyphae and body shrinkage as a result of sucking of body fluids in almost in same manner as parasitize *Scutellonema* spp. in laboratory conditions. Their mode of parasitism can be applied in biocontrol strategies of other economically important plant parasitic nematodes. *Paecilomyces lilacinus* (oviparasitic fungi) and *Pasteuria penetrans* (larval and female parasitic bacteria) were considered as potential bio control agents. But the present finding also suggests *Cladosporium cladosporioides* as one of potential bio control agent. Further research on towards devising the mass propagation and application techniques for harnessing bio control potential of this organism against other plant parasitic nematodes are suggested.

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Presumed trematode induced granulomatousuveitis in south India

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Background : Uveitis comprises a large group of diverse disease primarily affecting the uveal tissues with subsequent damage to the retina, optic nerve and vitreous[1-2]. The causes of Uveitis vary widely depending upon several factors, including geography, culture, local environment, and socioeconomic factors, and prevalence of causative agents[3-6]. According to the literature etiology of more than 30% Uveitis is unknown[3]. Most of the cases are idiopathic, but identifiable causes include various infections and systemic diseases, many of which are autoimmune. Granulomatous uveitis in children is potentially vision threatening condition and it is major infectious cause for uveitis**.** Subconjunctival and anterior chamber granulomatous uveitis in children of South India is one of the newly recognized ocular disease. It is a major infectious cause for pediatric uveitis and is potentially vision threatening condition. The cause of this disease was unknown to ophthalmologists and many children were treated either with antitubercular treatment or with steroid. At Aravind eye hospital, it was observed that patients had history of exposure to village pond or river water prior to the eye disease. In our previous study we demonstrated the presence of a tegument of trematode in the subconjunctival granuloma by histopathological analysis; this showed evidence possibly a trematode as a causative agent[7].Therefore, the proposed study aims to confirm the causative agent and their source of infection using molecular techniques.

Key words **:** *Granulomatous Uveitis, Trematode, PCR, Sequencing and ITS2*

Purpose

To confirm the etiology of subconjunctival and anterior chamber granulomatous Uveitis, which was clinically suspected to be due to the trematode in children from South India.

Methods

Clinical isolates and DNA preparation

In this study Children from different villages of Tamilnadu were brought to the ophthalmologist with a history of frequent red eye. On examination, all of them had good general health; there was no clinical evidence of any chronic systemic illness. Ocular examination of the 11 children revealed an anterior chamber granuloma (fig-1). Other three children had a subconjunctival granulom (fig-2). In all 14 patients, except the granuloma other ocular structures were normal. Only positive relevant past history was, swimming in their village pond and history of intermittent red eye since then.

The study protocol was approved by the ethics committee for human research at the hospital. All the procedures in this study adhered to the tenets of the declaration of Helsinki. Informed consent was obtained from all the subjects after explanation of the nature of the study. Eleven patients with anterior chamber granuloma =3 mm underwent aspiration of the nodule and three cases with subconjunctival nodule = 5 mm was excised. Prior to surgical intervention, the children received topical or oral corticosteroids or both for at least 15 days. Informed consent was obtained from the patients or their parents, and all patients were hospitalized for complete systemic examination. Under general anesthesia, with aseptic precautions, the anterior chamber nodules were aspirated with a 25-gauge needle passed through the limbus.

DNA was extracted from the all 14 granuloma biopsy using QIAamp Mini Kit –Qiagen,Tissue lysis procedure and DNA was stored at -80 ? c for further experiments.

Molecular analysis

DNA amplification

The rDNA region spanning the ITS regions was amplified from DNA obtained from the fluke by polymerase chain reaction (PCR) following the standard protocol (White 1993) with minor modifications by (Prasad et al. 2007; Tandon et

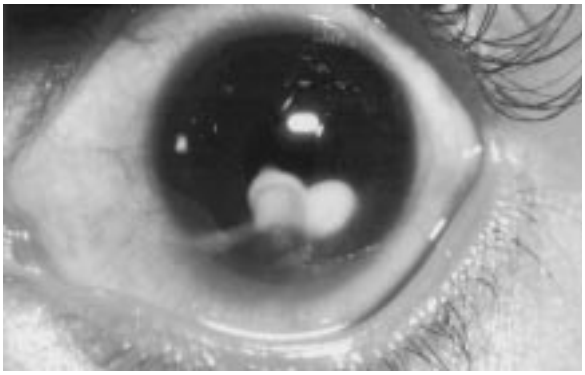


Fig 1. Showing Clinical manifestation of anterior chamber granuloma

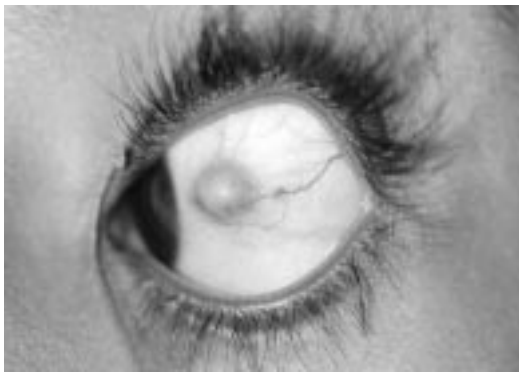


Fig 2. Showing Clinical manifestation of subconjunctival granuloma

al.2007). We used the universal primer based on conserved ITS2 sequences of Schistosoma species (Bowles et al. 1995) as detailed below :

ITS2 region—

3S (forward): 5'-GGTACCGGTGGATCACTCGGCTCGTG-3'

A28 (reverse): 5'-GGGATCCTGGTTAGTTTCTTTTCCTCCGC-3'

In each assay one positive control *Fasciola gigantica* DNA source Calf liver and one water (DNA-free) negative control were tested. For each PCR, 1ng of positive control (*Fasciola gigantica*)DNA was used in a 10 ìl mixture containing 10X PCR buffer contaning 15 mM $MgCl_2$, 10 mM dNTPs, 10 pmol of each primer, 1X Q-solution (Qiagen), and 3 U of *Taq* polymerase (Bangalore gene). PCR conditions (thermal cycler PTC 200; MJ Research) were as follows: five minutes at 94°C for denaturation, 38 second at 56°C for primer annealing and seven minutes at 72°C for extension; this cycle was repeated 30 times for adequate amplification.

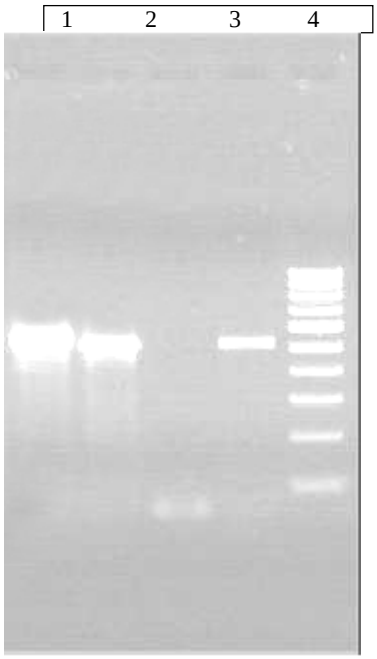
Molecular sequencing and sequence analysis

The amplified PCR products were loaded on the gel and purified using the biobasic PCR purification kit (Promega). Sequencing reaction was performed with Big-Dye Terminator Mix version 3.1 sequencing kit (Applied Biosystems). Sample were denatured at 96ºC for 2 minutes, and then cycled 28 times at 96 ºC for 10 seconds, 52ºC for 10 seconds and 60ºC for 4 minutes. Unincorporated nucleotides were removed using the $NaCO_3$, 125 Mm EDTA and absolute alcohol. HI-DYE was used to stabilize the single strand template before sequencing. All patient's samples and environmental samples with positive control were subjected to bidirectional sequencing in ABI Applied Biosystem 3130. The sequence alignment was performed using BLAST analysis.

Results

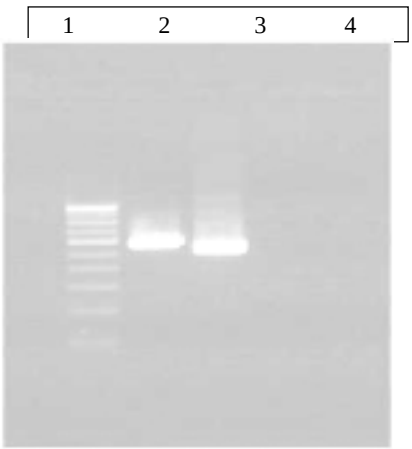
Out of 14 patients samples analyzed, in which two subconjunctival (Fig-3) and one anterior chamber granuloma (fig-4) were found to be confirmatory for trematode DNA and their maximum sequence similarity matched with *Procerovum species*

(Family- Heterophyidae) of trematode. Which were similar as environmental cercariae DNA which harvested from the snail (*Melenoid tuberculata)*



Lane 1. Positive control (Trematode DNA) – 539 bp.
2. Sample -1 (PCR product – 501 bp.)
3. Negative control (MQ water)
4. Sample – 2 (PCR product – 501 bp.)

Fig 3. PCR product of trematode DNA found in Sub conjunctival granulomatous tissues of human eye



Lane 1. 100 bp DNA Ladder.
2. Positive control (Trematode DNA)-539bp.
3. Sample (AC granuloma)-501bp.
4. Negative control (MQ water)

Fig 4. PCR Results of trematode DNA found in Anterior chamber granuloma of human eye

Discussion

The most striking features in this study are the distribution of cases in a coastal village, with the occurrence of conjunctival nodules and anterior chamber granuloma, who were exposed to a freshwater pond, and the histologic findings of nodules exhibiting a distinct zonal granulomatous inflammation, with some displaying evidence of a trematode infection by Raethiam *et al*. 2001& 2002. Because the village pond water was cited as the source of infection by several cases in which the water reservoirs were inspected. There were two types of ponds in the village, one for drinking and the other for bathing and cleaning purposes, Underground water was pumped into the ponds almost every day with an electrically operated motor, but the pond beds were never cleaned. Swimming and playing in the water of the second pond was the most important recreational activity for the children throughout the year. All patients gave a history of swimming in their village ponds.There present study confirmed the etiology of granulomatous Uveitis in children of South India could be a *Procerovum species* (family Heterophyidae) of trematode. The possible source of the infection is by taking bath in the snail infested water bodies, which is the main reservoir for this pathogenic trematode.

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Protoopalina andulensis and *Protoopalina* limnocharis (Protozoa : Slopalinida) with a note on their heterospecific association with anuran host of West Bengal, India

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Abstract : Two new species of opaline i,e *Protoopalina andulensis* and *Protoopalina limnocharis* is described from the gut of *Rana taipehensis* Van Denburgh and *Limnonectes limnocharis limnocharis* Weigman respectively collected from district Howrah, West Bengal, India. The two species have been considered as new by the author considering their morphological and morphometric parameters.

Key words **:** *Protoopalinav andulensis, Protoopalina limnocharis, Rana taipehensis, Limnonectes limnocharis limnocharis,* opaline

Introduction

India with its diverse ecological habitats affords suitable living condition to a variety of anuran amphibians. In nature ,almost all the amphibians display in the rear part of their gastro-intestinal tract, luxuriant fauna of opalines and ciliates. The opalines are large endocommensals and have at least one, but often hundreds of identical nuclei. However, the opalines pose a burning dilemma regarding their status as they are found only in the gut of tadpoles and young anurans who suffer from indigestion (Somsiri,1994).These suggests the adults develop some kind of immunity against the opalines. The opalines are variously shaped and the body is provided with numerous flagella oriented in oblique and parallel rows termed as kineties. The surface lies in delicate parallel folds that allows interference of reflected light and opalescence. The anterior end is defined as the direction of travel and anterior margin is termed as falx. There is no mouth, nutrition by pinocytosis and no contractile vacuole. According to Levine *et al* (1980) they have been ranked under,

Kingdom-Protista

Phylum-Sarcomastigophora Honigberg and Balamuth,1963

Subphylum-Opalinata Corliss and Balamuth,1963

Class-Opalinatea Wenyon,1926

Order-Opalinida Poche,1913

Later, Pattrtson(1989)included the opalines under order Slopalinida. The present paper deals with the binucleated genus *Protoopalina* Metcalf,1918 under family- Opalinidae Claus,1874;subfamily-Protoopalininae Metcalf,1920.These opalines are spindle shaped with two nuclei and characterised by a short ,broad, axial falx parallel to the antero-posterior axis of the cell. The binucleate opalines remained undiscovered in India till Bezzenberger(1904) who reported *Zelleriella macronucleata* Metcalf (1918) erected the genus *Protoopalina* and described a large number of species throughout the world (1932,1940).

The reports from the Indian subcontinent are as follows :

Nie (1935) reported *P.caudata microhyla* from *Microhyla ornata*. Uttangi (1952,1961) described nine species from Dharwar frogs. Mandal and Nair (1974, 1975) described *P.chauhani* and *P.cornata* from *Rana cyanophlyctis*. Roy, Tandon and Dutta (1991) observed their presence in frogs of Shillog, Meghalaya.

The present paper deals with the two new species of *Protoopalina* reported by the author from**,** *Rana taipehensis* and *Limnonectes limnocharis limnocharis* respectively collected from different regions of district Howrah, West Bengal, India in the month of July-September, 2001-2002.

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Materals and methods

The host specimen were taken alive to the laboratory and kept in the aquaria .Faecal samples of the host were collected by inserting micropipette through the cloacal aperture. Then the faecal matter was placed in 0.67% normal saline and placed on glass slides. The semi-dried smears were fixed in Schaudinn's fixative and stained with Heidenhain's iron-alum Haematoxylin. Photographs were taken with Carl Zeiss Axiolab Microscope using MC-80 Camera.

Results

Protoopalina andulensis Sp. Nov.

The body is elongated, cylindrical and very small in size. The anterior end is nearly rounded and the posterior end tapers gradually to a narrow point from the middle to the end .L:W=4.4:1.The falx area is restricted anteriorly. The pellicle is thin and covered with very small flagella which are more densely arranged at the anterior end. The somatic kineties run almost obliquely throughout the body. The cytoplasm is very clearly diffentiated into ectoplasm and endoplasm. The ectoplasm is hyaline and form distinctively wide strip around the central mass. No inclusion of any kind are present in these area. The endoplasm on the other hand is full of granules and vacuoles. The density of the granules are greater at the area from the anterior end to the second nucleus. The remaining part posses small vacuoles. The two ovoid nuclei are located along the longitudinal axis of the body, both of which are located at the anterior half. The first one is located very close to the anterior end whereas the second one lies just at the middle of the body.

All morphometric measurements have been given in Table 1.

Table 1 : Morphometric measurementsof *Protoopalina andulensis*.Measurements in micrometers : n=20

	R	AM	SD	SE	CV(in %)
Body length	58.50 - 121.5	91.35	17.76	3.97	19.44
Body width	13.50 - 31.5	20.70	5.40	1.20	26.08
Nuclear length	6.75 - 9	7.74	0.77	0.17	9.94
Nuclear width	4.50 - 5.40	4.95	0.34	0.07	6.86
Distance from cell apex to first nucleus	13.50 - 27	20.87	4.71	1.05	22.56
Distance between two nuclei	18 - 36	26.77	6.54	1.46	24.43
Distance from second nucleus to posterior end	27 - 58.50	41.07	11.84	2.64	28.82
Flagellar length	4.50 - 7.20	5.94	1.04	0.23	17.50
Flagellar interval	2.25 - 3.15	2.70	0.40	0.08	14.81
Ectoplasmic width	1.80 - 4.50	2.97	0.96	0.21	32.32

Taxonomic summary :

Type material : Holotype-Slide No.PC Zoo(Para)131

Paratype : Slide No.PC Zoo(Para)132,133

Type host : *Rana taipehensis* Van Denburgh

Type locality : Andul, Howrah, West Bengal, India

Prevalence : 2/28

Location : 22.35N/88.23E

The materials have been deposited in the collection of Parasitology Laboratory, Department of Zoology, University of Kalyani.

Protoopalina limnocharis Sp. nov**.**

The body is cylindrical, gradually tapering towards the posterior end. L:W=8.2:1.The pellicle evenly covered with short flagella. The somatic kineties run parallely from the anterior to the posterior end. The cytoplasm is well demarcated into thin ectoplasmic and thick endoplasmic zone. Numerous granules (well stained with iron- haematoxylin) were evenly distributed

throughout the endoplasmic region. The most distinguishing feature of the opalines lies in the shape of the nuclei which have a pear shaped appearance. The anterior nucleus is located at the middle point of the first half of the body while the posterior one is situated just below the middle point. The chromatin element of the nuclei is broken up into numerous bits.

All morphometric measurements have been given in Table 2.

Table 2 : Morphometric measurements of *Protoopalina limnocharis*.Measurements in micrometer n=20

	R	AM	SD	SE	CV (in %)
Body length	135-283.50	208.80	42.60	9.53	20.40
Body width	22.50-31.50	25.20	3.60	0.80	14.28
Nuclear length	13.50-20.25	16.65	2.29	0.51	13.75
Nuclear width	9-18	13.05	3.73	0.83	28.58
Distance from cell apex to first nucleus	36-90	56.70	15.61	3.49	27.53
Distance between two nuclei	54-90	67.95	12.31	2.75	18.11
Distance from second nucleus to posterior end	76.50-148.50	88.65	29.37	6.57	33.13
Flagellar length	6.75-9.45	8.37	0.92	0.20	10.99
Flagellar line interval	2.70-4.50	3.60	0.55	0.12	15.27
Ectoplasmic width	1.80-3.60	2.70	0.63	0.14	23.33

Taxonomic summary :

Type material : Holotype-Slide No.PC Zoo(Para)297

Paratype : Slide No.PC Zoo(Para)298

Type host : *Limnonectes limnocharis limnocharis* Weigmann

Type locality : Shibpur,Howrah,West Bengal,India

Prevalence : 5/70

Location : 22.35N/88.23E

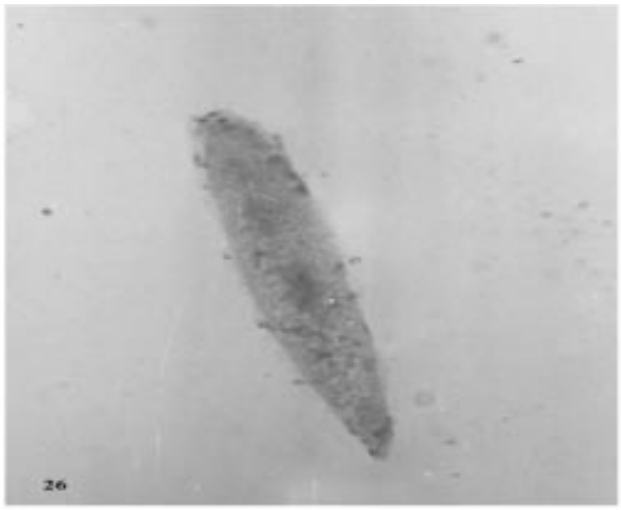
The materials have been deposited in the collection of Parasitology Laboratory, Department of Zoology, University of Kalyani.

Discussion

The first new species of opalines shows close proximity with *P .tenuis* (Raff,1912)Metcalf,1923 reported from *Crinia signifera* Girard from Australia in general body outline, nuclear shape and nuclear arrangement. But the great variation lies in the morphometric measurements. The former posses a body dimension of 530x36um. Considering the major differences the group of specimen has been proposed to be ranked under different species category under genus *Protoopalina*. Again it shows some resemblances with *P.dharwarensis* reported by Uttangi(1952)from *Microhyla ornata* in Karnataka,India. Both of them shows slender appearance with two nuclei placed at the anterior half of the body. But critical review shows that the exact body cotour and also the morphometric parameters varies greatly between them.

Table3 : Comparative mensural data of *P.dharwarensis* and *P. andulensis* All measurements in micrometers.

	P.dharwarensis	***P. andulensis***
Body length	207	91.35
Body width	25	17.76
L:W	8.02:1	4.4:1
Nuclear length	11.5-13.5	7.74
Nuclear width	9-11	4.95
Ectoplasmic width	2	2.97



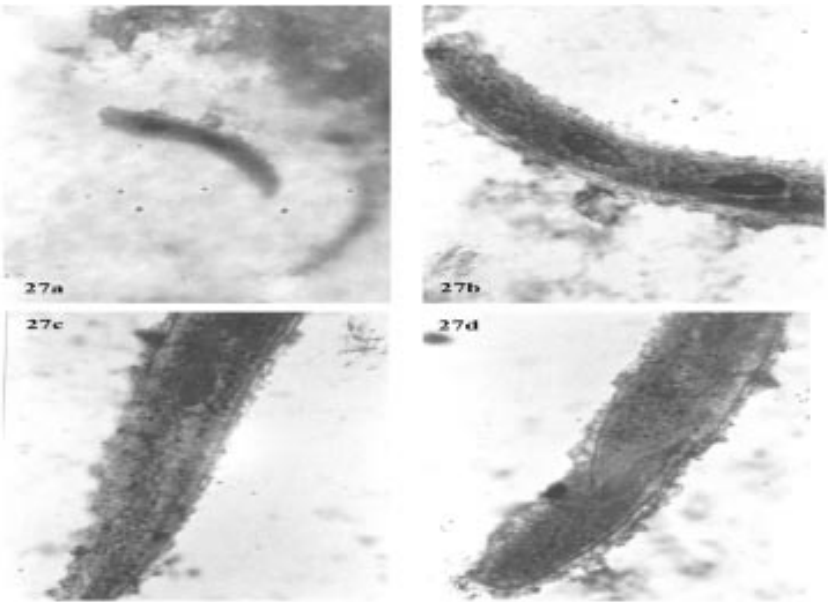


Fig. 1 : A trophozoite of P sp1 under high power objective of a microscope (bar = 20 u m)

Fig 2a : A trophozoite of P sp2 under high power objective of a microscope (bar = 20 u m)

Fig 2b : Anterior portion of the body of trophozoite of P sp2 under oil - immersion objective of a microscope (bar = 20 u m)

Fig 2c : Middle portion of the body of trophozoite of P sp2 under oil - immersion objective of a microscope (bar = 20 u m)

Fig 2d : Posterior portion of the body of trophozoite of P sp2 under oil - immersion objective of a microscope (bar = 20 u m)

Therefore, after a critical review it can be concluded that the present group of specimen falls under a new specie category under genus *Protoopalina and* named *Protoopalina andulensis* after the name of the type locality of the host..

The second group of specimen under present study shows very close proximity with *P.indica* Uttangi,1952 described from *Microhyla ornata* from Dharwar, Karnataka, India. The similarity lies in their body appearance, nuclear shape and nuclear position. The dense granulation is also observed in both of them. However, the pattern of granulation is different between them. Again, the major difference lies in their morphometric measurements.

Table 4 : Comparative mensural data of *Protoopalina indica* and *Protoopalina limnocharis*

	Protoopalina indica		***Protoopalina limnocharis***	
	R	**AM**	***R***	***AM***
Body length	135 – 415	275	135 – 283.50	208.80
Body width	40 - 75	57	22.50 –31.50	25.20
L : W	4.8 : 1		8.2 : 1	
Nuclear length	27 – 33	-	13.50 –20.25	16.65
Nuclear width	14 - 17	-	9 – 18	13.05
Ectoplasmic width	-	3	1.80 – 3.60	2.70

Considering the above facts, the author proposes that the present group of specimen falls under new species category under genus *Protoopalina limnocharis* after the name of the host species.

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Effect of chaetothyrium species, a causal agent of sooty mould on silk production of *Bombyx mori*

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Abstract : In West Bengal, an unusual black incrustation of fungal mass on mulberry leaves is common during winter (November-January) when temperature is low and relative humidity is considerable (19-21º C and 68-70%) in ambience. The first fungal forays become clear in November to reach epidemic in January. The disease characterizes presence of black velvety coating on dorsal surface of leaves. The infection starts from ground level affecting older leaves and soon spreads to entire foliage, except uppermost tender leaves. Microscopic examination of infected leaves has shown black incrustation, epiphytically growing mycellial threads and spores of chaetothyrium species which were later inhabited by *Curvularia affinis* Boedijin (Rao et al., 1992). Different species of *Chaetothyrium* and *Curvularia* causing various types of sooty mould and leaf spot diseases that have been reported from forest tree species. Occurrence of these two fungi has also been reported from mulberry.

Like most of the other economic plantation and field crops, mulberry is also prone to attack by a varied pest complex belonging to a large number of insect orders. Whitefly, a phytophagus insect was observed infesting mulberry. Whitefly sucks the juice from tender leaves of mulberry resulting chlorosis and leaf curl. The nymphs suck the juice and secrete honeydew, which acts as a medium for the growth of the *Chaetothyrium* species and ultimately forms a black coating on the dorsal surface of leaf. This sooty mould disease caused by *Chaetothyrium* species due to the severe infestation of whitefly on mulberry leaf. This species grows as an ecto parasite on mulberry leaf causes a leaf yield loss to the tune of 24% every year. All the economic parameters of silkworm rearing also deteriorate by feeding of the silkworm with sootymould infected leaf. The sooty mould disease has also got an impact on silk gland and ultimately reduces the production of silk.

Key Words **:** *Bombyx mori*, Sooty mould, Silk gland, Silk productivity

Introduction

Mulberry, *Morus alba* L is the sole food plant of silkworm, *Bombyx mori* L like most of the other economic plantation and field crops, mulberry is also prone to attack by a varied pests belonging to a large number of insect orders. Whitefly, a polyphagus insect was observed infesting mulberry. Whitefly sucks the juice from tender leaves of mulberry resulting chlorosis and leaf curl. The nymphs suck the juice and secret honeydew, which acts as a medium for the growth of the *chaetothyrium* species and ultimately forms a black coating on the upper surface of the mulberry leaves during winter (Nov-Jan) when temperature is low and humidity is optimum (19-22°C and 68-70 %) in ambience. The infection starts from ground level, affecting older leaves soon to spread to entire foliage, except uppermost tender leaves (Rao et al. 1992).

Different species of *Cheatothyrium* and *curvularia* causing varius types of sooty moulds and leaf spot diseases have been reported for forest tree species (Mukherjee and Bhasin,1986 ; Alexopoulous and Mims, 1985). In mulberry, the occurance of these two fungi was also reported (Rao et al, 1992). Effects of sooty moulds have been studied for economic parameters of silkworms. The present investigation brings forth the effects of the mulberry diseases on silkgland of *Bombyx mori* and silk production.

Materials and methods

Studies were conducted (December - January) using pure bivoltine hybrids of multivoltine x bivoltine & reciprocal and multivoltine hybrid. The silkworms were reared on healthy leaf maintained as control, sooty mould infected leaf maintained as treatment and sooty mould infected leaf with 0.2% bavistin (control measure) sprayed leaf of S1 mulberry variety, from date of brushing till spinning as infected control. Rearing was conducted in replicated manner having 100 worms. Rearing was conducted as per standard scheduled (Krishnaswami, 1978 ; Krishnaswami et al. 1973).

Weight of 10 matured larvae, weight of 20 green cocoons (10 each of male and female) were taken at random for each replication for assessment of varius cocoon characters like single cocoon weight, single shell weight and cocoon shell ratio. Other parameters like absolute silk content, non- breakable filament length, denier etc. were recorded. Significance was analysed through Analysis of Variance.

Results and discussion

Silkworm races / combinations fed with sooty mould infected leaf showed pronounced deterioration. Thus plate 1 (a) and 1 (b) show the distinctive characters of anterior, middle and posterior parts of the gland when stretched and loosened. Also, weight of total silkgland recorded showed that the gland collected from healthy worms weighing 1.0 gram,while it was 0.813 gm in worms fed on bavistin sprayed leaves (infected control) and 0.668 gm in respect of sooty mould infected leaf (treated).

The photograph of silk gland, plate 1 (a) & 1 (b) and the weight of silk gland as recorded, resolve the bavistin treatment though reflected revival tendency by about 21 percent over worms fed on sooty mould infected leaf, it is clear that specific fungicide needs to be explored, corroborative to above tends the most affected middle part of silk gland which has lost original pattern, besides narrowing down similar analysis were not projected, as associated with mulberry (Chaturia, 1968) or silkworm disease (Aruga, H.1957; Aruga et al, 1963 & Sidhu et al., 1968).

In the present study it is clear that lumen diameter of middle part of the silk gland narrowed down due to the mulberry disease. Whether, number of cells of the gland are affected is not yet studied. It is shown that the lumen and number of cells in silk gland are fixed (Akai, 1965; fayard, 1979) and content of silk is the function of the activity status of silkgland cells and elasticity potential of the gland (Akai, 1984; Sehnal and Akai, 1990)

These results showed that sooty mould disease of mulberry affects protein synthesis as similar in other diseases (Watanabe, et al., 1968), since posterior and middle part of the silkgland thus having been affected, fibroin and sericin patterns deranged. Derangement of metabolic functions due to pathogens have been shown for various animal groups (Weiser, 1976).

Table : Effect of feeding of sooty mould infected leaves, sprayed leaves (0.2% bavistin) and normal leaves on rearing performances of different races / combnations of silkworms.

A) Single Cocoon Weight (gm)

Treatments	Bivoltine	Bi x mul	Mul x Bi	Mul x Mul	Mean
Normal leaves (control)	1.565	1.639	1.910	1.419	1.633
Sprayed leaves	1.451 (7.28)	1.298 (20.81)	1.544 (20.81)	1.168 (17.69)	1.365
Sooty Mould infected leaves	1.160 (25.88)	1.171 (28.55)	1.200 (37.17)	1.050 (26.00)	1.145

B) Single Shell Weight (gm)

Treatments	Bivoltine	Bi x mul	Mul x Bi	Mul x Mul	Mean
Normal leaves (control)	0.280	0.258	0.307	0.196	0.260
Sprayed leaves	0.233 (16.79)	0.171 (33.72)	0.228 (25.73)	0.138 (26.59)	0.193
Sooty Mould infected leaves	0.150 (46.43)	0.139 (46.12)	0.144 (53.09)	0.116 (40.82)	0.137

C) Silk Ratio (%)

Treatments	Bivoltine	Bi x mul	Mul x Bi	Mul x Mul	Mean
Normal leaves (control)	17.890	15.743	16.053	13.787	15.868
Sprayed leaves	16.083 (10.10)	13.147 (16.49)	14.770 (7.99)	11.813 (14.32)	13.953
Sooty Mould infected leaves	12.927 (27.74)	11.860 (24.66)	12.000 (25.25)	11.083 (19.61)	11.968

d) Absolute Silk Content

Treatments	Bivoltine	Bi x mul	Mul x Bi	Mul x Mul	Mean
Normal leaves (Normal)	2557.270	2408.200	2678.670	1096.070	2335.050
Sprayed leaves	1710.370 (33.12)	1387.000 (42.41)	1355.200 (30.74)	1150.930 (32.14)	1525.880
Sooty Mould infected leaves	882.867 (65.48)	1143.730 (52.51)	997.933 (62.75)	960.867 (43.35)	996.350

CD at 5% for comparing treatment means and interaction effects

	Treatment	Interaction
Single cocoon weight	0.024	0.047
Single Shell weight	0.006	0.012
Silk Ratio	0.284	0.567
Absolute Silk Content	**99.270**	**198.540**

(Figures in parentheses are percent decreases over corresponding)

Fig. 1(a) : Silk gland fed with normal leaves

Fig. 1(b) : Silk gland fed with fungicide sprayed leaves

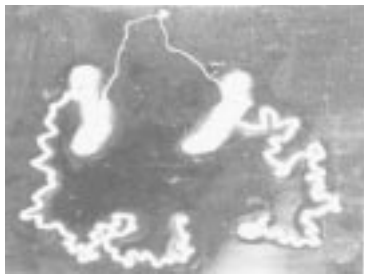

Fig. 1(c) : Silk gland fed with sooty mould infected leaves

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Trichodinid ectoparasites (Ciliophora : Trichodinidae) from the gills of fresh-water fishes in the Shitalakhsya River, Bangladesh

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Abstract : The systematic and taxonomy of ichthyotrichodinid parasites was investigated in the Shitalakshya River and local fish farm in the Kapasia Upazila of Gazipur District, Bangladesh between January and December, 2009. Smears of the gills of 12 host fish species, caught by fishing nets and obtained from fish farms revealed the presence of 14 species of *Trichodina.* The identified species of *Trichodina* are: *Trichodina acuta, T. anabasi, T. centrostrigeata, T. cancilae, T. domerguei, T. matsu, T. microspina, T. modesta, T. molae, T. mossambicusi, T. nigra, T. shitalakshyae, T. siluri* and *T. sylhetensis.* Out of these, *Trichodina cancilae, T. domerguei, T. matsu, T. microspina, T. molae* and *T. siluri* is reported as a first record in Bangladesh. One of the two unidentified species was published as *Trichodina shitalakshyae* from *Glossogobius giuris* based on the dry silver impregnated specimens. Photomicrographs and morphometric data are presented for each species. General prevalence of parasitic infection in specimens obtained from the Shitalakshya River is also recorded.

Key Words **:** *Bangladesh, Ciliophora, Trichodinidae*

Introduction

In Bangladesh, Asmat et al. (1997) made the first report of trichodinid ciliates. Since then scanty and infrequent information are available on the taxonomy of this particular group in this region. As a result, 17 species of trichodinid ciliates representing the genera *Trichodina, Paratrichodina, Tripartiella* and *Trichodinella* were identified from different freshwater and estuarine fishes by Asmat et al. (1997; 2003a,b,c; 2005; 2006) and Bhouyain et al. (1999), Asmat and Sultana (2005), Habib and Asmat (2008) and Kibria et al. (2009; 2010). During a survey on the species diversity of trichodinid ciliates from the Shitalakhsya River (Latitude 23° 49' 20.11" N Longitude 90° 33' 0.47" E), a branch of the River Brahmaputra, in the Kapasia Upazila of Gazipur district, 70km north-east of Dhaka (Fig. 1), during January 2008 to December 2009 fourteen species of *Trichodina* were identified from the gills of 38 host fish species.

Materials and methods

The host fishes were collected from one of the Shitalakhsya River in the Kapasia Upazila of Gazipur district, by seine nets and gill nets. Gill scrapings were made at the pond side; air-dried and then were transported to the laboratory. The slides with trichodinid ciliates were impregnated with Klein's silver impregnation technique (Klein, 1958) and examined under a research microscope, OSK 9712 T-2 at 10x100 magnifications. Measurements were made according to the recommendations of Lom (1958), Wellborn (1967), Arthur and Lom (1984) and Van As and Basson (1989; 1992). All measurements given are in micrometers, range in parentheses by the arithmetic mean and standard deviation. For statistical analysis, morphometric measurements of 20 specimens for both species were considered. Photomicrographs were made in order to have comprehensive morphological analyses of the ciliates. The level of infection was measured as low (1-5 ciliate/slide), medium (6-10 ciliate/slide) and high (more than 11 ciliates/slide).

Results and Discussion

Fourteen species of trichodinid ciliates were recorded from the gills of 12 commercially important finfish species of Bangladesh.

***Trichodina acuta* Lom 1961 (Fig. 3, 17; Table 1)**

Host: *Mystus bleekeri* (Day 1877) (Bagridae). Locality: Shitalakshya River, Kapasia Upazila of Gazipur district, Bangladesh. Location: gills. Prevalence: 19/41 (46.3%). Infection: low. Reference material: MB 1 and MB 2 (10/10/2009) of silver stained slides are deposited in the Museum of the Department of Zoology, University of Chittagong, Chittagong 4331.

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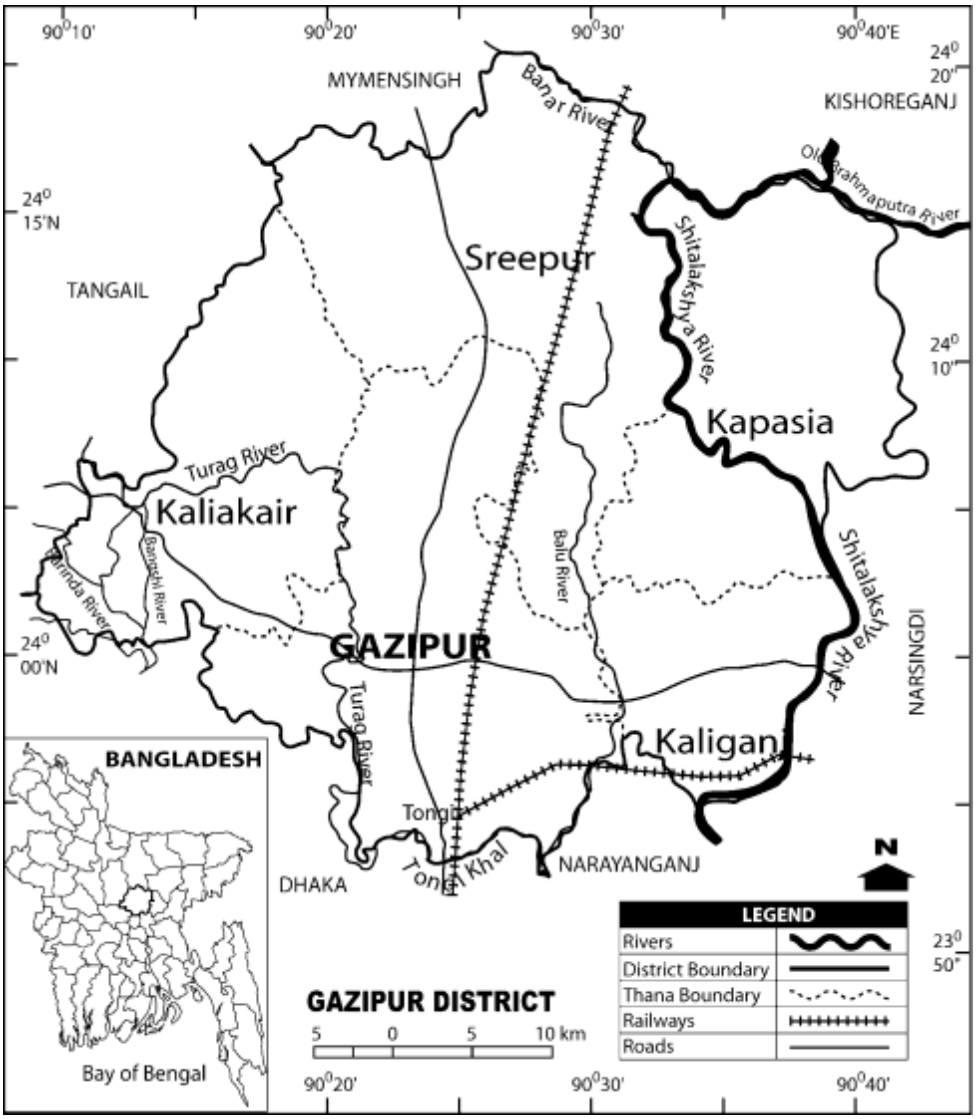

Figure 1. Map of sampling area from where fishes were collected from the Shitalakhsya River.

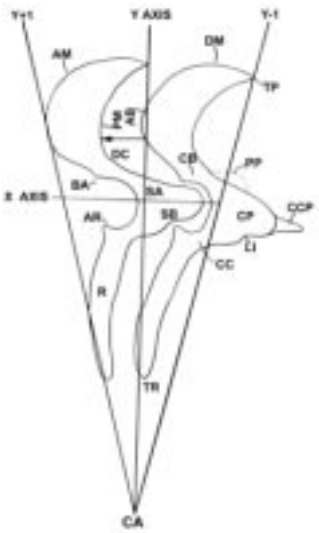

Figure 2. Denticle structure and construction of X and Y axes as fixed references for description of denticles (after Van as and Basson 1989). Explanations: **AB**, apex of blade; **AM**, anterior margin of blade; **AR**, apophysis of ray; **B**, blade; **BA**, apophysis of blade; **CA**, central area of adhesive disc; **CB**, section connecting blade and central art; **CC**, secton connecting central part and ray; **CCP**, central conical part; **CP**, central part of blade; **DC**, deepest point of semilunar curve relative to apex; **DM**, distal margin of blade; **PM**, posterior margin of blade; **PP**, posterior projection; **R**, ray; **SA**, section of central part above x axis; **SB**, section of central part below x axis; **TP**, tangent point; **TR**, tip of ray.

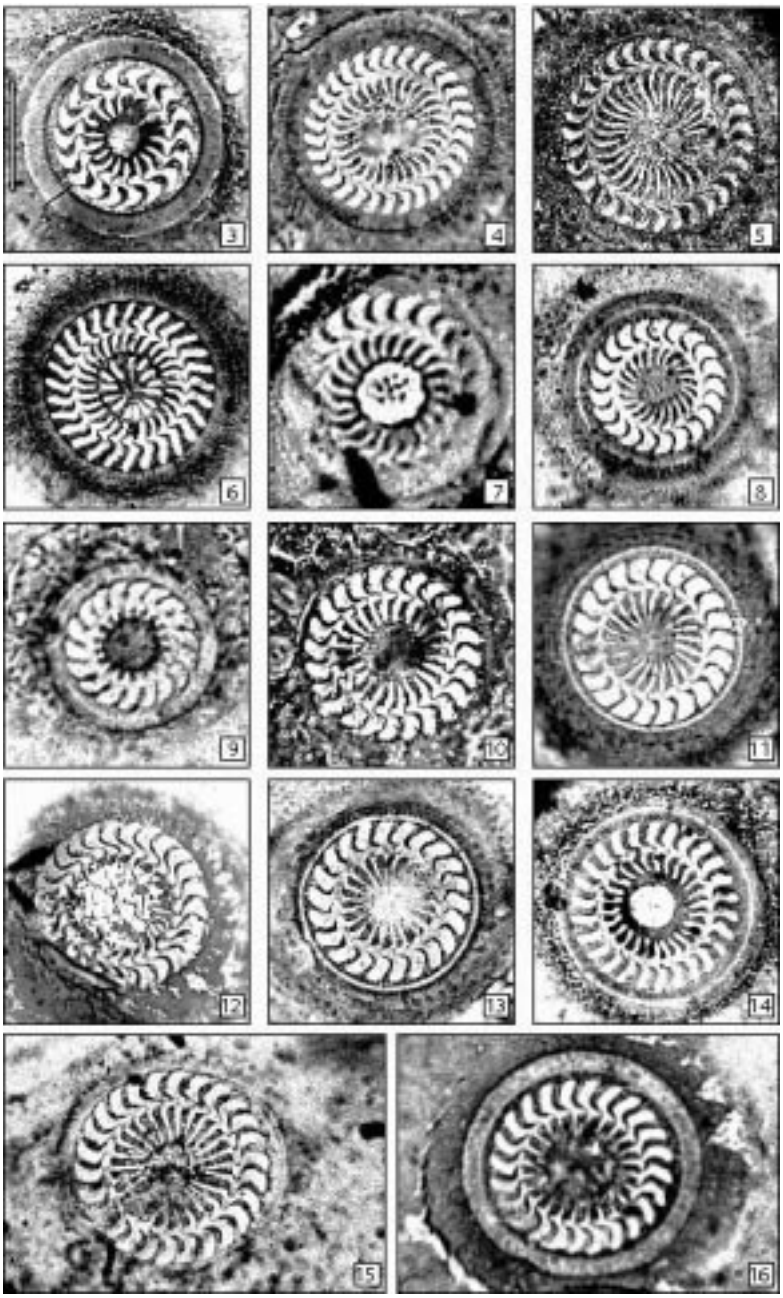

Figure 3-16. Photomicrographs of silver impregnated adhesive disc of trichodinid ciliates obtained during the study period in the Shitalakhsya River, Bangladesh. Scale bar 30 µm.

Trichodina acuta is one of most widely distributed trichodinid species from freshwater fishes. It was originally described by Lom (1961) from five species of freshwater fishes, viz., *Cyprinus carpio, Perca fluviatllis, Lucioperca lucioperca, Leucaspis delineatus* and *Rhodeus sericeus,* and from the skin of tadpoles belonging to several species of frogs in Czechoslovakia (Czech Republic). Since then, *T. acuta* has bees reported from China (Chen 1963; 1984a; 1984b; Anon. 1973), Russia (Kandilov 1964; Kashkovsky 1965; Stein 1968; Kulemina 1968), Poland (Kazubski and Migala 1968), the USA (Lom 1970), South Africa and Israel (Basson et al. 1983), Israel (Van As and Basson 1989), from the Philippines (Duncan 1977; Natividad et al. 1986; Bondad-Reantaso and Arthur 1989; Albaladejo and Arthur 1989) from Cuba (Arthur and Lom 1984b), Czech Republic (Navratil 1991), Finland (Halmetoja et al. 1992), South Africa (Burton and Merron 1985; Basson and Van As 1993), Germany (Grupcheva and Sedlaczek 1993), Turkey (Özer and Erdem 1998; 1999; Özer 2002), the United Kingdom (Gaze and Wooten 1998), India (Asmat 2000), Serbia (Nikoliæ et al. 2003), Brazil (Piazza et al. 2006), Australia (Basson 2010) and Bangladesh (Kibria et al. 2010).

At least 25 host fish species were infested by this trichodinid through out the world. The above results, therefore, confirm the presumption that host specificity in fish trichodinids is absent as expressed by Stein (1976), Hoffman and Lom (1967) and Van As and Basson (1987). During the present study *T. acuta* was obtained from *Mystus bleekeri*. The dimensions of measurements fall well within the range given for *T. acuta* by Lom (1961) and Duncan (1977). The denticle measurements are close to specimens presented by Van As and Basson (1989) from various species of *Oreochromis* from Israel and the morphology of denticles agree in most of the essential aspects with the summer specimens as recorded by Kazubski and Migala (1968) from *C. carpio* in Poland. During the present survey this ciliate was obtained mainly from October 2008 to February 2009, i.e., in pre-colder to post-colder period as was reported by Asmat (2000) from India. Basson and Van As (1993) stated *T. acuta* as the European species which parasitizes the European and Asian cyprinids and have spread via the translocation of their hosts. They also concluded the presence of this species in South Africa as a recent introduction which will also become part of the fish parasite fauna of that region in the future. In Bangladesh, due to lack of comprehensive survey of trichodinid ciliates from indigenous as well as exotic species, it is not possible to comment whether this ciliate is an introduced species or indigenous one. However, the present study reports an extension of the geographic range of *T. acuta* and *Mystus bleekeri* to be a new host.

***Trichodina anabasi* Asmat, Mohammad and Sultana 2003 (Fig. 4, 18; Table 1)**

Host: *Anabas testudineus* (Bloch 1795) (Anabantidae). Locality: Sarker Bari culture ponds in Kapasia Upazila of Gazipur district, Bangladesh. Location: gills. Prevalence: 10/25 (40.0%). Infection: low. Reference material: AT 1 and AT 2 (10/07/2009) of silver stained slides are deposited in the Museum of the Department of Zoology, University of Chittagong, Chittagong 4331.

Trichodina anabasi was established by Asmat et al. (2003) from the gills of *Anabas testudeneus*. It is characterized by large body dimensions and having uniformly stained, dark central area at the centre of the adhesive disc; broad blade with angular and slightly rounded distal margin and flat tangent point, but almost parallel anterior and posterior margin; stout and cylindrical central part with bluntly rounded point which extends slightly more than halfway to the y-axis; and the ray is slightly curved in the posterior direction with anterior margin parallel to the y+1 axis and distinct central groove (Asmat et al. 2003). The present specimens obtained from the gills of cultured *A. testudeneus* was identified on the basis of original description of *T. anabasi* given by Asmat et al. (2003). By the denticle morphology the present ciliate was quite similar to that of Asmast et al. (2003). However, the average size of *T. anabasi* of the present study appears to be slightly smaller than that of Asmat et al. (2003). *T. anabasi* seems to have rather narrow host range as such a distinct species has only been found from the climbing perch, a highly commercial fish species distributed widely in Bangladesh.

***Trichodina cancilae* Asmat 2001 (Fig. 5, 19; Table 1)**

Host : *Xenentodon cancila* (Hamilton 1822) (Belonidae). Locality: Shitalakshya River of Gazipur district, Bangladesh. Location: gills. Prevalence: 45/120 (37.5%). Infection: low, in mixed infection with *Tripartiella bursiformis*. Reference material: XC 9 and XC 10 (10/10/2009) of silver stained slides are deposited in the Museum of the Department of Zoology, University of Chittagong, Chittagong 4331.

Trichodina cancilae was established by Asmat (2001) from the gills *Xenentodon cancila* in West Bengal, India. *T. cancilae* is a large trichodinid having denticles with elongated and arched blades; 1 or 2 cut-like notches in the convex side of the blade and the presence of the single non-staining granule at the base of blades and the base of rays; moderate central part; and broad and greatly arched rays (Asmat 2001). The blade shapes and orientation of rays of *T. cancilae* obtained in the present investigation were identical, but one of the identifying characters, i.e., the presence of non-impregnable pearl-shaped glistening particles in interblade space and in between ray bases in many specimens of the Indian populations of *T. cancilae* as inserted by Asmat (2001) was absent in the present specimens.

***Trichodina centrostrigeata* Basson and Van As 1983 (Fig. 6, 20; Table 1)**

Host : *Oreochromis mossambicus* (Peters 1852) (Cichlidae). Locality: Sarker Bari culture ponds in Kapasia Upazila of Gazipur district, Bangladesh. Location: gills. Prevalence: 30/70 (42.8%). Infection: low to medium. Reference material: OM 4 and OM 5 (10/10/2009) of silver stained slides are deposited in the Museum of the Department of Zoology, University of Chittagong, Chittagong 4331.

Trichodina centrostrigeata was first described by Basson et al. 1983, from the gills, occasionally from the skin and fins, of *Oreochromis mossambicus, Pseudocrcnilabnis philander, Tilapia rendalli, T. sparrmanni and Cyprinus carpio* in South Africa. Subsequently, this species was reported Taiwan (province of China) (Van As and Basson 1986; Basson and Van As 1994), Egypt (El-Tantawy and Kazubski 1986), the Philippines (Natividad et al. 1986; Bondad-Reantaso and Arthur 1989), South Africa (Van As and Basson 1992). *T. centrostrigeata* appears to be highly specific to cichlid fishes. There are only two reports

of this species of trichodinid ciliates from non-cichlids, i.e., by Basson et al. (1983) and Abdel-Meguid (1995) from common carp and grass carp, respectively. The ciliate obtained from the gills of *O. mossambicus* in the present study agree well with the descriptions of Basson et al. (1983), Van As and Basson (1986), Van As and Basson (1992), Basson and Van As (1994) and Mitra and Bandyopadhyay (2006). Basson and Van As (1994) commented that *T. centrostrigeata* is a parasite endemic to Africa, which at some stage was translocated to Taiwan and Philippines. The origin of *O. mossambicus* is Africa. It is commonly known as Mossambique cichlid and was introduced in Bangladesh in 1974 from Thailand to control the aquatic insects. The species is now well adapted in nature. As a result, the present trichodinid populations are also became wide spread and highly commercial fish in Banglasdesh. During the present study *T. centrostrigeata* was obtained from the gills of *O. mossambicus* from the Dhaka division. The Bangladeshi population of *T. centrostrigeata* is larger in size as well as in denticle dimensions.

***Trichodina domerguei* (Wallengren 1897) Heider 1964 (Fig. 7, 21; Table 1)**

Host : *Johnius clitor* (Hamilton 1822) (Sciaenidae). Locality: Shitalakshya River of Gazipur district, Bangladesh. Location: gills. Prevalence: 6/62 (9.7%). Infection: low. Reference material: JC 1 and JC 2 (13/04/2009) of silver stained slides are deposited in the Museum of the Department of Zoology, University of Chittagong, Chittagong 4331.

T. domerguei was first recorded by Wallengren (1897) as *Cyclochaeta domerguei* from five fishes, *Pungitius pungitius, Gastrostens aculeatus, Carassius carassius, Phoxinus phoxinus* and *Leucaspis dilineatus.* This ciliate was reported from the Rumanian Black Sea coast (Lom 1962), SSR (Lom and Stein 1966), Germany (Grupcheva and Sedlaczeck 1993), UK (Gaze and Wootten 1998), Turkey (Özer 2000; 2003a; 2003b; Özer and Erdem 1998; 1999), India (Asmat 1999), Baltic Sea (Morozinska-Gogol 2000), Poland (Rolbiecki 2006). *T. domerguei* is similar to *T. jadranica* Raabe 1958, *T. cottidarum* Lom 1970 and *T. murmanica* Polyanski 1955 (Lom 1970a; Lom and Dyková 1992). These species are euryhaline or marine trichodinids which may suggest that *T. domerguei* is of marine origin (Gaze and Wooten 1998). Gaze and Wooten (1998) also opined that the smaller dimensions of the marine specimens described in this study agree with the findings of Lom (1970) who remarks on the small specimen size in marine populations. In India, Asmat (1999) collected only a single specimen of this species from *Mystus gulio*, an estuarine fish in Canning area of West Bengal. Host specificity in trichodinids seems to be highly variable, some species such as *T. domerguei*, *T. acuta* and *T. nigra* infest a large number of host species (Lom and Stein 1966; Lom 1970a; Calenius 1980). During the present study *T. domerguei* was recorded not from any estuarine or marine fish but from *Johnius coitor***,** a well known commercial freshwater fish from a freshwater river, the Shitalaskhya River. Due to the reduced quality of stain only a few good quality specimens were obtained from this population. However, the present study reports an extension of the geographic range of *T. domerguei* and *J. coitor* to be a new host.

***Trichodina matsu* Basson and Van AS 1994 (Fig. 8, 22; Table 1)**

Host : *Labeo rohita* (Hamilton 1822) (Cyprinidae). Locality: Sarker Bari culture ponds, Kapasia Upazila of Gazipur district, Bangladesh. Location: gills. Prevalence: 14/60 (23.3%). Infection: low. Reference material: LR 3 and LR 4 (08/03/2009) of silver stained slides are deposited in the Museum of the Department of Zoology, University of Chittagong, Chittagong 4331.

Trichodina matsu was first described by Basson and Van As (1994) from the gills of the frestwater fish *Crossoptoma lacustre* and from the gills, skin and fins of the fish *Leiocassis adiposalis* in Confluence of Nankang and Peikang Rivers in Taiwan (China). This species has been subsequently reported from the gills of catfish *Clarias gariepinus* inhabiting Damietta branch of the River Nile in Egypt by El-Tantawy and El-Sherbiny (2010). Although the body dimension and other features of *T. matsu* obtained in the present study closely resemble those described by Basson and Van As (1994) for the same species, some minor differences were also recorded. First, the blade apophysis of the present trichodinid is more prominent than that of Basson and Van As (1994). Secondly, Basson and Van As (1994) recorded indentation on the lower central part of the blade whereas in the present study none of these indentations were observed. Moreover, the blade length recorded by Basson and Van As (1994) appeared to be greater than of the present study. It should be emphasized that these differences are intraspecific variations between individuals of the same species. The present study reports the first record of *T. matsu* from Bangladesh and *Labeo rohita* to be a new host.

***Trichodina microspina* Van As and Basson 1992 (Fig. 9, 23; Table 1)**

Host : *Rita rita* (Hamilton 1822) (Bagridae). Locality: Shitalakshya River, Kapasia Upazila of Gazipur district, Bangladesh. Location: gills. Prevalence: 15/80 (18.7%). Infection: low. Reference material: RR 6 (26/10/2009) of silver stained slides are deposited in the Museum of the Department of Zoology, University of Chittagong, Chittagong 4331.

Trichodina microspina was established by Van As and Basson (1992) from the skin and gills of climbing perch *Ctenopoma multispine* of Lake Lisikili in South Africa. Since then this species has not been reported from any part of the world. However,

Table 1: Morphometrical data of the trichodinid parasites obtained from the gills of freshwater fishes in the Shitalakhsya River.

Species	*Trichodina acuta* (n=10)	*Trichodina anabasi* (n=15)	*Trichodina cancilae* (n=20)	*Trichodina centrostrigeata* (n=20)	*Trichodina domerguei* (n=20)	*Trichodina matsu* (n=20)	*Trichodina microspina* (n=20)
Host	*Mystus bleekeri*	*Anabas testudineus*	*Xenentodon cancila*	*Oreochromis mossambicus*	*Johnius coitor*	*Labeo rohita*	*Rita rita*
Locality	Shitalakshya River, Bangladesh	Culture Pond, Gazipur, Bangladesh	Shitalakshya River, Bangladesh	Culture Pond, Gazipur, Bangladesh	Shitalakshya River, Bangladesh	Culture pond , Gazipur, Bangladesh	Shitalakshya River, Bangladesh
Location	Gills	gills	gills	gills	Gills	Gills	Gills
Reference	Present study	Present study	Present study	Present study	Present study	Present study	Present study
Diameter of body	36.4-47.5(43.3±3.5)	55.5-70.7(61.9±4.4)	57.5-86.9(69.4±6.8)	41.9-53.5(47.2±2.8)	30.4-38.4(34.6±2.3)	34.3-48.5(40.5±3.7)	15.6-21.2(18.2±1.6)
adhesive disc	30.3-37.4(34.5±2.4)	50.5-65.7(57.0±4.4)	52.5-80.8(63.6±6.7)	33.3-43.4(38.4±2.6)	25.2-30.3(27.8±1.6)	28.3-36.4(32.7±2.6)	12.1-17.2(14.3±1.5)
denticulate ring	16.2-22.7(20.0±1.9)	33.8-43.4(37.2±2.5)	35.3-47.5(42.9±3.6)	18.7-27.7(23.9±2.2)	14.1-19.2(16.2±1.6)	15.1-21.2(18.1±1.9)	6.1-9.6(7.6±1.0)
central area	7.1-9.6(8.7±0.9)	13.1-20.2(17.7±2.3)	15.1-21.2(18.2±1.6)	10.6-15.1(12.8±1.4)	6.1-10.1(7.9±0.9)	5.5-11.1(7.9±1.3)	3.8-6.1(4.6±0.7)
Width of border membrane	3.0-5.0(4.4±0.7)	3.0-6.1(4.9±0.8)	5.0-6.1(5.8±0.4)	4.0-5.0(4.4±0.4)	2.0-4.0(3.4±0.7)	3.0-6.1(3.9±0.8)	1.8-2.3(2.0±0.2)
Number of denticles	18-21(19.5±1.2)	25-29(27.5±1.1)	28-33(30.7±1.4)	21-28(25.8±2.1)	21-23(22±0.6)	19-24(22.4±1.2)	18-20(18.9±0.7)
radial pins/denticle	7-9(8.0±0.8)	7-10(8.7±0.9)	8-11(9.7±0.9)	6-8(7.4±0.6)	6-9(7.0±0.9)	7-10(8.6±0.8)	5-8(6.7±0.8)
Span of denticle	10.1-12.1(10.9±0.7)	14.1-20.2(17.4±1.6)	17.2-24.2(20.7±1.9)	10.6-13.6(12.0±0.8)	7.1-9.1(8.1±0.6)	8.6-12.6(10.2±1.0)	4.0-5.5(4.7±0.5)
Length of denticle	4.5-7.1(5.7±0.7)	7.1-10.1(8.3±0.8)	5.0-9.6(7.1±1.3)	4.0-7.1(4.6±0.7)	3.0-5.0(4.2±0.6)	3.0-6.1(4.6±0.6)	1.8-2.3(2.0±0.1)
ray	4.0-5.0(4.6±0.3)	7.1-9.1(8.2±0.5)	7.1-12.6(10.5±1.4)	4.0-5.5(4.3±0.4)	2.5-3.5(3.0±0.3)	3.0-5.5(3.9±0.7)	1.0-1.5(1.2±0.1)
blade	4.0-5.0(4.7±0.4)	5.0-9.1(7.0±1.0)	6.1-8.6(6.9±0.6)	4.5-6.1(5.3±0.4)	3.0-4.0(3.3±0.4)	3.0-4.3(3.9±0.3)	1.8-3.1(2.6±0.4)
Width of central part	1.5-2.0(1.8±0.2)	2.0-3.0(2.4±0.4)	3.0-4.0(3.3±0.4)	2.0-3.0(2.3±0.3)	1.3-2.0(1.8±0.2)	2.0-3.0(2.3±0.3)	0.5-1.0(0.8±0.2)
°Adoral ciliature	390-400^0	———	400^0	410°	———-	about 420º	————

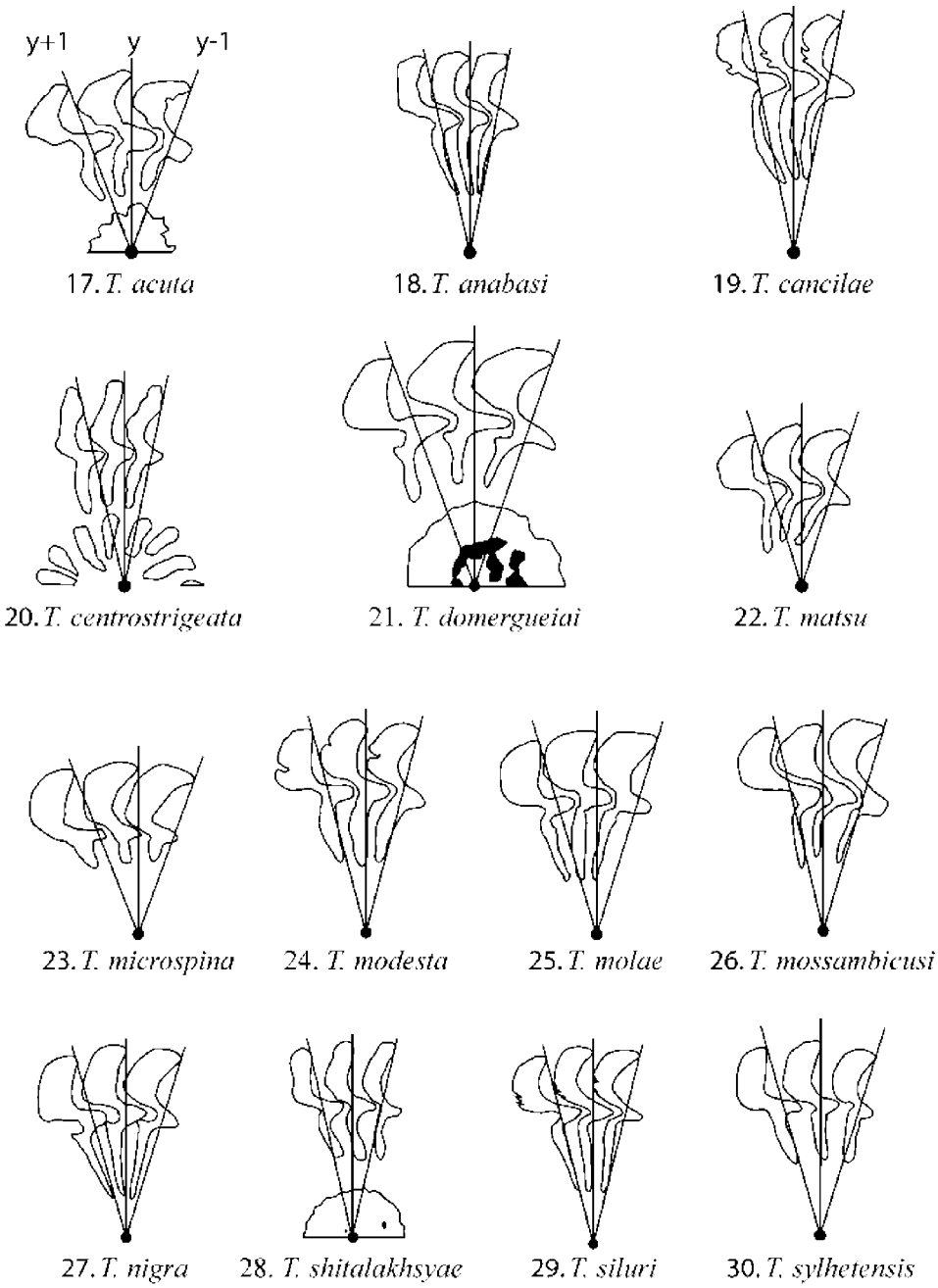

Figure 17-30. Diagrammatic drawings of denticles of trichodinid ciliates obtained during the present study in the Shitalakhsya River, Bangladesh.

the present study reports the occurrence of this species from Bangladesh as well as from a new host, *R. rita*. *T. microspina* is characterised by having small-sized body dimension with broad blade but small spine-like ray, spacious and argentophilic central area and narrow interblade space. Although based on the denticle morphology of the present population of the ciliate resemble those of Van As and Basson (1992), the average body size of *T. microspina* of the present study appears to be smaller than that recorded in the specimens of Van As and Basson (1992).

***Trichodina modesta* Lom 1970 (Fig. 10, 24; Table 2)**

Host : *Clupisoma garua* (Hamilton 1822) (Schilbeidae). Locality: Shitalakshya River, Kapasia Upazila of Gazipur district, Bangladesh. Location: gills. Prevalence: 02/70 (2.8%). Infection: low. Reference material: CG 2 (26/09/2009) of silver stained slides are deposited in the Museum of the Department of Zoology, University of Chittagong, Chittagong 4331.

Lom (1970) first described *Trichodina modesta* from the gills of *Vimba vimba* in Hungary. It is relatively small trichodinid having denticles with relatively delicate inner rays, straight or slightly curved posteriorly; elongated blades (Lom 1970) and gently curved with a noticeable thickening of the posterior border; the blades usually terminate with a rounded anterior margin, but sometimes form a flattened distal surface (Gaze and Wootten 1998). Since its discovery, *T. modesta* was reported from the skin, fins and gills of cyprinid and silurid fishes in the eastern Europe (Stein 1982, Arthur and Lom 1984), Poland (Wierzbicka 1997), United Kingdom (Gaze and Wootten 1998), Taiwan (province of China) (Basson and Van As 1994), Turkey (Özer 2007; Ozturk and Özer 2007), and China (Zhou et al. 2007; Liu and Zhao 2010). In Bangladesh, *T. modesta* was first reported by Kibria et al. (2009) from the gills of *O. mossambicus* in various culture ponds of Chittagong and Cox's Bazar districts. The presently described populations of *T. modesta* are identical to those of Lom's (1970) nominate population in size but host fish species is different, i.e. *Clupisoma garua.* The extent of morphological variation in this species in Bangladesh is unknown.

***Trichodina molae* Mitra and Haldar 2005 (Fig. 11, 25; Table 2)**

Host : *Amblypharyngodon mola* (Hamilton 1822) (Cyprinidae). Locality: Shitalakshya River, Kapasia Upazila of Gazipur district, Bangladesh. Location: gills. Prevalence: 04/56 (7.1%). Infection: low. Reference material: AM 1 and AM 2 (11/01/2009) of silver stained slides are deposited in the Museum of the Department of Zoology, University of Chittagong, Chittagong 4331.

Trichodina molae is characterized by its medium size and by the adhesive disc components, which include strongly rectangular, paintbrush-like blade; slender, tubular central part; and leaf-like flattened ray with constriction at the base, well developed central groove and sharply pointed tip. *T. molae* was named and described as a separate species by Mitra and Haldar (2005) from the gills of *Amblypharyngodon mola* in Ranaghat of West Bengal, India. The specimens described in the present paper exhibits a slightly lower range of body dimensions than those of Mitra and Haldar (2005). Other variations could be noted in Bangladeshi *T. molae* from the Indian populations are, the specimens described in this paper exhibits a slightly higher range of body dimensions than those of Mitra and Haldar (2005). Secondly, the distal margin of blade of the present study is flat whereas in the Indian population is slightly rounded. Thirdly, in the Bangladeshi specimens the anterior margin of blade forms acute angle whereas slopes down gradually in Mitra and Haldar (2005). Moreover, the ray apophysis in the present study is present, whereas in the originally described specimens the ray apophysis is absent. The mentioned minor morphological variations can be due to different geographical conditions etc.

***Trichodina mossambicusi* Asmat 2005 (Fig. 12, 26; Table 2)**

Host : *Oreochromis mossambicus* (Peters 1852) (Cichlidae). Locality: Sarker Bari culture ponds in Kapasia Upazila of Gazipur district, Bangladesh. Location: gills. Prevalence: 05/70 (7.1%). Infection: low. Reference material: OM 7 (06/06/2009) of silver stained slides are deposited in the Museum of the Department of Zoology, University of Chittagong, Chittagong 4331.

Trichodina mossambicusi was originally described by Asmat (2005) from the gills of *Oreochromis mossambicus* in and around Kalyani town of Nadia District, West Bengal, India. Since then it is a new country record for Bangladesh from the same host. The denticle morphology of silver impregnated adhesive disc and denticle morphology of the species in both the Indian and Bangladeshi populations are identical. However, the average size of *T. mossambicusi* of the present study appears to be smaller than that recorded by Asmat (2005).

***Trichodin nigra* Lom 1961 (Fig. 13, 27; Table 2)**

Host : *Notopterus notopterus* (Pallas 1767) (Notopteridae). Locality: Shitalakshya River of Gazipur district, Bangladesh. Location: gills. Prevalence: 20/74 (27.0%). Infection: low to medium. Reference material: NN 6 (08/09/2009) of silver stained slides are deposited in the Museum of the Department of Zoology, University of Chittagong, Chittagong 4331.

Trichodina nigra was established by Lom (1961) from several freshwater fish, like *Cyprius carpio, Scardinius erytrophthalmus, Rutilus rutilus, Abramis brama, Perca fluviatilis, Tinca tinca, Albnnnis albitnnts, Leitciscus cephalus* and some tadpoles of unidentified frogs in Czechoslovakia. *T. nigra* could be characterized as a trichodinid in which the blade is broad and nicely rounded or angularly curved; the central part is more robust with bluntly or sharply rounded point; the ray base is fused with the central part and broad; a central axis extends through the length of ray; the ray is straight and tapers to a blunt point; and the central area of the adhesive disc is small (Asmat 2002). *T. nigra* is a common parasite of cyprinids in Europe. This species has been recorded from Czechoslovakia (Lom 1961), UK (Gaze and Wootten 1998), USA (Hoffman and Lom 1967), Poland (Lyubarskaya and Stein 1967; Kazubski and Migala 1968; and Migal, 1970; 1978), USSR (Kulemina 1968; Stein 1968; 1982; Konovalov 1971; Urazbaev 1971; Kostenko 1972; Kashkovsky 1974; Akhmetova and Diarova 1975; Bragina 1975; Diarova 1975; Smirnova and Kairova 1975; Vismanis et al. 1975; Mikailov and Ibragimov 1980; Stein 1984; Arthur and

Table 2 : Morphometrical data of the trichodinid parasites obtained from the gills of freshwater fishes in the Shitalakhsya River.

Species	*Trichodina modesta* (n=05)	*Trichodina molae* (n=05)	*Trichodina mossambicusi* (n=20)	*Trichodina nigra* (n=15)	*Trichodina shitalakhsyae* (n=20)	*Trichodina siluri* (n=20)	*Trichodina sylhetensis* (n=20)
Host	*Clupisoma garua*	*Amblypharyngodon mola*	*Oreochromis mossambicus*	*Notopterus notopterus*	*Glossogobius giuris*	*Notopterus notopterus*	*Nandus nandus*
Diameter of body	28.3-40.4(35.2)	50.5-57.1(52.9)	36.4-44.4(40.3±2.8)	52.0-60.3(54.5±2.5)	36.4-47.5(43.3±3.5)	48.5-62.1(53.0±3.7)	29.8-56.6(44.2±7.6)
adhesive disc	24.2-39.4(31.1)	45.4-50.5(48.1)	29.3-35.3(32.3±2.1)	46.5-54.5(49.2±2.5)	30.3-37.4(34.5±2.4)	43.4-56.6(48.0±3.5)	25.2-46.5(35.9±6.0)
denticulate ring	13.1-24.2(17.9)	29.8-35.3(32.4)	16.7-21.2(19.1±1.1)	30.3-35.3(31.7±1.6)	16.2-22.7(20.0±1.9)	24.2-36.4(30.4±3.3)	15.1-30.8(21.8±3.9)
central area	5.0-10.1(7.4)	13.1-4.6(13.9)	7.1-10.1(8.6±0.9)	10.1-13.1(12.1±1.0)	7.1-9.6(8.7±0.9)	9.1-15.2(12.1±1.7)	8.1-13.1(9.4±1.6)
Width of border membrane	4.0-4.5(4.1)	4.5-5.0(4.8)	3.0-5.0(4.0±0.6)	4.5-5.8(5.2±0.3)	3.0-5.0(4.4±0.7)	4.0-5.6(5.0±0.4)	2.3-5.0(4.1±0.9)
Number of denticles	17-23(20)	26-29(27.8)	20-26(22.7±1.5)	23-27(25±1.3)	18-21(19.5±1.2)	22-27(25.0±1.2)	23-26(25±0.9)
radial pins/ denticle	7-9(8.2)	8-10(9.2)	7-9(7.7±0.7)	7-9(8±0.7)	7-9(8.0±0.8)	9-10(9.7±0.5)	6-9(7.7±0.9)
Span of denticle	9.1-13.4(11.5)	14.6-17.7(16.6)	10.1-12.6(11.4±0.8)	15.1-19.2(16.9±1.2)	10.1-12.1(10.9±0.7)	14.1-20.2(17.2±1.7)	7.8-14.1(11.7±1.9)
Length of							
denticle	4.3-6.1(5.0)	6.1-7.1(6.7)	3.8-5.0(4.3±0.4)	4.0-5.5(4.8±0.5)	4.5-7.1(5.7±0.7)	6.6-7.6(7.1±0.4)	3.5-6.1(4.8±0.7)
ray	3.0-6.0(4.5)	7.1-8.6(7.6)	3.8-6.1(4.3±0.6)	7.1-9.1(7.8±0.6)	4.0-5.0(4.6±0.3)	6.1-9.1(7.8±0.9)	3.0-7.1(4.9±1.0)
blade	4.0-5.0(4.7)	5.0-6.3(5.9)	3.8-5.5(4.8±0.5)	6.1-7.6(6.6±0.5)	4.0-5.0(4.7±0.4)	5.1-8.1(6.4±0.7)	3.0-5.3(4.5±0.6)
Width of central part	2.0-3.0(2.3)	2.5-3.5(3.1)	1.5-2.8(2.2±0.3)	2.5-3.0(2.5±0.3)	1.5-2.0(1.8±0.2)	2.0-4.0(3.0±0.5)	1.5-3.0(2.3±0.5)
°Adoralciliature	—-	—-	400°	480°	390-400	380-390^0	400^0

Lom 1984), the Philippines (Albaladejo and Arthur 1989), China (Chen 1963; 1984a; b), Taiwan (Basson and Van As 1994), Denmark (Buchmann et al. 1995; Buchmann and Bresciani 1997), Finland (Rintamaki-Kinnunen and Valtonen 1997), and from India (Mukherjee and Haldar 1982; Saha et al. 1995; and Asmat (2002). Most workers mentioned above have assigned the materials to *T. nigra* or to any of its subspecies (except Arthur and Lom 1984a) adding confusion to identifying the species (Asmat 2002). To avoid further confusion in the present work the described species is also compared with the original description and photomicrographs of *T. nigra* by Lom (1961). The appearance of the adhesive disc and denticle morphology of *T. nigra* obtained in the present study is quite identical to that of Lom (1961), Arthur and Lom (1984a) and Asmat (2002). However, the Bangladeshi specimens coincide with the Indian population in morphometry (Asmat 2002), while the body size and denticle dimensions of the Czech Republic (Lom, 1961) and Russian Federation (Arthur and Lom 1984a) are considerably larger. Incidentally, *T. nigra* is a new country record for Bangladesh and recognised as host specific on *Notopterus notopterus* in Indo-Bangla subcontinent.

***Trichodina shitalakhsyae* Kibria, Islam, Habib and Asmat, 2010 (Fig. 14, 28; Table 2)**

Host : *Glossogobius giuris* (Hamilton 1822) (Gobidae), Locality: Shitalakhsya River of Gazipur district, Bangladesh. Location: gills. Prevalence: $^{06}/_{10}$ (60%). Infection: low. Reference material: GG 1 (11/02/2009) of silver stained slides are deposited in the Museum of the Department of Zoology, University of Chittagong, Chittagong 4331.

Kibria et al. (2010) established *Trichodina shitalakhsyae* from *Glossogobius giuris* in the Shitalakhsya River of Gazipur District. The species is characterised by having an undivided clear central area in the adhesive disc with a rounded or slightly undulated perimeter containing a few dark granules which form patches; elongated and rectangular blade with large interblade space and blunt tangent point; indistinct anterior blade apophysis and a shallow apex at the base of blade that never extends beyond the y+1 axis; moderately wide and triangular central part with blunt point; and space between tip of ray and central clear area forms a wide impregnated ring. Based on these characters and the unique shape and absence of variability of the denticles among the silver impregnated specimens of the present species, it resembles *Trichodina porocephalusi* Asmat, 2001.

***Trichodina siliuri* Lom 1970 (Fig. 15, 29; Table 2)**

Host : *Notopterus notopterus* (Pallas 1767) (Notopteridae). Locality: Shitalakshya River of Gazipur district, Bangladesh. Location: gills. Prevalence: 14/74 (18.9%). Infection: low to medium. Reference material: NN 1 and NN 2 (06/05/2009) of silver stained slides are deposited in the Museum of the Department of Zoology, University of Chittagong, Chittagong 4331.

The trichodinid *T. siluri* was originally described from the gills of *Silurus glanis* from Hungary and Czechoslovakia by Lom (1970). Thereafter, this species was recorded by Bondad-Reantaso and Arthur (1989) from the skin of cultured Nile tilapia with the mixed infection of *T. heterodentata* in the Philippines. *T. siluri* may be characterised by having dark-centred adhesive disc with considerably arched blades which sometimes bear small notches or frayed areas at their anterior margins; and the rays are stout, about the same width throughout their length and extend backwards. The present report of *T. siluri* extends its known geographical distribution as well as *N. notopterus* appears to be a new host for it.

***Trichodina sylhetensis* Asmat, Hafizuddin and Habib 2003 (Fig. 16, 30; Table 2)**

Host : *Nandus nandus* (Hamilton 1822) (Nandidae). Locality: Shitalakshya River, Kapasia Upazila of Gazipur district, Bangladesh. Location: gills. Prevalence: 6/35 (17.1%). Infection: low. Reference material: NN 1 (12/12/2009) of silver stained slides are deposited in the Museum of the Department of Zoology, University of Chittagong, Chittagong 4331.

Asmat et al. (2003) established *Trichodina sylhetensis* from the gills of freshwater fish *Nandus nandus* from the Tanguar Haor at Sunamganj in Sylhet Division. The species is characterized by having medium body dimensions; slim, rectangularly-angular blade with slightly curved distal margin and blunt tangent point; bluntly rounded, slender central part having no indentation and posterior blade apophysis; broad ray with equal thickness and bluntly rounded tip; and dark-stained centre of the adhesive disc (Asmat et al. 2003). The denticle morphology of the silver impregnated adhesive disc of this species match with report of Asmat et al. (2003). However, the present population of *T. sylhetensis* is larger in size than forms recorded by Asmat et al. (2003) from the Tanguar Haor.

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A preliminary survey on entomophagous nematodes of Manipur

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Abstract : Nematodes are morphologically, genetically and ecologically diverse organisms occupying various habitats. Some parasitize man and other vertebrates while others parasitize insects and other invertebrates. In the last century it has been recognized as having an important role in regulating key ecological processes in soil food webs. Among them Entomophagous nematodes are recognized as insect regulators. Steinernematids and Heterorhabditids are already being exploited for managing not only the insect pest of agricultural crops, but also household and veterinary pests. Other similar nematodes are also reported as parasitizing the larvae of land and fresh water arthropods, including, mosquitoes, and are placed in the Superfamily Mermithoidea, family Mermithidae (Chitwood, 1950). In the present work, a survey was conducted to examine the prevalence of Entomophagous nematodes in various areas of Manipur. The entomophagous nematodes encountered are *Mermis* sp., *Hexamermis* sp., *Heterorhabditis* sp. and *Diplogaster* sp. The first two genera are recovered from grasshopper while *Heterorhabditis* sp. and *Diplogaster* sp. are isolated from soil by insect trap method. The present study deals with the isolation of indigenous entomophagous species to study their potentiality in the control of agricultural and veterinary insects.

Key words **:** *Entomophagous, Manipur, nematodes.*

Introduction

Nematodes are very common and widespread group of invertebrates occurring in large numbers in different habitats. Most of them are free living in water or soil or as parasites of plants and animals. Among them a number of nematodes are also associated with insects. These associations always intrigued scientists, particularly, zoologists and entomologists and in recent times nematologists. These nematodes vary in their size, shape and in their associationship with insects. All the major families of insects involved in crop production, transmission of human or livestock diseases or those of nuisance value are susceptible to nematode parasitism. The first record of insect nematodes was in 1623 by Aldrovandi (also referred as Aldrovandus) and the first nematode reported was probably a mermithid. However, *Mermis nigrescens*, the type species of insect nematodes, was systematically described by Dujardin in 1842. Since then much work has been done on the insect nematodes and their associations.

In the present work, a random survey was conducted to isolate indigenous entomophagous nematodes in various areas of Manipur. The entomophagous nematodes encountered are *Mermis* sp., *Hexamermis* sp., *Heterorhabditis* sp. and *Diplogaster* sp. A laboratory work on the biocontrol potential using wax moth was also done.

Materials and methods

About 70 soil samples were collected from selected hill areas of Manipur. Two isolates of entomophagous were extracted from soil samples by using *Gallaria* baiting. The collected nematodes populations were maintained on *Galleria mellonella* larvae in the laboratory. The adult were obtained by dissecting infected *G. mellonella* larvae periodically in Ringer solution. Infective Juveniles (IJs) were collected by White Trap method (White, 1927).Insects (larval and adult stages) were also collected by hand picking and using net from different areas of Imphal district. Collected insects were dissected in Ringer's solution and observed to detect the presence of Mermithids.

Nematodes were killed by applying gradual heat, fixed and processed to anhydrous glycerine using the methods described by Hominick *et al.* (1997). Measurements of all specimens were taken by an ocular micrometer.

Results and discussion

Populations of four entomogenous nematodes were detected. Two belongs to the family Mermithidae (*Mermis* sp. and *Hexamermis* sp.), *Dilopgaster* sp. belong to the family Diplogasteridae and *Heterorhabditis* sp.belong to the family Heterorhabditidae. Descriptions of the identified entomophagous nematodes are given hereunder.

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Nematodes in this order are parasite of invertebrates. The main morphological characteristic of the order is the presence of a stichosome. Early juvenile stages bears a protrusible stylet while it is absent in the adults.

Mermithid species were encountered from grasshopper collected from orchid farm, Khonghampat. The specimens are in Post parasitic stage. They are long, slender, upto 10 -20cm in length, color brownish white. Cuticles containing faint criss-cross fibers near the outer layers. Head containing six cephalic papillae and amphids reduced. Tail tip with appendage. Due to presence of appendages the species is identified as *Hexamermis* sp.

***Mermis* sp.** was also recovered from grasshopper collected from Porompat, Imphal East district. Cuticle smooth, with criss-cross fibres below. Body tapering in front. Head slightly offset, rounded in front. Oesophagus comparatively short and indefinite. Immediately behind head an orange-red coloured area is found in egg-laying female. Tail rounded or sub-conical. Vulva equatorial. Egg globular, 54 µm in diameter and 50 - 53 µm from pole to pole. Male with arcuate spicules. Males are apparently much less common than females.

***Diplogater* sp.** was isolated from the soil of Siroy hill, Urkhrul district, Manipur. Labia seldom well developed; a hexaradiate symmetry is distinct. External circle of labial sensilla setose but always short, never long and hairlike. The stoma may be slender and elongate or spacious or any gradation between these two. Stoma armed or unarmed; the armature may be movable teeth, fossores, or a pseudostylet. Corpus muscled and distinct from the postcorpus, divisible into isthmus and glandular posterior bulb. The metacorpus valved present. Female reproductive system may have one or two ovaries and males may or may not have caudal alae; however, gubernaculum always present. Male tail with nine pairs of caudal papillae; three pre-anal and six caudal.

***Heterorhabditis* sp. : Adult :** Head truncate or slightly rounded, having six distinct lips well developed, with one labial papilla each. Amphidial apertures inconspicuous pore-like. Stoma short wide. Cheilostom short with retractile rhabdions. Posterior part of stoma collapsed, with reduced pro, meso, and metarhabdions. Base of stoma surrounded by oesophagous. Oesophagous rhabditoid. Nerve ring distinct, surrounding isthmus. Excretory pore located posterior to nerve ring.

Hermaphrodite (first generation) : Body curved into 'C' shape. Vulva located near middle of body. Female with sperms in proximal portion of ovotestes and functional vulva. Vulval lips slightly protruding. Post-anal swelling well developed. Tail 64.6-84 µm long, terminus blunt and mucronate.

Female (second generation) : Amphidelphic. Body C-shaped upon fixation. Females with didelphic amphidelphic reflexed gonad. Vulval lips slightly protruding. Tail conoid, and about 65-67.2 µm in length. Postanal swelling well developed.

Males (second generation) : Monorchic. Body ventrally curved with more curvature in the posterior region when relaxed. Oesophageal structure but small in size. Testis single and reflexed anteriorly, spicules paired and separate, symmetrical, slightly curved with pointed tips. Spicule head short, setoff from lamina by a constriction. Gubernaculum present Bursa open, peloderan with nine pair of genital papillae.

Third-stage infective juvenile (IJ): Third stage juvenile in cuticle of second stage juvenile (J_2). Body tapers more in the posterior region to a long acutely pointed tail. IJ with prominent cuticular dorsal tooth. The oesophagous narrow with a weak basal bulb having weakly developed valve plates. Mouth and anus closed.

Locality : Chiru Hill, Bishnupur district, Manipur. Height of the location is around 6265 ft. (MSL).

In a trial of the different entomophagous nemtodes *Heterorhabditis* sp. was found to be highly effective. It was designated as *Heterorhabditis* sp. EPN-MU1. The pathogenic potential of *Heterorhabditis* sp. EPN-MU1 using wax moth larvae was found to have very high biocontrol potential, since it induces insect mortality within 24-42hrs and the infective juveniles emerged from the cadavers within 7-8 days after inoculation at room temperature (15-25^{0}C). So, there is strong possibility that the same strain could be effective in biocontrol of the agricultural pests of economic importance.

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Influence of abiotic factors and parasitoid, *Eritmocerus adustiscutum* on the incidence of whitefly, *Dialeuropora decempuncta*

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Abstract : Whitefly, *Dialeuropora decempuncta* (Quaintance & Baker) (Homoptera : Aleyrodidae) is the most important major pest of mulberry eco-system causing a considerable yield loss by inflicting chlorosis, leaf curl and sooty mould disease. These infested leaves are not preferred by silkworms resulting in large economic losses to sericulturists of West Bengal. The nymphal stages of this whitefly are parasitized by *Eritmocerus Adustiscutum* (Krishnan & David) (Hymenoptera : Chalcidoidea). It is well established that environmental factors affect the occurrence and intensity of a pest. An understanding of the role of critical abiotic factors conducive for field occurrence of a pest will facilitate the forecasting of the outbreak in advance. The percent parasitism level on *D.decempuncta* (Quaintance & Baker) was correlated with the abiotic factors and the host population. While establishing the correlation between the total population of *D. decempuncta* and the abiotic factors, it was found that the population is positively correlated with all the abiotic factors considered i.e. maximum temperature, minimum temperature, maximum relative humidity, minimum relative humidity and rainfall. When the whitefly population was correlated with percent parasitism to find out the effect of parasitoids in influencing the whitefly population, it was found to be positive, but non significant with r value as 0.109. The multiple regression analysis has revealed that the most significant time period showing the effect of the abiotic factors of previous 42-48 days and the natural enemy population of previous 21 days was found to have significant effect in influencing the population of *D.decempuncta*.

Key words **:** *Correlation coefficient, multiple regression, parasitoid, population dynamics, whitefly,*

Introduction

Environment in nature rarely, if ever, remain constantly favourable or unfavourable but fluctuate irregularly between two extremes. Innate capacity of animals for increase fluctuates correspondingly, being sometimes positive and sometimes negative. If the conditions remain favourable, the capacity of a species to increase in number remains positive and indefinitely the species would continue to multiply (Andrewartha and Birch, 1954). The role of natural enemies and other biotic factors was also emphasized among the forces which control the abundance of organisms (Thompson, 1929a & 1939). The emphasis created a critical discussion of the relative importance of biotic factors in contrast to other factors of the environment which are conceived by some ecologists as deserving equal or great importance in determining abundance (Bodenheimer, 1938, Thompson, 1956). Hence, there is a need for integrating these roles and relating the status of natural enemies in the entire complex of forces involved in determining the abundance of populations in nature. The knowledge of insect population dynamics is essential for developing sustainable crop protection strategies and for safeguarding the health of the agricultural environments. The ecological factors affecting insect population are of major importance in insect pest control. All available knowledge about the biotic and abiotic characteristics of the environment affecting the pest should be used in weaving a pattern of insect control for a specific pest in a specific place or area. In the present study attempts were made to study the influence of abiotic factors and a native parasitoid *Eritmocerus adustiscutum* (Krishnan & David) (Hymenoptera : Chalcidoidea) on the incidence pattern of whitefly, *Dialeuropora decempuncta* (Quaintance & Baker) (Homoptera : Aleyrodidae). The whitefly used to heavily infest the lower surface of leaves of a wide variety of plants resulting in chlorosis, yellowing, leaf fall and impairment of growth. Extensive sucking of phloem juice from the lower surface of the leaves causes upward curling and 24% leaf yield loss (Bandyopadhyay et al., 2000). The honey dew excreted by nymphs favoured development of sooty mould fungi, *Curvularia affinis* and *Chetothyrium sp.* Boedijin. These fungi form a black velvety coating on the upper surface of mulberry leaves, which results in the reduction of photosynthesis and the leaves will become unfit for silkworm rearing.

Materials and Methods

For studying the population dynamics of whitefly, *D.decempuncta* and its native parasitoid, *E.adustiscutum* weekly surveys were conducted in the Mulberry fields of Central Sericultural Research & Training Institute, Berhampore, West Bengal from 20 mulberry plants selected randomly(Schuster, 1998 and Assad et al., 2006). From each plant, adult whitefly population was collected from top two mulberry leaves, early nymphal counts were made from two leaves of middle region and the late nymphs from bottom two leaves (Purohit and Deshpande, 1991, Naranjo and Flint, 1995, Karut and Sekeroglu, 2003). For recording the parasitoid, *E.adustiscutum*, the whitefly infested leaves specially the middle and bottom leaves with good number of parasitized whitefly nymphs were plucked and brought to the laboratory for observation. The peduncles of the leaves were wrapped with moist cotton swabs to retain the freshness and were placed in petridishes. The infested leaves were then screened under stereomicroscope and the records of the number of parasitized nymphs of *D.decempuncta* were made. Number of successfully developed and emerged parasitoids was determined basing on the color of the nymphs and their emergence holes (Van Dreische et al., 2001b; Hoddle and Van Dreishe, 1996). The parasitized nymphal cases were counted and recorded basing on the change in the puparial colour to black (Gill, 1992 and Michelakis, 1995). For establishing the correlation between the whitefly population and the biotic and abiotic factors, coefficient of correlation was worked out. Further, to pinpoint the previous effect of meteorological factors and biotic factor in influencing the whitefly population, data was subjected to multiple linear regression analysis.

Results

The first phase of three year study on the seasonal incidence of the whitefly, *D. decempuncta* showed that in the year 2004-2005, the population of the whitefly was 19.76 per plant during the fourth week of August [Fig. 1(a)]. This number is almost close to the economic threshold level (ETL) which is 20 per plant. While following the seasonal trend of the population of the whitefly, it was observed that the population build up in the month of August is fair enough to cause a significant damage to mulberry. This number was observed to be the highest in the particular year of study i.e. 2004-2005. During this year, the least number of the whitefly was recorded during fourth week of March, as 0.94 per plant [Fig. 1(a)].

During the second year i.e. 2005-2006, it was observed that the population of this species was above the economic threshold level from the very first week of the study [Fig. 1(b)]. Unlike the previous year, during this year the population reached a number of 50.46 per plant in the month of August [Fig. 1(b)]. The population of *D. decempuncta* was observed to be above ETL till the end of November [Fig. 1(b)]. The highest population was recorded as 80.24 per plant in the second week of November [Fig. 1(b)]. The, higher range of population was observed from November till first week of February. The whitefly number reached a minimum of 1.01 per plant in the first week of June.

In the third year of the study (2006-07) also it was observed that the population of the pest started building up from August. The maximum incidence of the pest was recorded as 88.07 per plant in the second week of September [Fig. 1(c)]. The minimum population of whitefly was observed as 0.6 per plant during the first week of March. Thus, the seasonal incidence of *D. decempuncta* (Quaintance and Baker) during the last year of the study was found to be different from the previous two years of the study. In the last year the population remained below ETL for a maximum span of time from November to June. Whereas in the previous two years the population of the pest was below ETL from February to June.

During 2004-2005, the percent parasitism by *E.adustiscutum* on nymphs of *D. decempuncta* (Quaintance and Baker) was found to be maximum during 56.58 during second week of April and it was nil during last week of February to first week of March [Fig. 2a]. In the second year of study i.e. 2005-2006, the highest percent parasitism was observed to be 63.3% per plant during the second week of May, 2006. Like the previous year the percent parasitism came down to nil in the first week of March. [Fig. 2b]. During the third year of observation i.e. 2006-2007, the percent parasitism reached to the highest of 68.49 during last week of August, 2006 [Fig. 2c]. The percent parasitism was observed to be nil during fourth week of May, 2007. It is evident from the fig. 2 a, b and c that the percent parasitism on nymphal stages of whitefly is highly fluctuating during three years of study.

Discussions

The statistical analysis i.e. the coefficient of correlation was determined in phases relating the whitefly (*D.decempuncta*) population with meteorological factors and natural enemy, *E.adustiscutum*.

While establishing the correlation between the total population of species of *D. decempuncta* and the abiotic factors, it was found that the population is positively correlated with all the abiotic factors considered i.e. maximum temperature,

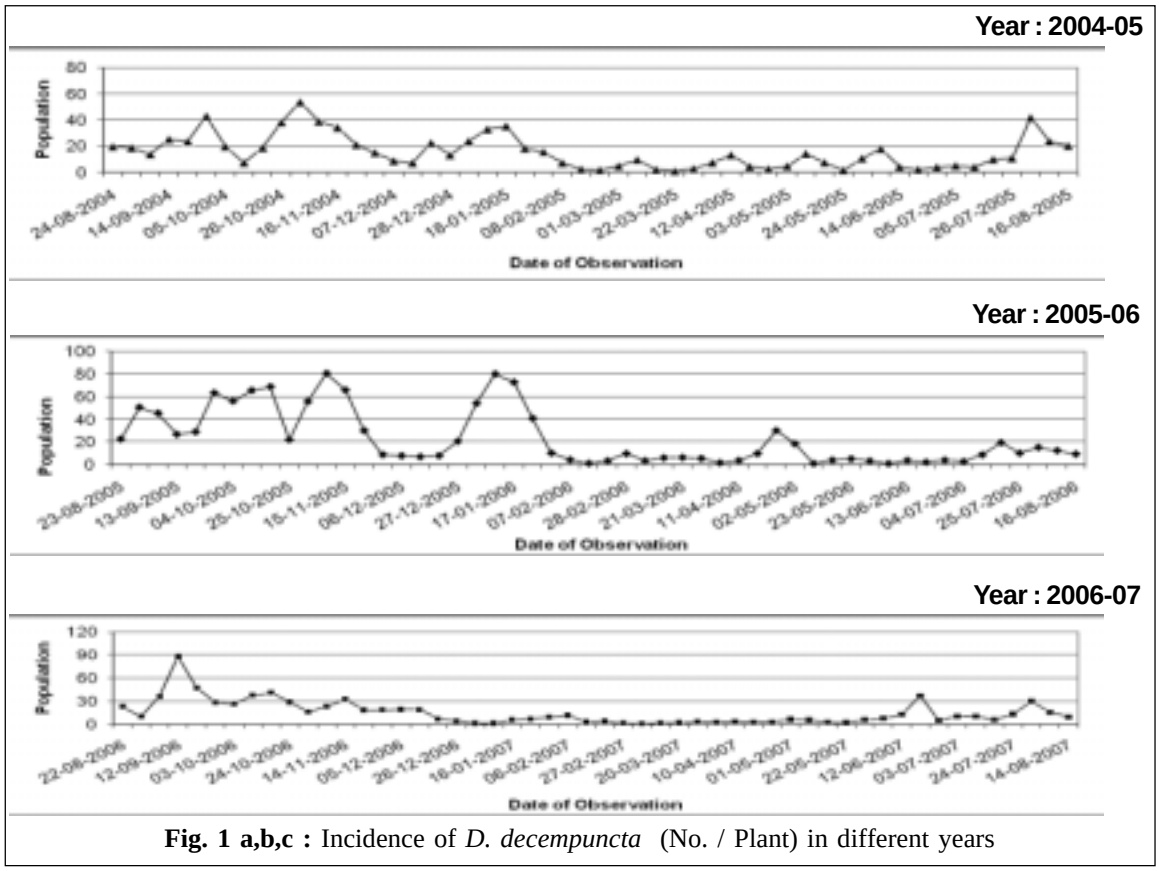

Fig. 1 a,b,c : Incidence of *D. decempuncta* (No. / Plant) in different years

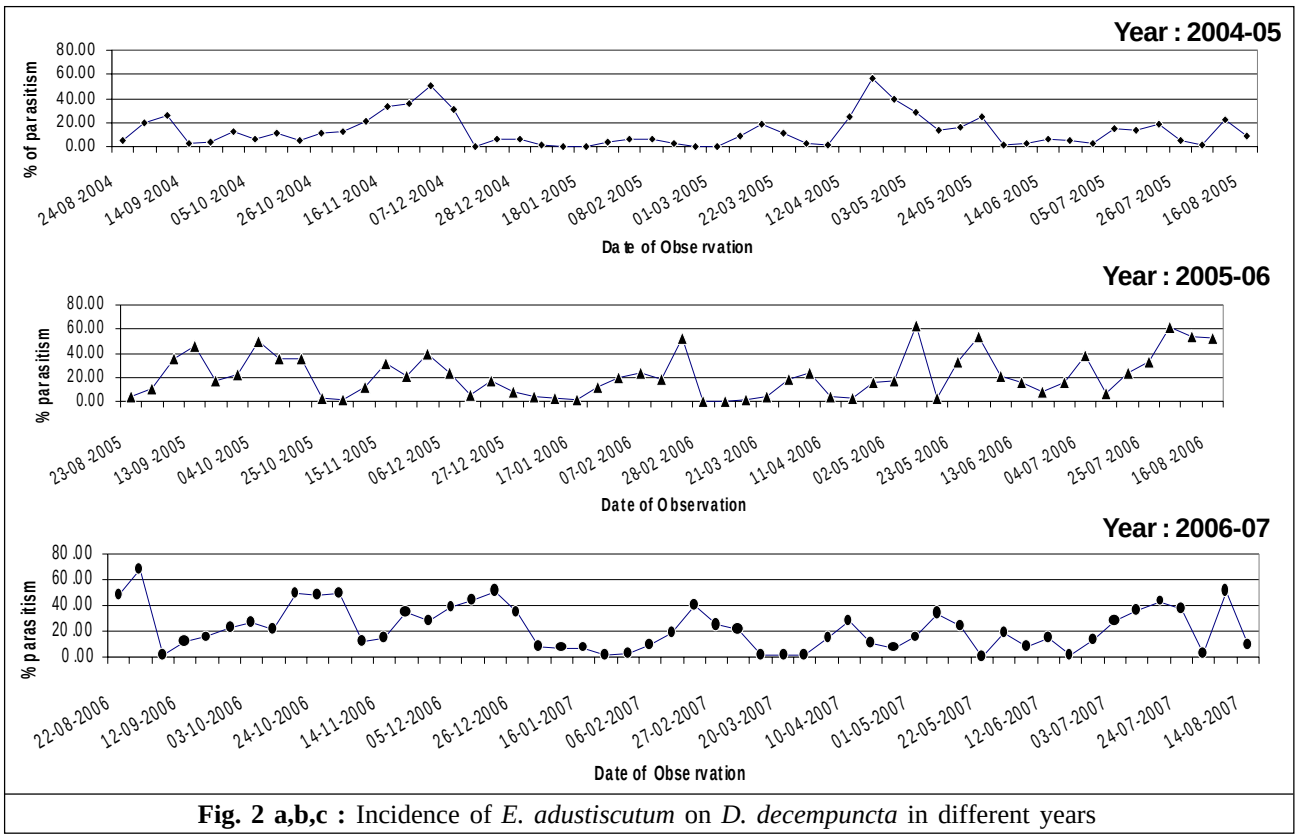

Fig. 2 a,b,c : Incidence of *E. adustiscutum* on *D. decempuncta* in different years

minimum temperature, maximum relative humidity, minimum relative humidity and rainfall. In cotton whitefly, *Bemisia tabaci* (Gennadius), it was found that rainfall is negatively correlated to the population (Gupta *et al.*, 1998). Significant but negative correlation of whitefly population with rainfall was also reported in winter as well as summer by Shyam Prasad and Logiswaran (1997). Negative and non significant correlation of population of whitefly with rainfall was also reported by Nandihali *et al*., 1993. But in case of maximum temperature, though the correlation is positive but it is not found to be significant with r value of 0.143. Gupta *et al.* (1998) reported that the correlation with minimum temperature was negative but significant in case of cotton whitefly, *B. tabaci* at 5% level. Correlation with the other abiotic factors viz., minimum temperature, maximum relative humidity, minimum relative humidity and rainfall was significant at 1% level as well as positive with r values, 0.309, 0.256, 0.457 and 0.223 respectively. The population of *B. tabaci* was reported to have positive and significant correlation with maximum temperature and relative humidity and negative and non significant with minimum temperature in winter (Shyam Prasad and Logiswaran, 1997). The observation related to relative humidity was in agreement with that of Nandihali *et al*., (1993), who had observed positive significant correlation of whitefly population with mean morning relative humidity. However, Hirano *et al.* (1993 & 1995), studying population dynamics of *B. tabaci* in soybean and mung beans in Java and Indonesia, respectively, reported that the weather was not among the principal factors to regulate the population of this insect.

When the whitefly population was correlated with percent parasitism to find out the effect of parasitoids in influencing the whitefly population, it was found to be positive, but non significant with r value as 0.109 .

Multiple linear regression analysis was performed to draw inference related to forecasting the pest incidence in the mulberry eco-system. The regression equations were worked out for the whitefly population with respect to the abiotic and biotic factors. While analyzing the data with various methods, the best and the most significant time period showing the effect of the abiotic factors of previous 42-48 days have influence on the total population of *D. decempuncta* (Quaintance and Baker) and the regression equation for it was computed as

$Y = -186.188 + 3.980\ X_1 - 2.495X_2 + 0.626X_3 + 1.225X_4 - 0.431X_5$.

Where Y= Population of *D. decempuncta*,

X_1 = Maximum temperature,

X_2 = Minimum temperature,

X_3 = Maximum Relative Humidity,

X_4 = Minimum Relative Humidity,

X_5 = Rainfall.

With coefficient of determination $R^2 = 0.288642$ ($P = 1.06 \times 10^{-9}$).

Similarly the effect of the biotic factor i.e. the natural enemy population on the whitefly population was worked out and it was found that the natural enemy population of previous 21 days was found to have significant effect. The regression equation for it was –

$Y = 10.652 + 0.161\ X_1$.

Where Y= Population of *D. decempuncta*,

X_1 = Percent parasitism on whiteflies.

With coefficient of determination $R^2 = 0.193349$ ($P = 6.22 \times 10^{-6}$).

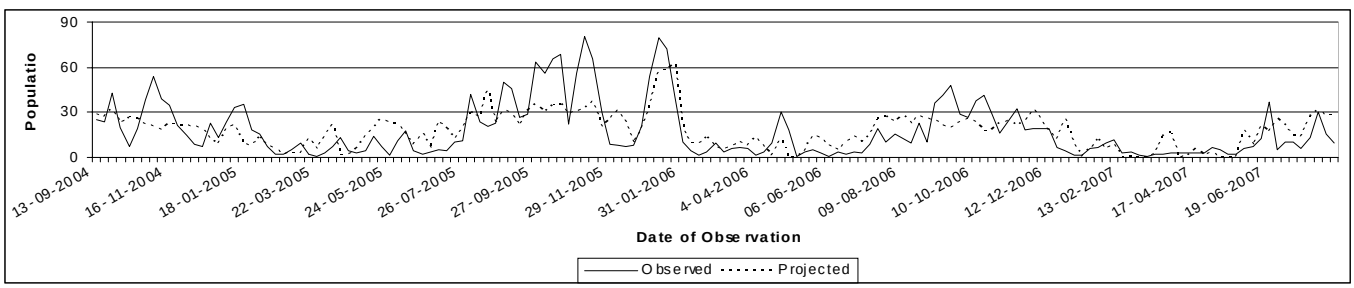

Fig. 3 : Correlation between seasonal incidence of *D. decempuncta*, natural enemies and abiotic factors

The regression equation showing the combined effect (Fig.3) of the abiotic factors (previous 42-48 days) and the natural enemy population (previous 21 days) on the total population of the whitefly was found as :

$Y = -144.158 + 2.787\ X_1 - 1.44\ X_2 + 0.462\ X_3 + 0.962\ X_4 - 0.247\ X_5 + 0.047\ X_6$

Where Y= Population of *D. decempuncta*,

X_1= Maximum temperature,

X_2 = Minimum temperature,

X_3 = Maximum Relative Humidity,

X_4 = Minimum Relative Humidity,

X_5 = Rainfall

X_6 = Percent parasitism on whiteflies

With coefficient of determination $R^2 = 0.458276$ ($P= 7.19 \times 10^{-15}$).

These findings are helpful to the researchers for formulating computerized forecasting models which will be of direct applicability to the extension functionaries in predicting the probable pest and natural enemy population. Accordingly the extension functionaries will be advising the policy makers and farmers' community to take prophylactic measures for preventing the probable outbreaks of whitefly.

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A report on effective treatment with ciprofloxacin along with vitamin C and E to EUS infected snake head fish *Ophiocephalus gachua* (Hamilton, 1822)

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Abstract : The dreaded fish disease, Epizootic Ulcerative Syndrome (EUS) has become one of the major threats to the inland aquaculture for the last few decades. It is a seasonal disease primarily affects on the wild fish including snake heads and eventually causes death of ulcerated fish. The EUS has a multiple and complex etiology. A number of opportunistic bacteria and a fungus *Aphanomyces invadus* are associated with necrotic ulcers of diseased fish. In the present experiment Ciprofloxacin in combination with vitamins (vitamin C & E) and lime took shortest time for recovering the ulcers of fish due to EUS. The findings of the study will provide the useful information regarding the application of Ciprofloxacin in the control of fish disease like EUS.

Key Words **:** *Ciprofloxacin, EUS, Ophiocephalus gachua, Vitamin C & E*

Introduction

Epizootic Ulcerative Syndrome (EUS) or Ulcerative Disease Syndrome (UDS) is a dreaded fish disease of epizootic nature and of complex infectious aetiology characterized by dermatitis, myostitis and mycotic granuloma (Mohan and Shankar,2000). It causes sudden and often mass mortalities in wild freshwater and some brackish water fishes.This seasonal necrotic disease initially was reported from wild waters, rivers and canals but later with the entry of flood waters the infestation gradually expanded to almost the entire flood plain including low-lying water logged areas, beels/baors, ponds, paddy fields etc. Since1972, this disease affects several wild to culturable species fish and thus causing a serious damage to the inland fishery of different parts of Asia (Kumar and Dey, 1992; Vishwanath et al, 2000). More than hundred fish species with a wide geographical distribution have been reported for being affected by EUS (Lilley et al, 1992). In India, among the susceptible species, the most severely affected are *Puntius* sp., *Channa* sp., *Clarias batrachus*, *Heteropneustes fossilis* and *Mastacembelus* sp. and mildly affected species include Indian major carps which suffer high mortality in fingerling stage (Roberts et al, 1989 ; Kumar and Dey, 1992).

Recently it has been confirmed that a highly invasive and specific slow growing monoclonal species of fungus *Aphanomyces invadans* is the principal factor in EUS (Willoughby et al, 1995; Lilley et al, 1998; Chinabut and Roberts, 1999), but it is also believed that the fungal invasion is secondary to primary skin damage and /or immuno-suppression (Raj et al, 2003). EUS has a multiple and complex etiology. Besides one or more invasive fungi, a number of opportunistic bacteria have been isolated from necrotic ulcer of fishes with EUS outbreaks, which cause eventual death of ulcerated fish (Roberts et al, 1993; Vishwanath et al, 2000 ; Raj et al, 2003). As the definite primary pathogen has not yet been identified, the effective control and treatment of EUS is the main problem today. Unfortunately, in view of its complex infectious etiology, it is yet to be accurately defined.

In the present study an attempt was made on the effective use of Ciprofloxacin along with two natural anti-oxidants (vitamin C and E) to snake head fish Ophiocephalus gachua (Hamilton, 1822) for the recovery from the disease, Epizootic Ulcerative Syndrome (EUS). The findings of the study will provide the useful information regarding the application of Ciprofloxacin in the control of fish disease like EUS.

Materials & methods

Mature dwarf snake head fish *Ophiocephalus gachua* (12.5 $\pm$ 1.5 cm; 17 $\pm$ 2 gm) irrespective of sex were collected from EUS infected pond connected with Kangsabati cannal irrigation systems at Indpur in the district Bankura, West Bengal by random sampling in the month of October 2003. The fishes were severely affected and lethargic. The major clinical signs were

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the presence of brown to dark red haemorrhagic lesions to large deep necrotic ulcers of various sizes on the caudal region of fish (Photograph I). Some fishes were also with small or large grey or red shallow ulcers in the ventro-cephalic region.

At the time of fish collection, physico-chemical parameters of the pond water were measured following the methods of APHA (1992).

Collected fishes were allowed to bath treatment in 1% NaCl solution for 2-3 minutes and then were kept in glass aquaria (24" x 12" x 12") containing 15 litres of unchloronited tap water (Tempt. 27.0 $\pm$ 0.5 °C, pH 6.8 $\pm$ 0.1, Dissolved oxygen 5.4 $\pm$ 1.0) with proper aeration system under laboratory condition. In this condition fishes were acclimatized for 3-4 days. The bioassays were conducted to observe fish behavior and ulcer condition due to EUS during different days of treatment. During the present experiment six different treatments were made along with sufficient replicates (four replicates for each treatment). Eight infected fishes were exposed to each treatment (two per aquarium). The treatments details of the present experiment are given in table I.

Table I. Details of the treatment schedules maintained during experiment

Sl. No.	Treatment No.	Treatment schedule
1.	T-1 (Control)	Feed (5% of fish body weight)
2.	T-2	Feed (as T-1) + Lime (25 ppm)
3.	T-3	Feed(as T-1) + Ciprofloxacin (200 mg kg-1 feed for first 3 days followed by 100 mg kg-1 feed for next 2 days)
4.	T-4	Feed(as T-1) + Ciprofloxacin (as T-3) + Lime(as T-2)
5.	T-5	Feed(as T-1) + Ciprofloxacin (as T-3)+ Vitamin C (100 mg kg-1 feed for 7 days) and Vitamin E (50 mg kg-1 feed for 7 days)
6.	T-6	Feed(as T-1) + Ciprofloxacin (as T-3)+ Vitamin C&E (as T-5) + Lime (as T-2)

The feed was used as egg custard given twice a day @ 5% of the fish body weight in split doses. The Ciprofloxacin (the most potent first generation quinolone antimicrobial active against broad range of bacteria including *Escherichia, Pseudomonas, Klebsiella, Vibrio, Salmonella, Staphylococcus* etc.) in the form of CIFRAN tab. (containing Ciprofloxacin hydrochloride, marketed by Ranbaxy) @ 200 mg kg-1 feed for first 3 days and @ 100 mg kg-1 feed for next 2 days together with two natural antioxidants vitamin C in the form of CELIN tab. (containing Ascorbic acid IP and Sodium ascorbate IP, marketed by Glaxo SmithKline) @ 100 mg kg-1 feed and vitamin E in the form of EVION cap. (containing Tocopheryl acetate IP, marketed by Merck Ltd.) @ 50 mg kg-1 feed for 7 days were added to the egg custard following the schedule given in the table I.

The uneaten feed was siphoned out from the aquaria in the everyday morning to avoid organic pollution. Aquariums were scrubbed, cleaned and dried at every week for preventing the accumulation of harmful pathogens and to minimize the variation in the result to be obtained. Moreover, at every change, the lime was added to the aquarium @ 25 mg/l of water to maintain steady pH and alkaline level during the experiment. The experiment was continued for 30 days. The behavior of fish and the ulcer conditions during the experiment were recorded daily.

Results

The physico-chemical parameters of the pond water were measured during the collection of diseased fish and recorded in table II.

Table II. Physico-chemical parameters of the EUS infested pond water

Parameters	Value
Colour	Light brown
Temperature (°C)	25.8± 0.3
pH	5.9± 0.7
Dissolved oxygen (mg/l)	4.9± 1.0
Free CO_2 (mg/l)	20.7± 2.2
Total alkalinity as $CaCO_3$ (mg/l)	35.2± 3.2
Hardness as $CaCO_3$ (mg/l)	25.9± 4.2
Ammonia nitrogen ((mg/l)) [NH4 - N]	0.9± 0.1

(N.B. Values are mean of four replicates ±SD)

The physico-chemical parameters of the treated waters recorded during experiment have been given in table III.

Table III. Physico-chemical parameters of treated waters during experiment

Treatment	Temperature (°C)	pH	Free CO_2 (mg/l) $CaCO_3$ (mg/l)	DO (mg/l) (mg/l)	Total alkalinity as	Hardness as $CaCO_3$
T 1	27.4 ± 0.2	6.97 ± 0.12	20.2 ± 2.50	5.4 ± 0.52	134 ± 4.1	110 ± 3.9
T 2	27.3 ± 0.1	8.33 ± 0.15	0	5.5 ± 0.43	175 ± 5.0	126 ± 5.0
T 3	27.4 ± 0.1	6.81 ± 0.22	25.2 ± 3.40	5 ± 0.60	146 ± 3.8	107 ± 3.9
T 4	27.5 ± 0.1	8.4 ± 0.12	0	5.6 ± 0.41	170 ± 5.1	123 ± 5.0
T 5	27.3 ± 0.2	6.82 ± 0.26	20.15 ± 2.60	5.3 ± 0.56	132 ± 5.3	98 ± 4.2
T 6	27.3 ± 0.2	8.3 ± 0.30	0	5.7 ± 0.50	172 ± 4.1	124 ± 4.3

(N.B. Values are mean of four replicates ±SD)

The progress of recovery from EUS after treatment of Ciprofloxacin and Vitamin C&E was recorded during the experiment and has been summarized in table IV. The recovery of ulcer was observed in all treatments except control. In control (T-1) all the fishes died within 15-20 days of experiment. The recovery from the disease was faster i.e. within 5-7 days in the treatment (T- 6) where Ciprofloxacin was used along with vitamin – C and E in presence of lime (Photograph II). Such efficacy of Ciprofloxacin to combat bacterial fish pathogens was also in agreement with Rahman et.al., (2009). The lowest progress for the recovery of the disease i.e. within 21-30 days was found in the treatment (T-2) where fishes were treated with lime only. The intermediate progress in curing of disease was recorded where Ciprofloxacin was used alone (T-3) or in combination with vitamin C and E (T-5) or lime (T-4). It has been observed that the vitamin C and E along with lime accelerate the activity of Ciprofloxacin and causes faster recovery from the ulcer and development of the scale in the healing part (Photograph II).

Discussion

In the present study, the values of temperature, pH, dissolved oxygen, free carbon dioxide and ammonia nitrogen of pond water obtained during the disease outbreak period which did not reveal any noticeable differences from their optimum values (table 2). However, there was a severe fall in total alkalinity and hardness values of the pond water prior to and during EUS outbreak which probably caused ‘stress’ condition for fishes in the water and making them susceptible to attack by the pathogens leading to the outbreak of EUS. Similar phenomenon was also recorded by Kar and Dey (1990).

In the present experiment vitamin C & E in presence of lime accelerate the activity of ciprofloxacin leading to faster recovery from ulcer due to EUS and development of scales in the healed part. Perhaps vitamin C plays an important role in the regulation of growth, RNA synthesis and tissue repair by collagen synthesis in fish (Reddy, 1992; Mitra, 1998) and vitamin E along with C prevents muscular dystrophy (Paulraj, 1995). Application of lime increased the total alkalinity and hardness of the test water and caused faster recovery from the disease reducing predisposing ‘stress’ factor aroused due to low alkalinity.

The present findings will no doubt provide the useful information regarding the application of Ciprofloxacin in controling fish disease like EUS and also provide a support in making a package in favour of proper fish health management.

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Table IV. Observation on fish behavior and ulcer condition of *Ophiocephalus gachua* due to EUS during different days of treatment.

Treatments	DAYS							
	0	1-2	3-4	5-7	8-10	11-15	16-20	21-30
T -1 (Control: only feed)	1) Brown to dark red haemorrhagic lesions to large deep necrotic ulcers of various sizes on the caudal region were recorded. (Photograph I) 2) Small or large grey or red shallow ulcers in the ventro-cephalic region were also found in some fish. 3) Lethargic movement was recorded	i) Lethargic ii) Took rest in the corner iii) Did not take any food	i) Motion-less ii) Did not take food iii) Ulcers extended	i) Disturbed movement with Jumping property ii) Spreading of ulcers iii) Did not take food	i) Erratic and disturbed movement ii) Did not take food iii) Ulcers became reddish	i) Loss of balance was recorded ii) Ulcers were deeply exposed	i) All fish died	
T – 2 (Feed + Lime)		i) Body colour darken ii) Low feeding rate iii) Fish restricted at corner	i) Loosening of scales from the side of ulcers were found ii) Low feeding rate iii) Ulcers turned to dull whitish iv) Slow movement	i) Erratic swimming observed ii) Ulcers in caudal region became whitish iii) Caudal fin was broken down in some fishes	and large sized i) Jerking movement was recorded ii) Reddish colour of ulcers turned to whitish	i) Ulcers changed to whitish colour	i) Ulcers started to heal up ii) Red ulcers in ventro-cephalic part were disappeared	i) Ulcers healed up ii) Normal move-ment and behave-or was found
T – 3 (Feed + Cipro-floxacin)		i) Some fish showed jerk movement ii) Low feeding rate	i) Slow movement on the surface level was recorded ii) Body colour was slightly whitish	i) Reddish ulcers turned to whitish ii) Caudal fin rays was broken down in some fishes	i) Ulcers started to disappear	i) Healing was in progress but full recovery was not recorded	i) Wound healed up ii) Normal swimming was found	-
		i) Irregular movement was recorded ii) Reddish coloured ulcers turned to dull white	i) Reddish or brownish colour of ulcers changed to whitish	i) Ulcers on cephalic region gradually disappeared ii) Wounds healed up in caudal region	i) Wounds healed up ii) Scales on the ulcers did not regenerate			
-		i) Some fished showed jerk movement ii) Body colour was slightly whitish	i) Free movement was recorded ii) Reddish wounds changed to whitish	i) Ulcers getting healed up ii) Feeding behavior normal	i) Recovery of ulcers was in progress	i) Ulcers on cephalic region disappeared ii) Wounds on caudal part healed up	i) Scales on ulcer parts started to regenerate ii) Normal behavior was recorded	

Treatments	DAYS							
	0	1-2	3-4	5-7	8-10	11-15	16-20	21-30
T – 6 (Feed + Ciprofloxacin + Vitamin C&E + Lime)		i) Most of the fishes started jerk movement ii) Body was slightly white in colour iii) Some caudal fin rays were broken down in some fishes iv) Reddish coloura-tion of wound slightly turned to whitish	i) Reddish colour in ulcers disappeare, turned to whitish ii) Ulcers were healed up iii) Reddish patch of ulcers on cephalic region gradually disappeared	i) Reddish colour of wound on cephalic region gradually disappeared ii) Wounds on caudal part healed up iii) Caudal fin brittled down in some fishes iv) Scales on the ulcers parts started to regenerate (Photo-graph (II)	i) Scales re-generated ii) Normal movement and behavior were recorded iii) Ulcer condition was much more improved	-	-	-

Photograph I **:** EUS infected fish (*Ophiocephalus gachua*) before treatment.

Photograph II **:** EUS infected fish (*Ophiocephalus gachua*) after treatment (T-6).

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Distribution of worm infestation in naturally grazing garole sheep of Sundarbans region

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Abstract : Garole sheep of Sundarbans, West Bengal, India is one of such sheep breed which is well adapted to harsh climate and serves as an alternative source of income. Worm infestations greatly affect the performance of garole sheep which in-turn influence the economy. Thus, the present study was carried out to indentify the worm infestation of sheep, so that a parasitological map can be developed in and around Sundarban district for garole sheep. Five blocks namely Joynagar-I, Joynagar-II, Mathurapur-I, Mathurapur-II and Pathar Pratima of South 24-Parganas, West Bengal were selected purposively for the present study. Sixty faecal samples from each block were collected in a moisture tight container and were fixed by mixing it with 10% formalin for the present study. Faecal samples were examined for the presence of parasitic eggs through sedimentation by Gravitation method and Floatation by Levitation method by Willi's technique. It was found that 87.33% faecal samples were positive for worm infestations. Mixed types of infestations were found among the animals. 68.66%, 51.66%, 14.00%, 10.66% and 56.33% positive cases for *Amphistome, Strongyle, Strongyloides, Trichuris* and *Coccidia* infestations respectively were documented. No faecal sample with *Fasciola* infestation was found.

Key words **:** *garole sheep, sundarban, worm infestation.*

Introduction

The world heritage delta, Sundarbans, falls under the complex-diverse-risk-prone agro-eco system. The entire South 24-Parganas district of West Bengal as a whole and especially Sundrbans is one of the most agriculturally under developed region due to poor drainage and irrigation facilities, low lying area accentuated with high salinity in the soil. The climatic condition of this coastal saline zone is hot and humid. Thus, animal husbandry holds a pivotal role being alternative source of income, stabilizing their meager revenue from the land based mono-cropped system. Sheep occupy a special niche in this husbandry practices and are important for this rural economy acting as "mortgage lifter" (Gopalkrishnan and Lal, 1985). Garole (*Ovis aries*), is one of the largest and most abundant meat breed of high prolificacy found in this area. They are reputed for multiple births and these hardy breed can thrive well in coastal agro-climatic zone of Sundarbans. They are also habituated with knee-dip grazing in water logging area and having high disease resistant property (Ghosh et al., 1999). But due lack of knowledge about scientific managemental practices and husbandry concept, the performance of this bred in poor in this locality along with fear of improper conservation of such lucrative breed which can only be possible under strict disease monitoring system emphasizing on reproductive disorders and adoption of improved sheep husbandry practices. This can be achieved by careful study of important haematological, serum bio-chemical and parasitological parameters controlling the reproductive efficiency of the sheep. The reproductive disorders mainly the anoestrus and repeat breeding in garole sheep is a multi-factorial syndrome which includes the factors like age, breed, environment, nutritional status, micro and macro-minerals present in the body fluid, parasitic load and behavioral attitude and consciousness of sheep owners. With the above objective the present experiment was conducted for three years including follow-up period.

Materials and methods

Selection of experimental site and animals

Ten Garole sheep each from twenty five villages were selected randomly. The age of the experimental animals considered were more than two years and all were maintained as the normal rearing practice adopted by the sheep owners. Total 250 animals

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were purposely divided into two groups, viz normal cyclic garole sheep (Gr-I) and reproductively disordered garole sheep (Gr-II). A survey was conducted to assess the normal characteristic features of garole sheep, adoption behaviour and communicating ability of garole sheep owners.

Methods

Laboratory changes in the level between the two groups in respect of haemoglobin, and changes in biochemical parameters like total serum protein, serum albumin, serum globulin, plasma calcium, plasma inorganic phosphorus, micro minerals like copper, zinc, iron and manganese in the normal sheep and reproductively disordered garole sheep were measured. The trace minerals were estimated as per methods described by Sandel (1950) and modified by Arneza *et al.* (1977) in Atomic Absorption Spectrophotometer. Serum calcium and magnesium level were measured by as per the method of Trudean *et al.*, 1967. Serum phosphorus levels were measured by the method of Verley, 1991. Serum total protein and albumin in each sample were determined by Biuret methods of Reinhold (1953) in a photoelectric colorimeter using a yellow green filter. Level of haemoglobin concentration was measured by the method of Verly (1991).

Determination of Parasitic Infestation

The faeces of the animal was collected in a moisture tight container and kept covered except when the sample was removed for microscopical examination. The faeces containing eggs were fixed by mixing them with sufficient quantity of 10% formalin and then these were kept in a refrigerator for few hours. The standard Sedimentation by gravitation, Sedimentation by centrifugation and floatation (Willi's Technique and Lane's Method) method were conducted for examination of the faecal samples.

Result

Table-1. Comparatives of some characteristics of garole sheep between two groups

Category	10-12 months age (kg) body wt.	Gestational characters		Post partum oestrus (days)	Age at puberty (days)
		Conception rate (%)	% of abortion		
Gr-I	11.39 ± 0.83	65.23±0.01	03.01±0.12	92.36 ± 07.29	272.85 ± 22.78
Gr-II	8.95± 0.52	18.02±0.08	12.32±0.24	113.15±21.06	289.75 ± 12.78

It is observed (Table-1) from the present study that normal cyclic animals (Gr-I) attains puberty early with more body weight compared to reproductively disordered animals (Gr-II). Normal cyclic animals are also having more conception rate and the abortion percentage is more in Gr-II. The most significant finding is that, in Gr-I post partum oestrus period in much shorter than in Gr-II which is more advantageous for profitable and sustainable farming.

Table-II. Comparison of serum trace elements between normal cyclic and reproductive disordered garole sheep

Sl.No.	Item	Normal cyclic (Mean ± SE)	Reproductive disordered (Mean ± SE)
1	Copper (µgm/ml)	0.571 ± 0.034	1.653 ± 0.065
2	Zinc (µgm/ml)	2.129 ± 0.088	0.714 ± 0.061
3	Iron (µgm/ml)	1.835 ± 0.126	2.465 ± 0.093
4	Manganese (µgm/ml)	0.099 ± 0.009	0.125 ± 0.016

In the present study, it is evident that the level of Zinc is high in Gr-II than Gr-I where as all other parameters are just reverse (Table-II).

Table-III. serum macro elements status of normal cyclic and reproductive disordered garole sheep

Sl.No.	Item	Normal cyclic (Mean ± SE)	Reproductive disordered (Mean ± SE)
1	Calcium (mg/dl)	11.464 ± 0.229	8.398 ± 0.224
2	Phosphorus (mg/dl)	6.831 ± 0.611	5.142 ± 0.411

Table-III shows macro elements status of normal cyclic and reproductive disordered garole sheep indicating higher values of both the parameters in Gr-I than Gr-II.

Table-IV. Comparison between normal cyclic and reproductive disordered garole sheep in relation to serum bio-chemical constituents

Sl.No.	Item	Normal cyclic (Mean ± SE)	Reproductive disordered (Mean ± SE)
1	Total protein (gm/dl)	6.584 ± 0.118	5.920 ± 0.185
2	Albumin (gm/dl)	2.583 ± 0.104	4.255 ± 0.184
3	Globulin (gm/dl)	4.001 ± 0.132	1.664 ± 0.239

In this experiment, total protein content was comparatively higher in fertile group than the infertile, anoestrus and repeat breeding groups of animals (Table-IV).

Table-V. Estimation of haematological parameters of blood in normal cyclic and reproductive disordered garole sheep

Sl. No.	Item	Normal (Mean ± SE)	Reproductive disordered (Mean ± SE)
1	Haemoglobin (gm %)	12.902 ± 0.437	11.379 ± 0.504

In the present findings, the significant difference of haemoglobin value between the normal cyclic and reproductive disordered garole sheep was found (Table-V).

Table-VI. Showing the worm infestation of naturally grazing garole sheep of Sundarbans

Normal cyclic sheep					Reproductive disordered sheep				
Total sample taken	No. of positive sample	Parasite	Positive sample	%	No. of sample taken	No. of positive sample	Parasite	Positive sample	%
150	123	Amphistome	48	32	50	49	Amphistome	21	42
		Strongyle. spp	24	16			*Strongyle* spp.	10	20
		Strongyloides spp.	12	8			*Strongyloides* spp.	5	10
		Trichuris spp.	9	6			*Trichuris* spp.	2	4
		Coccidia	21	14			Coccidia	8	16
		Mixed	9	6			Mixed	3	6

A total 150 number of faecal samples from normal cyclic sheep and 50 numbers from reproductive disordered garole sheep were taken to observe the parasitic infestation and it has been observed from the Table-VI that out of 150 number of faecal samples from normal cyclic garole sheep, 123 numbers were positive (82%) in different worms of which Amphistome – 32 percent, Strongyle – 16 percent, Strongyloides – 8 percent, Trichuris – 6 percent, Coccidia – 14 percent and mixed infection – 6 percent. On the other hand, 49 samples out of 50 samples were positive (98 percent) in different worms in case of reproductive disordered garole sheep. The percentage of Amphistome, Strongyle, Strongyloides, Trichuris, Coccidia and mixed infection were 42, 20, 10, 4, 16 and 6 respectively in case of reproductive disordered garole sheep.

Discussion

Trace elements play an important role in body metabolism, protein synthesis, haemopoiesis, immuno competence, maintenance of vascular and skeletal integrity, structure, function of central nervous system. The imbalance of copper or deficiency of trace elements leads to inactive ovaries and repeat breeding in dairy animals (Hidiroglou, 1979). Increased copper level had significant positive co-relation with Estradiol-17B activity and influenced the gonadal hormones and pituitary gonadotropins affecting the ovarian activity with more occurrence of reproductive disorderness mainly infertility. Besides these,

copper has significant role in maintaining the optimum fertility as copper behaves in a regular way to be used as an indicator for FSH, LH and Oestrogen activities (Desai et al., 1982). Optimum level of zinc is also essential to maintain the activity of FSH and LH and thereby facilitating normal reproductive performance. Besides these, prostaglandin binds zinc and facilitates its transport. A reduction in zinc level might interfere with prostaglandin receptor-mediated phase and consequently the luteolytic process which causes the reproductive pathology (Carlson et al., 1982). Moreover, zinc influences certain enzymatic activities such as lactate dehydrogenase, DNA and RNA polymerase, carbonic anhydrase, alkaline phosphatase etc. which might have certain role in reproductive performance. The low level of iron could possibly result in improper tissue oxygenation to the uterus resulting in impaired nutrition to the uterus for the conceptus causing death of embryo. A deficiency of iron might also interfere with enzymatic reaction on the release of luteinizing hormone which is highly essential for the maintenance of pregnancy in cows reported by Reddy and Reddy (1988) but Maynard and Loosli (1969) suggested that iron was of little importance in reproduction as compared with copper and zinc. In the present study there is non-significant difference in manganese level between the two groups of garole sheep were found which was also supported by Das et al. (2002).

The low level of serum calcium level in the reproductive disordered sheep might be due to failure of endocrine system to mobilize the body calcium leading to reproductive failure. Though, deficiency of calcium does not have direct bearing on reproduction (Morrow, 1977) but calcium has a definite role in sensitizing the tubular genitalia for the action of hormones which is required to enhance the reproductive performance of animals. The significant ($p<0.05$) difference between the two groups of sheep were found in the present study which was corroborative with the findings of Shrivastava and Kadu (1995), Dutta et al. (2001), Das et al. (2002), Chandrahar et al. (2003) and Shah et al. (2004). It is well defined that the most prevalent deficiency affecting reproduction in animal appeared to be due to the lack of phosphorus because inorganic phosphorus has been reported to be essential for energy transformation at cellular level and is associated with the maintenance of sperm glycolysis and respiration which causes the higher number of services per conception.

It is well known that protein is very much important for the release of amino acids required for synthesis of protein hormones, gonadotrophins responsible for augmentation of reproduction of animals. Low protein level therefore will put the animal in negative nitrogen balance and continued status of such nitrogen deficiency obviously will affect on reproductive ability. Kavani et al. (1987), Dutta et al. (1988) and Yadav et al. (2004) observed that there was significant decrease in total protein level in anoestrus crow as compared to normal cyclic animals which was also corroborative with the present findings.

In the present findings, the significant difference of haemoglobin value between the normal cyclic and reproductive disordered garole sheep might be due to more parasitic load which was found in the reproductive disordered garole sheep in the present observation. This observation is in agreement of the findings of Garcia et al. (1994) who reported significant low level of haemoglobin and Packed Cell Volume (PCV) were due to higher faecal count of nematodes.

From the table presenting the different worm infestations (Table-VI), it can be revealed that in case of reproductive disordered garole sheep the percentage of worm load is much more than that of the normal cyclic sheep where Amphistome followed by Strongyle and Coccidia were prevalent for both the groups. As gastrointestinal parasites are one of the most important factors affecting the productive and reproductive traits of domestic animals especially in sheep and goats, the more worm load associated with low level of certain micro elements have direct impact on reproductive ability in garole sheep. High worm load resulting to intermittent diarrhoea causes the loss of certain important minerals of body hindering the animal to come in oestrus cycle in proper time as well as to maintain the pregnancy. The present findings are corroborative with the findings of Makhdoomi et al. (1995) who recorded that the Trichostrongylus and Strongyloides spp. were predominant in temperate Himalayan region but in the present study Amphistome showed the highest level of predominancy. The present study was also corroborative with the findings of Purohit (2002) who observed Paramphistomiasis is one of the most important worm infestations in garole sheep of West Bengal, India. The present study was also inconsistent with the findings of Maichamo et al. (2004). He reported the overall prevalence of GI nematodes in sheep (80 percent) and goat (82 percent) along with the coccidial oocysts occurred in sheep (44 percent) and in goats (45 percent). The coccidial oocysts observed in the normal cyclic and reproductive disordered garole sheep as 14 percent and 16 percent respectively which were lower than that of the observation of Maichomo et al. (2004). This variation might be due to the region, season and breed etc. However, the present study was in full corroborative with the observation of Choudhuri (2004) where major species of GI parasites of sheep were Paramphistomes. Trichostrongylus, Strongyloides and Trichuries spp. Nwosu et al. (2007) observed that nematode infestation in small ruminants of Nigeria were Strongyles sp. (22.5 percent), Trichuries sp. (5.9 percent) and Strongyloides sp. (4.9 percent) which was also in agreement with the findings of present study.

Conclusion

The study helps to understand the different etiological factors which directly or indirectly influence the reproductive performances of garole sheep in Sundarban. The study also lend a hand to institute the off-putting feature for reproductive disappointment and hence steer to correct those oddities ultimately boarding to better recital vis-à-vis higher fiscal revisit.

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Structural peculiarities of the eggs of nematodes-their significance in taxonomy

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Abstract : The paper analyses various peculiarities of the nematode eggs and elaborates their significance in taxonomy. The structure of the egg-shall of nematodes varies from species to species within the genus and among the genera within a family/sub family. Photomicrography of the egge-shell provides a first hand information about the status of the specimen within a heirarchial system of classification. The mode of attachment of eggs, the shape and the mode of egg laying also varies from one species to another.

Introduction

Nematodes are very often unsegmented, spindle-shaped roundworms with bilateral symmetry. The egg shell structure also provides first hand information while identifying a species. Precise identification of the cause of an infection is an essential for treatment in case of animal parasitic nematodes and plays a major role in our understanding of the epidemiology of the disease and the implementation of control measures. In such case, the egg shell provides one of the important characters. The egg-shall structure is widely used not only by classical taxonomist but also by other human/animal related fields comprising both basic and applied biological sciences.

Both free living and parasitic species take this form and the vast majority has a highly conservative developmental sequence. In addition to those species which are causative agents of human diseases, a number of other nematodes are of significance either because they infect agricultural animals/plants or because they have been utilized extensively as laboratory animals. Among the developmental stages, the eggs of nematodes are important as without them the life cycle will not be completed. The shell forms the general contours of the egg. The shell is ideally suited as a transmission stage and by virtue of the physico-chemical properties of its resistant shell, is able to withstand the rigors of the external environment for considerable periods of time. The character of individual helminth egg-shell is sufficiently specific to warrant a correct diagnosis. Some of the major human parasitic nematodes that are identified by their peculiar egg-shell structure are *Trichuris trichiura*, *Ancylostoma duodenale*, *Enterobius vermicularis*, *Ascaris lumbricoides*, etc. The eggs of parasitic nematodes are variously modified to adapt them to their complicated life cycles. Some of the major specializations are presence of **1.** Byssi (Examples: *Mermis subnigrescens*), **2**. Filaments (Examples: *Spinitectus*, *Metabronema* and *Citellina marmotae*,Tetrameres, *Pseudonymus*, *Cameronia*, *Binema*, *Chitwoodiella*, etc. **3**. Operculate shells (e.g. *Cyathostoma ,Pharyngodon spinicola*, *Oxyuris equi*, *Dermatoxyx veligera*, *Heterakis papillosa*, *Metabronema magnum*, *Trichuris ovis*, etc. **4**. Mammillation of shell (e.gs *Ascaris lumbricoides*, *Metastrongylus salmi*, *Hedruris siredonis*, *Physaloptera ortleppi ortleppi* **5**. Protuberances/spines like outgrowths (*e.g. Diploscaptor coronata*, *Rhabditis filiformis*, some cephalobids, *Gryllophila skrjabini*, etc.). Chitwood & Chitwood (1937) points out that species with filaments on the eggs are basically associated with an aquatic habitat and that the filaments may function by entangling the egg in the vegetation thus preventing it from settling into the debris of the substratum which would reduce its chances of survival. Van Beneden (1871) was the first to observe polar filaments in species of ***Ascarophis*** and stated that the eggs are distinguished from those of other nematodes by the presence of two filaments which garnish one of the poles. Polar filaments have also been observed In the genera *Spinitectus*, *Metabronema* and *Citellina marmotae*

Materials and methods

Nematodes collected from the gut of *Periplaneta Americana*, *Gryllotalpa africana* and *Hydrophilus triangularis* were fixed in warm TAF (Triethanol amine formalin), dehydrated using glycerine alcohol with calcium chloride(anhydrous) as medium

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for absorbing water vapour released from the nematodes. By using very fine needles, the nematodes were teased in normal saline in order to separate the eggs contained within the uterus. The eggs were mounted on glass slides using anhydrous glycerine. Glass wool of suitable thickness was used while mounting the eggs in order to avoid flattening. The prepared slides containing eggs were photomicrographed using digital camera.

Results and discussion

The eggs of the species of *Binema* and *Mirzaiella* are encapsulated (Figs.A,B,C,D and N) The number of eggs within the capsule varies from species to species (Shah & Rizvi,2004; Shah,2008). The eggs of *Pseudonymus basiri* and *Zonothrix alata* (Shah &Rizvi,2004) have filaments coiled around the eggshell (Figs. M,P). The eggs shell of *Protrellus shamimi* (Shah & Rizvi,2005) contains a crest at one pole(Fig.O). The eggs of *Chitwoodiella longicardia* Shah,2008 are attached to one another in strings (Fig.L) by polar filaments. In *Cameronia triovata* (Fig.I), three eggs are fused with ridges and furrows to one another along their flattened surfaces, the attachment between eggs is brought about by alternate arrangement of ridges and furrows (Shah,2007). In case of *Cameronia biovata* Basir,1948 (Fig.K) two eggs fused with ridges and furrows to one another along their flattened surfaces. The eggs of Camerinia *manipurensis* Shah,2007(Fig.J) are simply attached to one another forming a chain (Shah,2007). The eggs *Thelastoma periplaneticola* Leibersperger, 1960(Fig.H) are oval in shape while the eggs of *Leidynema appendiculatum* (Leidy, 1850) Chitwood, 1932 (Fig.F) are elliptical in shape and laind singly (Shah,2007). The eggs of *Gryllophila skrjabini* (Sergiev,1923) Basir, 1956 (Fig.E) possess spine-like outgrowths, deposited in strings held together by uterine secretions(Shah,2007). Lastly, the eggs of *hammerschmidtiella diesingi* (Hammerschmidt, 1838) Chitwood, 1932 (Fig.G) are elongate and ellipsoidal in shah (Shah,2007). From the above observations it has been observed that , the mode of attachment of eggs, the presence and absence of spine on eggs shell, the presence and absence of filaments, presence of cuticular crests, etc. provide a very significant information while identifying a species. The photomicrographs these shows actual figure of the egg shell as compared to line drawings.

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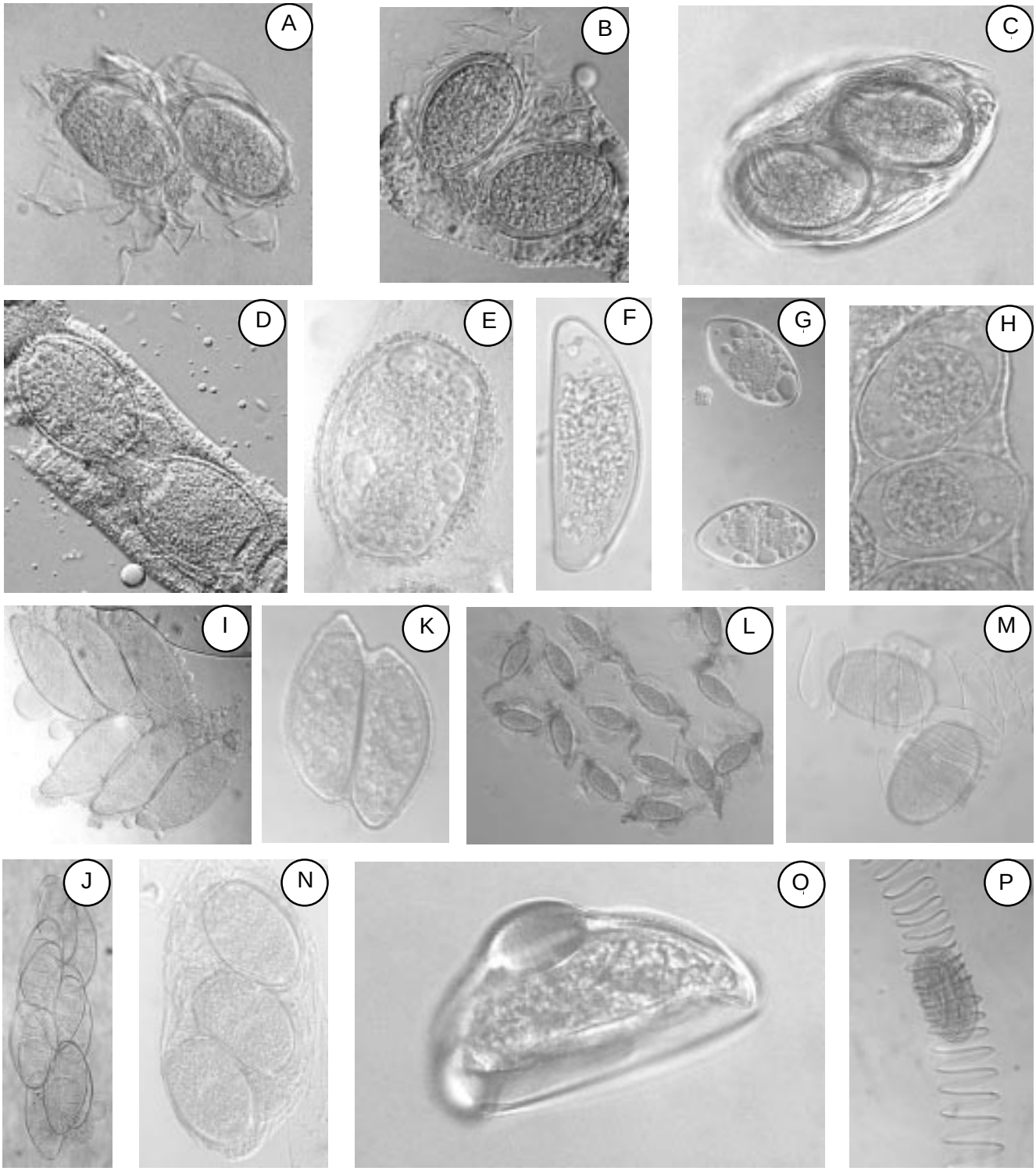

Plate-1. A-***Binema ornata*** Travassos, 1925; B-***Binema korsakowi*** (Sergiev,1923) Basir,1956; C-***Binema mirzaia*** (Basir,1942) Basir, 1956; D-***Binema anulinervus*** Shah & Rizvi, 2004; E-***Gryllophila skrjabini*** Basir, 1942; F-***Leidynema appendiculatum*** Schwenk (in Travassos,1929); G-***Hammerschmidtiella diesingi*** Chitwood,1932; H-***Thelatoma periplaneticola;*****I-*Cameronia triovata*** Shah & Rizvi, 2004;J-***Cameronia manipurensis*** Shah & Rizvi,2004; K-***Cameronia biovata*** Basir 1948 ;L-***Chitwoodiella longicardia*** **Shah,2008**; M-***Zonothrix alata*** Shah & Rizvi, 2004;N-***Mirzaiella asiatica*** Basir, 1942; O-***Protrellus shamimi*** Shah *et al*., 2004;P-***Pseudonymus basiri*** Shah & Rizvi, 2004

On a new species, *Oxysomatium teraensis* sp.nov. (Nematoda : Oxyuridae) from *Hyla annectens*, from Manipur, India

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Abstract : A new nematode species, *Oxysomatium teraensis* sp.nov. (Oxyuridae) is described from *Hyla annectens* from Manipur, India. The new species is characterized by the presence of body cuticle marked by coarse, unevenly spaced striations ; males with numerous and variable caudal papillae, absence of gubernaculum and comparatively longer spicules (longer than O. *hylae*, the most closely related species).

Keywords **:** *Hyla annectens, Manipur, nematode, new species, Oxysomatium teraensis.*

Introduction

Manipur lies in the Indo- Burma region, which is one of the 34 Global Hotspots (Myers et al. 2000) and Manipur alone harbours 30 species of amphibia (Bhanu,Y.2000). In the course of investigative study of nematode infestation of this rich amphibian fauna, a new nematode belonging to the genus *Oxysomatium* Railliet et Henry, 1913 was recovered from the rectum of *Hyla annectens*. The genus *Oxysomatium* was erected by Railliet and Henry, 1913 for its type species *O. brevicaudatum* (Schneider,1866).

During the past decades, a few species of *Oxysomatium* have been reported from different parts of India : *O. macintoshii* (Stewart, 1924) Karve, 1927 ; *O.srinagarensis* Fotedar, 1960 ; *O.annurae* Biswas and Chakravarty,1963 ; *O. stomatici* Biswas and Chakravarty, 1963 ; *O.manipurensis* Gambhir and Tarnita,2005 ; *O. hylae* Gambhir and Tarnita, 2005 and *O. striatus* (Tarnita et al.Personal communications). A systematic study on the present species collected from *Hyla annectens* appeared to differ from all known species of the genus and is described herein as belonging to a new species, giving the name *Oxysomatium teraensis* sp. nov.

Materials and methods

Amphibian host, *Hyla annectens* were collected from Tera locality, in Imphal West district of Manipur. They were then brought to laboratory and examined. The nematodes were recovered from the rectum of *Hyla annectens*. The parasites recovered were fixed in warm A.F.A. (Alcohol Formaline Acetate), preserved in 10% Glycerine alcohol (Seinhorst's (1959) rapid glycerine method for dehydration process) and permanent slides were prepared in dehydrated glycerine. Nematodes were studied, photographs were taken with the help of Olympus digital camera ; camera lucida drawing was made and measurements were taken with ocular micrometer. All measurements are in mm unless otherwise indicated.

Results

Oxysomatium teraensis sp. nov. (Fig.1)

Descriptions : Body cylindrical, stout and attenuated at both the ends. Mouth with 3 inconspicuous lips leading into a small buccopharyngeal region followed by a long tubular oesophagus terminating in a posterior valvated end-bulb. Cuticle is marked by unevenly spaced striations. Excretory pore anterior to bulb. 2-3 pairs of rectal gland present around the rectum.

Male : The caudal papillae are numerous and variable.The tail bears 9 pairs of post-anal papillae and 10-15 pairs of pre-anal papillae. Of post-anal papillae, 6 are lateral papillae and 3 are ventral papillae. The post-anal lateral papillae are very small in size of which two pairs are present near the tail tip and the remaining are distributed around middle of tail and at the level of the cloaca. Of the pre-anal papillae, 5-8 are ventral papillae and 5-7 are lateral papillae. The papillae arrangement of pre-anal papillae is irregular. The first ventral papillae is closely situated to the anal opening and the 2^{nd}, 3^{rd} and 4^{th} ventral papillae

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are situated very close to each other. The distance between the 5th, 6th and 7th ventral papillae are almost equal. The 3rd lateral papillae always lie at mid-point of the 4th and 5th ventral papillae. The rest of the ventral papillae and lateral papillae are irregularly spaced and varies. Spicules paired, pointed at distal end and nearly of equal length. Gubernaculum is absent.

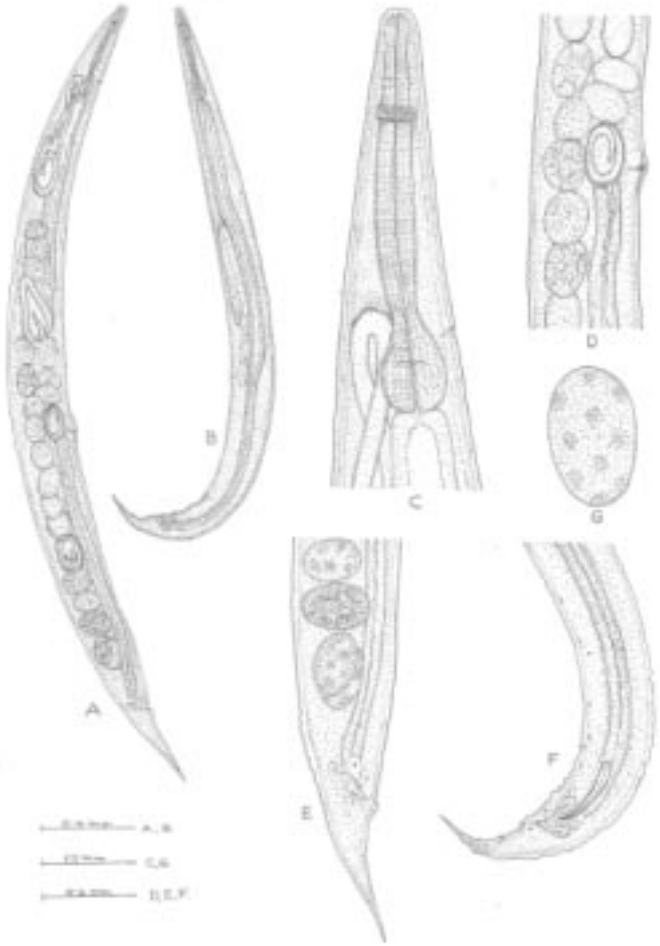

Fig.1 : *Oxysomatium teraensis* sp.nov. A, Entire Female. B, Entire Male. C, Anterior End of Female.D,Vulval Region.E, Posterior End of Female. F, Posterior End of Male.G, Egg.

Female : Reproductive system amphidelphic. Vulva post equatorial with slightly protruding vulva. Uterus with eggs in different developing stages and presence of juveniles in uterus. Tail is conical and pointed.

Taxonomic summary

Genus : *Oxysomatium* Railliet et Henry, 1913.

Species : *Oxysomatium teraensis* sp. nov.

Type host : *Hyla annectens* Jerdon.

Site of infection : Rectum.

Type locality : Tera, Manipur, India.Lat.24º30´N – 25º00´N ; Long. 93º45´E – 94º15´E.

Holotype : $Hy_2 R_1$ ♂ ; Deposited in the Collection of Parasitology, Department of Life Sciences, Manipur University, Canchipur, India.

Paratypes : Hy $_2R_{(2,3,4)}$ ♂, Hy $_2R_{(1,2,3)}$ ♀; 6 in Nos.

Date of collection : 23 June, 2009.

Etymology : The specific name of the nematode refers to Tera, the locality where the host of this parasite was collected.

Discussion

Oxysomatium teraensis sp.nov. is characterized by male having 9 post-anal papillae and 10-15 pre-anal papillae at variable positions, absence of gubernaculum,comparatively longer spicule, cuticular striations which are more prominent at cloacal region and female having long conical tail, post-equatorial vulva and presence of juveniles in maternal uterus. The cuticular striations are more prominent around vulval region.

Oxysomatium teraensis sp.nov. is morphologically most similar to *O.hylae* in the structure of tail, shape and size of oesophagus, position of nerve ring from anterior end and position of excretory pore from the anterior end. Gubernaculum is absent in both species. However, the present species differs from *O.hylae* in having coarse unevenly spaced cuticular striations compared to faint or indistinct cuticular striations in *O.hylae*; comparatively larger body size and anal body diameter. A pair of short cuticular extension protruding forwards at the anterior tip in female of *O.hylae* is absent in *O.teraensis* sp.nov. Another striking difference is the number and arrangement of the caudal papillae. *O.hylae* possess 9 pairs of caudal papillae while *O.teraensis* sp.nov. possess 19-24 pairs of caudal papillae. *O.teraensis* sp.nov. have comparatively longer spicules (0.16-0.17) than *O.hylae* (0.13-0.14). Compared to *O.hylae*, the present species have numerous and smaller sized eggs and juveniles are present in the maternal uterus which is not reported in *O.hylae*.Comparative chart is given in Table I.

Table I. Comparision of *Oxysomatium teraensis* sp.nov. with *O. hylae* Gambhir & Tarnita, 2005

Characters	*Oxysomatium teraensis* sp.nov.		*O. hylae* Gambhir & Tarnita, 2005	
	♂	♀	♂	♀
1. Body length	2.52 – 2.7	3.29 – 3.31	1.41 – 2.22	2.73 – 2.93
2. Body width	0.15 – 0.16	0.21 – 0.24	0.07 – 0.10	0.12 – 0.14
3. Total length of Oesophagus	0.40 – 0.42	0.41 – 0.43	0.35 – 0.42	0.43 – 0.47
4. Muscular oesophagus (Length & width)	0.33 – 0.35 x 0.02 – 0.03	0.35 – 0.37 x 0.03- 0.05	0.29 – 0.35	0.36 – 0.40
5. Glandular oesophagus or end bulb (Length & width)	0.06 – 0.07 x 0.05 – 0.06	0.06 – 0.08 x 0.05 – 0.06	0.05 – 0.07 x 0.05	0.07 – 0.08 x 0.06 – 0.07
6. Nerve ring	0.15 – 0.17	0.13 – 0.16	0.13 – 0.16	0.14 – 0.20
7. Excretory pore from the anterior end	0.31 – 0.39	0.32 – 0.33	0.25 – 0.35	0.32 – 0.36
8. Anal opening or cloacal aperture from the anterior end	2.32 – 2.55	3.0 – 3.01	1.24 - 1.99	2.40 – 2.70
9. Tail length	0.19 – 0.22	0.26 – 0.29	0.12 – 0.23	0.28 – 0.33
10. Spicules length	0.16 – 0.17		0.13 – 0.14	
11. Vulva from anterior end		1.76 – 1.81		1.57 – 1.65
12. Eggs		0.08 – 0.15 x 0.05 – 0.09		0.16 - 0.21 x 0.10
13. Anal Body Diameter (ABD)	0.08 – 0.09	0.08 – 0.09	0.05 – 0.06	0.05 – 0.06
14. Caudal papillae	Postanal – 9 pairs Preanal – 10 to 15 pairs		5 Postanal 4 Preanal	
15. Cuticularisation	Anterior –1.95 – 3.9 µm Middle – 3.9- 5.85 µm Caudal – 7.8- 27.3 µm	Anterior – 3.9-7.8 µm Middle – 7.8 µm Vulva – 19.5-27.3 µm Caudal – 7.8 µm	Cuticle with faint or indistinct striations	

The new species resembles *O.variabilis* Harwood (1930) in the presence of coarse cuticular striations and in the patterns of caudal papillae. But it differs from *O.variabilis* in the absence of gubernaculum, in number of caudal papillae, in having comparatively shorter spicules and in the absence of tail spike which is characteristic of *O.variabilis. O.teraensis sp.nov.* also have relatively smaller size oesophagus and differs also in nerve ring position. *O.teraensis* sp. nov. differs from some similar species like *O.tibetanum* Baylis (1927) ; *O.macintoshii* (Stewart, 1924) Karve, 1927 ; *O. ranae* Walton (1931)in the absence of gubernaculum, body measurements, size of spicules and in the number and arrangement of caudal papillae.

Fotedar (1960) described a new species, *O.srinagarensis* from rectum of *Bufo viridis* from Srinagar, Kashmir. *O.teraensis* sp. nov. differs from *O.srinagarensis* in having coarse cuticular striations (smooth in *O.srinagarensis*), in the absence of lateral alae, gubernaculum and body papillae which are present in *O.srinagarensis*. The position of vulva is post-equatorial in the present species while in *O.srinagarensis* the vulva is nearly median in position. The present species also differs from *O.annurae* and *O.stomatici* found in the Indian sub-continent in body measurements, cuticular striations, size of spicules and in the number and arrangement of caudal papillae.

Geographically *O.teraensis* sp.nov. is close to *O. manipurensis* and *O. striatus. O.teraensis* sp.nov. differs from *O. striatus* in the body width, greater oesophagus length and smaller end-bulb, position of excretory pore (which lies behind the end-bulb in *O. striatus*), shape of the tail and in having only cuticular striations (*O.striatus* have striations all over the body except the anterior region). *O.teraensis* sp.nov. differs from *O.manipurensis* in nerve ring position, in absence of post- anal membranous lip in the female and absence of warts which are characteristic of *O. manipurensis*. Cuticular striations are absent in *O. manipurensis* and it have longer tail, lesser number of caudal papillae (10 pairs) and their mode of arrangement.

On the basis of the above mentioned differences and comparison with the earlier reported ones, the species is considered to be a new species to which the name *Oxysomatium teraensis* is given. We anticipate that the rich amphibian fauna of Manipur and other regions in Indo-Burma Hotspot harbours numerous species of *Oxysomatium* that still await their discovery.

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On the nematofauna inhabiting mosses available at Indian Botanic Garden, Howrah, West Bengal, India and description of *Thornenema thornei* n.sp.

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Abstract : The study of moss associated nematofauna is the first of its kind in India except one report of Gadea (1968). The collected nematofauna constitute 18 species belonging to 14 genera in 7 orders. Among this, omnivores were the most common and dominant trophic group and Dorylaimida was the most dominant order in the nematocoenosis of mosses at Botanical Garden, Kolkata. *Mesodorylaimus chamoliensis* Ahmad, 1995 and *Microdorylaimus* n.sp. were the most common and prevalent species in mosses of this area, while *Mesodorylaimus chamoliensis* Ahmad, 1995 was the most prominent species. But *Aporcelaimellus* n.sp 2 was the most dominant and important species in the nematode community of mosses of Botanic Garden, Howrah, West Bengal. A new species *Thornenema thornei* n.sp. is described here.

Key words **:** *nematofauna, Botanic garden, nematocoenosis of mosses, Mesodorylaimus, Thornenema*

Inroduction

Mosses harbour a characteristics fauna of nematodes which are able to withstand periodic desiccation and extremes of temperature (Menzel, 1920; Burkhalter, 1928). Throughout the world numbers of studies have been done on nematode fauna of mosses. (Steiner, 1916; Gadea, 1970, 1972; Zullini, 1971, 1975; Lazarova *et al.*, 2000; Barbuto *et al.*, 2006), but practically nothing is known in this field in India except one papers by Gadea (1968) on this aspect from East Ghates (Orissa) and another by Duggal and Koul (1985) on plant parasitic nematodes associated with bryophytes and pterydophytes in north-west India.

The present work is the humble effort to analyze the distribution of nematodes from mosses in Indian Botanic Garden at Shibpur, West Bengal. This paper will be the first contribution to the knowledge of moss inhabiting nematofauna from West Bengal and also that from Botanic Garden.

Materials and Methods

Nematodes were collected from mosses found in Indian Botanical Garden, Howrah ($22^{o}33'$N, $80^{o}22'$E) lying on the west bank of river Hooghli and covers 109 hectares of land. The species of mosses collected were of genera *Bryum, Fissidens, Drepanocladus, Thuidium*.

The moss samples collected from soil, walls and trees during June and September 2007 were immersed in water for 24 hours and then nematodes were extracted with the help of modified Baermann funnel technique (Christie and Perry, 1951). Nematodes were fixed with hot F.A solution (Formalin: Acetic acid, 4:1) (Baqri, 1990), processed to an anhydrous glycerine (Seinhorst,1959), mounted on slides and identified to species level using a high magnification microscope following schemes of Andrassy (1976,1984) for orders Monhysterida, Araeolaimida, Chromadorida, Rhabditida, Tylenchida, Enoplida and Jairajpuri & Khan (1982) for Mononchida and Jairajpuri & Ahmad (1992) for Dorylaimida. The moss dwelling nematode genera were assigned to trophic groups according to classification of Yeates *et al.* (1993). The various trophic groups considered were predators, omnivores, bacteriovores, hyphal feeders and unicellular eukaryote feeders. All the ecological parameters were calculated by the methods given by Norton (1978) and Boag (1993).

Results

Vertical distribution of nematodes in mosses

The average pH of the collected moss samples was found to be 5.7 and vertical distributions of nematodes in these mosses were studied. Out of 26 moss samples, collected from different substrates and different heights from ground in Botanic Garden,

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Table I. Distribution of nematodes in mosses collected from different sites in Indian Botanic Garden, Shibpur, Howrah

Sample No.	*Site of collection*	*Abundance/10gm*
1	Bark of *Casuarina* at 5.5ft from ground	0
2	Bark of *Polyalthia* at 3.0ft from ground	5
3	Bark of *Swietenia* at 4.5ft from ground	0
4	Bark of *Samanea* at 3ft from ground	0
5	Bark of *Swietenia* at 4.5ft from ground	100
6	Bark of Palm tree at 4.0ft from ground	0
7	Bark of *Swietenia* at 1.0ft from ground	0
8	Bark of *Swietenia* at 0.75ft from ground	474
9	Bark of *Polyalthia* at 1.5ft from ground	42
10	Bark of *Tabebuia* at 4.5ft from ground	0
11	Rock at ground	0
12	Soil around Cactus plant	18
13.	Bark of *Tamarindus* at 2.5ft from ground	59
14	Bark of *Samanea* at 4.5ft from ground	45
15	Bark of Palm tree at 1.5ft from ground	39
16	Bark of *Tabebuia* at 3.5ft from ground	20
17	Bark of *Casuarina* at 5.0ft from ground	10
18	Bark of *Swietenia* at 5.5ft from ground	39
19	Brick at ground	91
20	Bark of Palm tree at 1.0ft from ground	82
21	Bark of *Dalbergia* at 2.5ft from ground	128
22	Soil around Cactus plant	13
23	Bark of Palm tree at 1.0ft from ground	48
24	Bark of *Casuarina* at 5.5ft from ground	63
25	Bark of *Adenanthera* at 2.5ft from ground	0
26	Bark of *Eriophyton* at 5.0ft from ground	5

18 samples i.e., 69.23 % of the samples contain nematodes. The total number of nematodes varied widely among the samples (0-474 individuals/10g of moss). There were atleast one and atmost four nematode species per sample. But no definite relation was found between the vertical height of site of moss sampling and abundance of nematodes in these sites. (Table I.)

Percentage of nematode order

A total of seven orders of nematodes have been encountered in the present study, of which Dorylaimida was the most dominant order (90.04%) among the moss inhabiting nematodes of this area followed by Enoplida (3.46%), while Mononchida was least frequent order (0.3 %) (Table II). A total of 18 species belonging to 14 genera in 7 orders were identified from different moss samples of this area. These included 11 species belonging to 7 genera in order Dorylaimida, 2 species belonging to 2 genera in order Araeolaimida and 1 species from each of order Enoplida, Mononchida, Rhabditida, Chromadorida and Monhysterida.

Table II. Percentage of different orders of nematodes inhabiting mosses of Botanical Garden, Kolkata, West Bengal

Serial Number	*Different orders of nematodes*	*Percentage (%)*
1.	Dorylaimida	90.04
2.	Araeolaimida	3.00
3.	Chromadorida	0.60
4.	Rhabditida	0.60
5.	Monhysterida	2.00
6.	Mononchida	0.30
7.	Enoplida	3.46

Table III. Community analysis of moss dwelling nematodes of Indian Botanic Garden, Howrah, West Bengal.(N- Frequency, P- Prevalence, I- Mean Intensity, PV- Prominence value, TG-Total biomass, FT- Feeding type, IV-Importance value)

Sl. No	*Nematode Taxa*	*N*	*P*	*I*	*PV*	*TG µgm*	*IV*	*FT*
	Dorylaimida							
1.	*Aporcelaimellus* n. sp1	1	3.85	12.00	23.55	3.75	9.27	o
2.	*Aporcelaimellus* n.sp2	4	15.38	51.75	202.95	30.86	56.56	o
3.	*Aporcelaimellus chauhani* Baqri et Khera, 1975	1	3.85	4.00	7.85	0.99	4.49	o
4.	*Mesodorylaimus chamoliensis* Ahmad, 1995	6	23.08	57.67	277.06	9.72	37.76	o
5.	*Mesodorylaimus* n.sp	1	3.85	19.00	37.28	2.26	8.83	o
6.	*Thornenema* n.sp.	3	11.54	27.67	93.9	2.28	15.60	o
7.	*Tylencholaimus* n.sp	1	3.85	72.00	141.27	1.53	7.02	hf
8.	*Microdorylaimus* n.sp.	6	23.08	21.67	132.93	5.98	26.54	o
9.	*Eudorylaimus* n.sp.	1	3.85	75.00	147.16	3.27	20.58	o
10.	*Eudorylaimus brevis* (Altherr, 1952) Andrassy,1959	4	15.38	34.00	133.34	17.39	37.26	o
11.	*Thornia juvenilis* (De Coninck,1935) Meyl, 1954	1	3.85	14.00	27.47	0.12	5.35	o
	Enoplida							
12.	*Alaimus acutus* Thorne, 1939	3	11.54	22.33	75.86	2.01	14.28	b
	Araeolaimida							
13.	*Plectus parietinus* Bastian, 1865	1	3.85	14.00	27.47	1.68	7.20	b
14.	*Cylindrolaimus communis* De Man, 1880	1	3.85	43.00	42.57	1.05	11.91	b
	Mononchida							
15.	*Mylonchulus lacustris* (Cobb in Cobb, 1915), Cobb, 1917	2	7.69	2.50	6.93	0.53	6.23	p
	Rhabditida							
16.	*Eucephalobus oxyroides* (De Man, 1876), Steiner, 1936	1	3.85	7.00	13.73	0.12	4.03	b
	Chromadorida							
17.	*Achromadora ruricola (*De Man, 1880) Micoletzky,1925	1	3.85	20.00	39.24	0.71	7.18	u
	Monhysterida							
18.	*Geomonhystera villosa* (BÜTSCHLI 1873) Andrassy, 1981	1	3.85	33.00	64.75	0.27	9.11	b

Feeding Types : bacterial feeders (b), omnivores (o), predators (p), unicellular eukaryote feeders (u), hyphal feeders (hf).

Community analysis of moss dwelling nematodes of Indian Botanic Garden

Mesodorylaimus chamoliensis Ahmad, 1995 and *Microdorylaimus* n.sp. were the most common and prevalent species in mosses of Botanic Garden, Howrah. *Eudorylaimus* n. sp. showed the highest mean intensity followed by *Tylencholaimus* n.sp., while *Mylonchulus lacustris* (Cobb in Cobb, 1915) Cobb, 1917 showed least mean intensity. *Mesodorylaimus chamoliensis* Ahmad, 1995 was the most prominent species among the mosses of this area followed by *Aporcelaimellus* n.sp 2, while *Mylonchulus lacustris* (Cobb in Cobb, 1915) Cobb, 1917 was least prominent. *Aporcelaimellus* n.sp 2 was found to be the most dominant species among the moss associated nematodes of this area followed by *Eudorylaimus brevis* (Altherr, 1952) Andrassy 1959. *Aporcelaimellus* n.sp 2 had the highest importance value while *Eucephalobus oxyroides* (de Man, 1876) Steiner, 1936 had lowest importance value.(Table III).

Trophic relation of the nematodes

Among the 18 species of nematodes encountered from the moss samples of this area, 10 species were omnivorous, 5 species were bacteriovorous, 1 species was predator, 1 species was hyphal feeder and 1 species was unicellular eukaryote feeder. Omnivores were found to be the most dominant and frequent group in the nematocoenosis of mosses at Indian Botanic Garden, Howrah, followed by bacterial feeders while predators were least frequent group. (Table IV). All the omnivores encountered in the present study belong to Order Dorylaimida and Superfamily Dorylaimoidea.

Table IV. Percentage of trophic groups of nematodes inhabiting mosses of Botanical Garden, Kolkata, West Bengal

Serial Number	*Feeding group*	*Percentage (%)*
1.	Omnivores	89.80
2.	Bacterial feeders	6.00
3.	Unicellular eukaryote feeders	0.50
4.	Hyphal feeders	3.50
5.	Predators	0.20

Description of *Thornenema thornei* n. sp. (Fig.1)

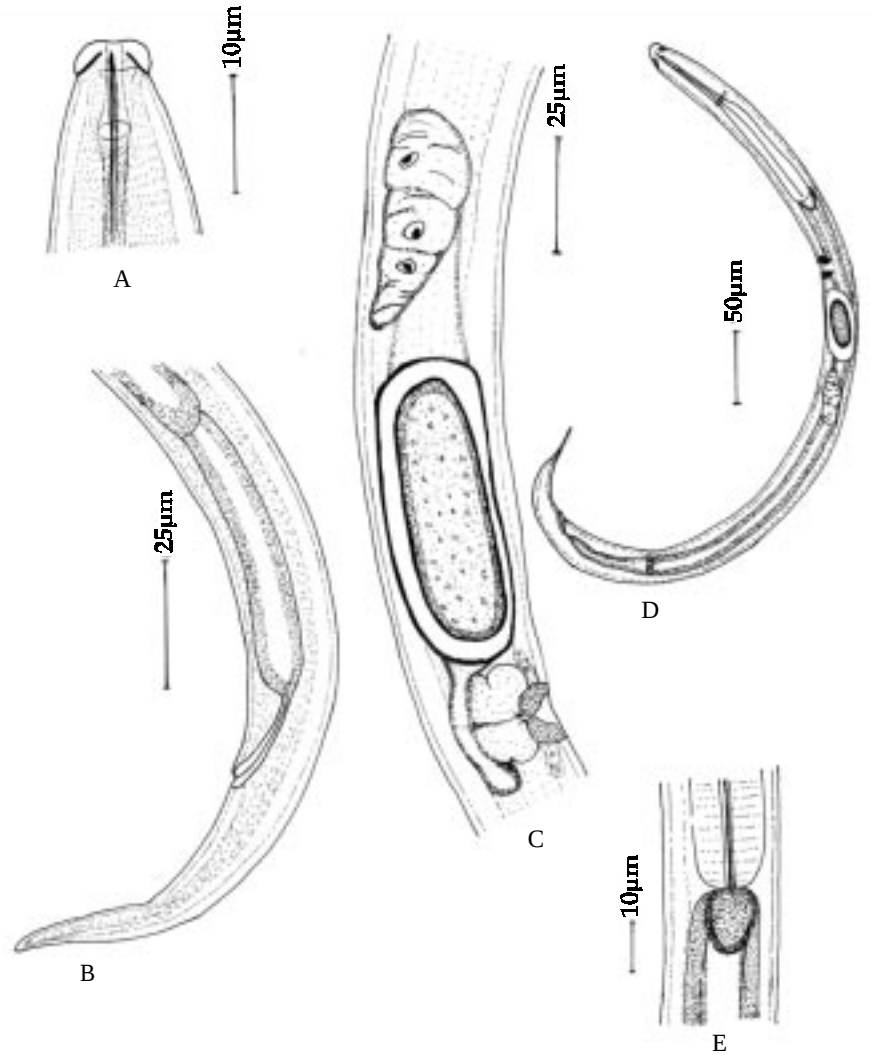

Figure 1. *Thornenema thornei* n. sp. (Camera lucida drawing) Female. A- Head region, B- Tail region, C- Gonad, D- Entire body, E- Oesophago-intestinal junction

Order- Dorylaimida Pearse, 1942

Suborder- Dorylaimina Pearse, 1936

Superfamily- Dorylaimoidea De Man, 1876

Family- Dorylaimidae De Man, 1876

Subfamily- Thornenematinae Siddiqi, 1969

Genus- *Thornenema* Andrassy, 1959

Species- *Thornenema thornei* n.sp.

Measurements

Female (paratypes; n = 2): L = 0.65-0.66 (0.65) mm; a = 23.6-26.9 (25.2); b = 4.0-4.3 (4.2); c = 10.3-10.8 (10.5); $c^{/}$ = 3.8-4.0 (3.9); V = 32.6-34.1 (33.3); G_2 = 26.7-29.8 (28.2); odontostyle = 10.0-12.5 (11.3) µm; odontophore = 18.75 µm; oesophagus = 150.0-165.0 (157.5) µm; prerectum = 40.0-52.5 (46.3) µm; rectum = 20.0-24.0 (22.0) µm; tail = 60.0-64.0 (62.0) µm; ABD = 16.0 µm.

Female (holotype): L = 0.66 mm; a = 23.6; b = 4.4; c =11.0; $c^{/}$ = 3.8; V = 31.8; G_2 =30.9; odontostyle = 10.0 µm; odontophore = 18.8 µm; oesophagus = 165.0µm; prerectum = 40.0 µm; rectum = 20.0 µm; tail =60.0 µm; ABD = 16.0 µm.

Female : - Body small sized, ventrally curved upon fixation, 24-28 µm wide at mid region. Cuticle with fine transverse striations, 2.5µm at mid region and at tail end. Labial region 6.3 µm wide, amalgamated, rounded and offset by a constriction; framework sclerotised; post-labial sclerotisation present and slightly sigmoid in optical section. Body at posterior end of oesophagus 3.8 times as wide as head. Amphid stirrup-shaped; 5.0µm wide. Odontostyle cylindrical, but more or less tapering towards tip, 10.0-12.5µm, 1.6-2.0 lip region widths long. Aperture occupying about one-fourth of the stylet length. Guiding ring single and plicate, at 7.5-8.8 µm from anterior end. Odontophore rod-shaped, 1.9 times the odontostyle length. Odontophore region of pharynx spindle-shaped; followed by a long slender portion which becomes narrower at nerve ring level and gradually widens to the basal enlarged portion; the latter about 34.6 - 36.4 % of total length. Nerve ring encircling the anterior slender part of pharynx at 44.0% of total neck length. Pharyngeal gland nuclei are located as D = 68.9-72.0 % , AS_1 = 26.8-29.0% , AS_2 = 26.8-29.0% % , PS_1 = 41.5% , PS_2 = 51.2%. Cardia conoid with rounded terminus, about 0.42-0.53 times as long as corresponding body width. Reproductive system mono-opisthodelphic, anterior uterine sac absent. Posterior gonad normal, 176-204 µm long or 6.9-7.3, occupying 26.7-30.9% of body length. Ovary reflexed with oocytes arranged in a single row. One uterine egg observed in one female specimen measuring 64x24 µm. Vulva pore-like with moderately sclerotised pars refringes. Vagina 11.3 µm long or 1/2-1/2.5 of the corresponding body width long. A pair of glands present on both sides of vagina. Pars distalis vaginae 3.8 µm long; pars refringes 1.3 µm long; pars proximalis vaginae 6.3 µm long. Uterus 40-64 µm long. Prerectum 2.8-3.3 anal body width long. Rectum 20-24 µm long, 1.3-1.5 anal body width long. Tail elongated convex-conoid, 60-64µm long, 3.8-4.0 times as long as anal body diameter, and occupying 9.1-9.7% of entire body length.

Male : - Not found.

Remarks : Presently there are eighteen valid species, *Thornenema andrassyi* Khan *et al*., 1989, *T. baldum* (Thorne, 1939) Andrássy, 1959, *T. coomansi* Ahmad & Ahmad, 2003, T. *elaboratum* Baqri & Jana, 1986, *T. elegans* Carbonell & Coomans, 1986, *T. garhwalicum* Srivastava *et al*., 2000, *T. laevicapitatum* (Cobb in Thorne & Swanger, 1936) Andrássy, 1959, *T. lissum* (Thorne, 1939) Andrássy, 1959, *T. maraouense* Coomans & Carbonell, 1981, *T. mauritianum* (Williams, 1959) Baqri & Jairajpuri, 1967,

T. oryzae (Ahmad & Jairajpuri, 1982) Carbonell & Coomans, 1986, *T. paraconurum* (Heyns, 1963) Goseco *et al*., 1976, *T. pseudosartum* Carbonell & Coomans, 1986, *T. punjabsinghi* Azmi & Ahmad, 1993, *T. samsoeni* Coomans & Carbonell, 1981, *T. shahi* Khan & Saeed, 1986, *T. shamimi* (Baqri & Jana, 1980) Carbonell & Coomans, 1986 and *T. spicatum* Shaheen & Ahmad, 2005 in the genus *Thornenema*.

Among these eighteen valid species of this genus described so far, the present species closely resembles *T. lissum* (Thorne, 1939) Andrássy, 1959, but differs from it in having smaller body length (*vs.*1.2mm), smaller value of c' (*vs.*7.0), larger value of c (*vs.*9.0), pore-like vulva and differently-shaped tail.

It also closely resembles *T. paraconurum* Heyns, 1963 but differs from the latter in having smaller body length (*vs.*1.3-1.6mm), larger value of c (*vs.*5.9-9.5), and smaller value of c' (*vs.*7.0-12.0), pore-like vulva and differently-shaped tail.

***Type locality and habitat* :** The new species was collected from moss (*Bryum* sp.) at Indian Botanic Garden, Howrah, West Bengal, India.

***Type specimens* :** One female specimen as holotype in one slide under Accession number 000041N/10 and 2 female specimens as paratype in one slide under Accession number 000042N/10 are deposited at present to the nematode collection of Parasitology Research Unit, Department of Zoology, University of Calcutta, 35 Ballygunge Circular Road, Kolkata-700019, India.

Discussion

The total number of nematodes associated with mosses varied greatly between samples and substrates, probably due to micro environmental conditions. It is also possible that the variation in both species abundance and community composition can be related to seasonal changes in temperature and precipitation, as has been demonstrated by Zullini (1971) and Steiner (1994), although Spaull (1973) found no direct correlation between water content of mosses and total number of nematodes.

Mesodorylaimus chamoliensis Ahmad, 1995 was found to be the most common, prevalent and prominent species among the moss inhabiting nematodes of Botanical Garden, Kolkata. But *Aporcelaimellus* n.sp 2 was found to be the most dominant and important species among the moss dwelling nematodes of this area due to its highest mean total biomass. The mean total biomass *Aporcelaimellus* n.sp 2 *was* higher than other prevalent species because of their larger body size. Gadea (1968) found *Mesodorylaimus bastiani* (Bütschli, 1873) Andrássy, 1959 as the dominant species among the moss dwelling nematodes of Eastern Ghates, India followed by *Rhabdolaimus terrestris* de Man 1880.

Omnivores were found to be most dominant and frequent group in the nematocoenosis of mosses in Botanic Garden, Howrah as also observed by Zullini and Peretti (1986), Lobaton (1991), Gadea (1968), and Gonzales (1972) from various moss samples from different countries. It has further been found that all the omnivorous nematodes encountered in the present study belong to order Dorylaimida. According to some authors (Johnson *et al*., 1974, Zullini and Peretti 1986, Bongers, 1990), dorylaimids are considered k-strategists in the broad sense, being very sensitive to disturbances. Thus great number of collected dorylaimids in the present study could also be the indicative of nematode community structure in undistributed environment ie, it confirms the good environmental condition of the investigated station.

The present species, *Thornenema thornei* n. sp. is morphologically close to *T. lissum* and *T. paraconurum* but differs from them in a number of morphological characters. Considering all these differences the authors suggest its name as *T. thornei* after an eminent nematologist, Prof. Gerald Thorne in recognition of his contribution to the taxonomy of nematodes.

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Impact of host population and abiotic factors on the percent parasitism by *Eretmocerus adustiscutum*, a parasitoid of *Aleuroclava pentatuberculata*

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Abstract : The dynamic role of parasitoids in a natural host-parasitoid interaction can be resolved into two components: The degree of depression in the host population density caused by parasitism and the degree of stability conferred on the interaction in the long term. Both of these components are likely to vary from one host-parasitoid system to another. The importance of parasitoids within the assortment of natural mortality factors affecting host populations can only be evaluated by detailed field studies of both host and parasitoid over a number of generations. For understanding the interactions between whitefly, *Aleuroclava pentatuberculata* (Sundararaj & David) (Homoptera: Aleyrodidae) and its parasitoid, *Eretmocerus adustiscutum* (Krishnan & David) (Hymenoptera : Chalcidoidea) population dynamics studies were conducted during 2004-2007 and their relation with abiotic factors were worked out. The observations have revealed that percent parasitism was positively and significantly correlated with maximum and minimum temperature. The significance level for maximum temperature was at 1% level whereas that of minimum temperature was at 5% level. The correlations of percent parasitism with that of maximum and minimum relative humidity and rainfall were observed as negatively significant with maximum relative humidity and non significant with that of minimum relative humidity and rainfall. The r values for maximum and minimum relative humidity and rainfall were found to be -0.295, -0.043 and -0.030 respectively. The correlation of the host population with the percent parasitism was noted to be negative and non-significant with r value as -0.080. Multiple regression analysis was performed to draw inference related to forecasting the probable incidence of the parasitoid in relation to host population. The observations revealed that the abiotic factors of previous 27-33 days and the host population of same day were found to influence the incidence of the parasitoid.

Key words **:** Abiotic factors, *Aleuroclava pentatuberculata*, biotic factor, correlation coefficient, *Eretmocerus adustiscutum*, multiple linear regression, parasitism.

Introduction

In insects, the population dynamics is the result of interaction between physical conditions, population movements and both density dependent and independent factors. The relative importance of each of the factors might vary among populations or even within a population from time to time (Huffaker et al., 1984). It was reported that the climatic factors have dominating influence on various aspects of an individual insect's life cycle such as reproduction, development, fecundity and longevity (Howe, 1953). Fluctuation in number of insects has been closely correlated with changes in weather and the population densities were determined coupled with relatively small degree of mortality caused by parasitoids, predators and diseases have commonly been regarded as establishing the importance of the physical factors in determining the abundance of insects in a given population (Cook, 1930). In any ecosystem, populations of different organisms interact with biotic and abiotic environments with the limiting factors most likely to maintain population densities in equilibrium (Berryman, 1993). These factors which limit population growth and regulate population dynamics can be used as parameters to make quantitative forecasts of population fluctuations (Berryman, 1997). Accordingly in the present study interactions between population of whitefly, *Aleuroclava pentatuberculata* (Sundar raj & David) (Homoptera : Aleryrodidae) and their native parasitoid, *Eritmocerus adustiscutum* (Krishnan & David) (Hymenoptera : Chalcidoidea) were worked out in order to facilitate the forecasting the native parasitoid population for effective biological control of this serious pest.

Materials and methods

The population size of whitefly was studied in the mulberry fields of Central Sericultural Research and Training Institute, Berhampore during 2004-2007. Population was recorded at weekly intervals in randomly selected 20 mulberry plants. The

vertical population of whitefly was assessed by considering top (1-3), middle (4-7) and bottom (7-14) strata (Lynch and Symmons, 1993). To reduce disturbance that might have interfered with an accruable census, counts were done on two leaves (top, middle, bottom). The investigations were made during cooler hours of the day preferably 6.00 A.M. – 7.00 A.M. (Naranjo and Flint, 1995). Counts of whitefly were made carefully turning the leaf over by rotating the petiole (Naik and Lingappa, 1992). The incidence of *E.adustiscutum* was noted by collecting the middle and bottom positioned leaves from all 20 plants infested with nymphal population of whitefly. These nymphs were screened in the laboratory under stereomicroscope that whether they are parasitized or not by following the methods suggested by (Van Dreishe *et al.*, 2001b). For understanding the relationship between the parasitoid, whitefly and meteorological factors, the data was subjected to analysis by Coefficient of Correlation. To develop forecasting models of this parasitoid the data was analyzed by multiple linear regression analysis and fittings of the regression equations were adjudged by the significance of R^2 (the coefficient of determination).

Results

The seasonal incidence studies of whitefly, *A. pentatuberculata* in the mulberry ecosystem had revealed that, during the first year of the study (2004-05), it was noticed that the population (23.93 per plant) was above the economic threshold level (20/plant) from the very first week of the observation i.e. during fourth week of August, 04. The population of the pest after showing an increasing trend during the first week suddenly came down for two consecutive weeks in September (Fig.1a). The whitefly population was maximum (41.83 per plant) during second week of October, 2010. The count of the whitefly remained below the threshold level during November, 2004 – August, 2005.

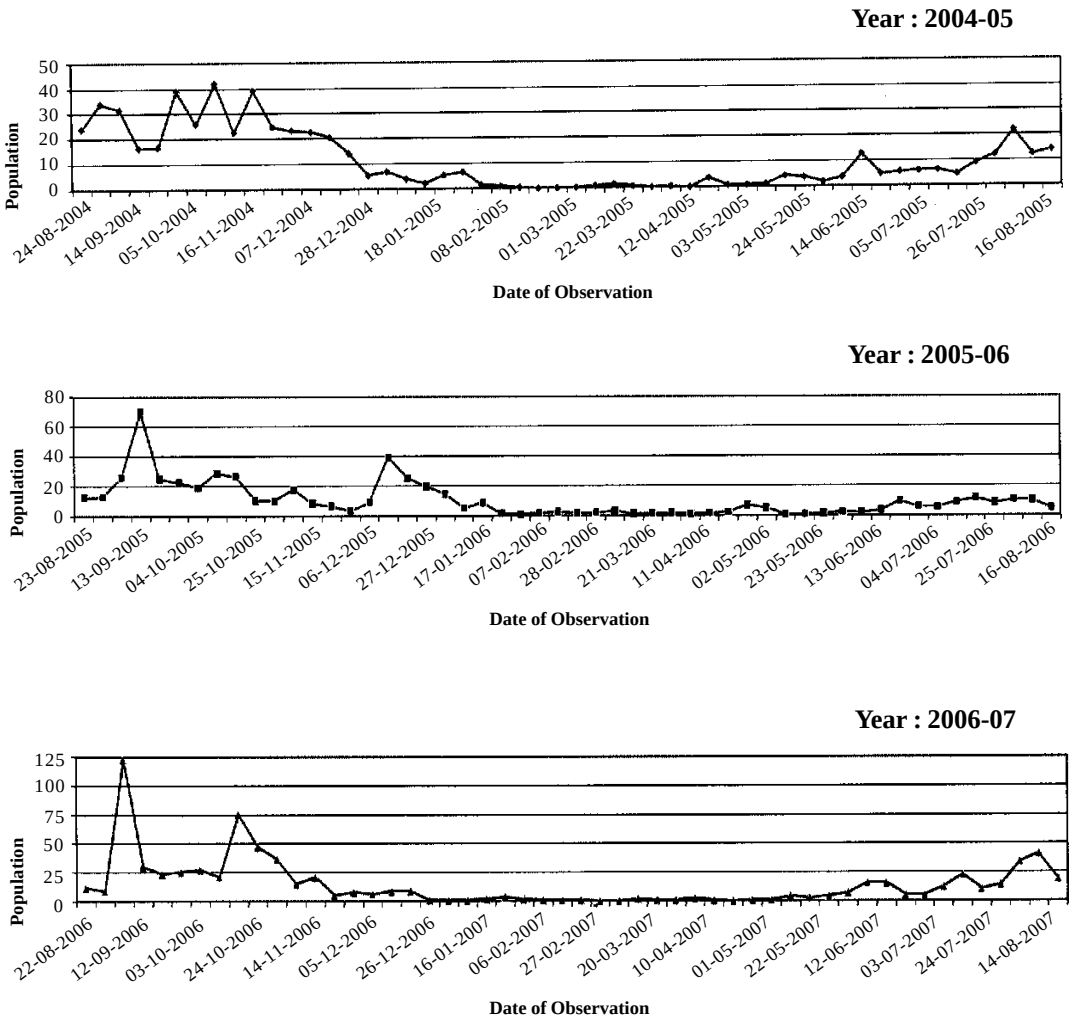

Fig. 1(a, b, c) : Incidence of *A. pentatuberculata* (No./Plant) in different years

During the second year (2005-06), the population of *A. pentatuberculata* was highest (70.05 per plant) during second week of October, 2005 (Fig. 1b], one month earlier in comparison to the previous year of the study. The population of *A. pentatuberculata* lowered down from the end of December and was found to be below 10 per plant till August, 2006. The minimal population was found to be 0.83 per plant during second week of May, 2006 (Fig. 2b). Thus, the trend of seasonal incidence was almost same as that of the previous year with very low population from March to May.

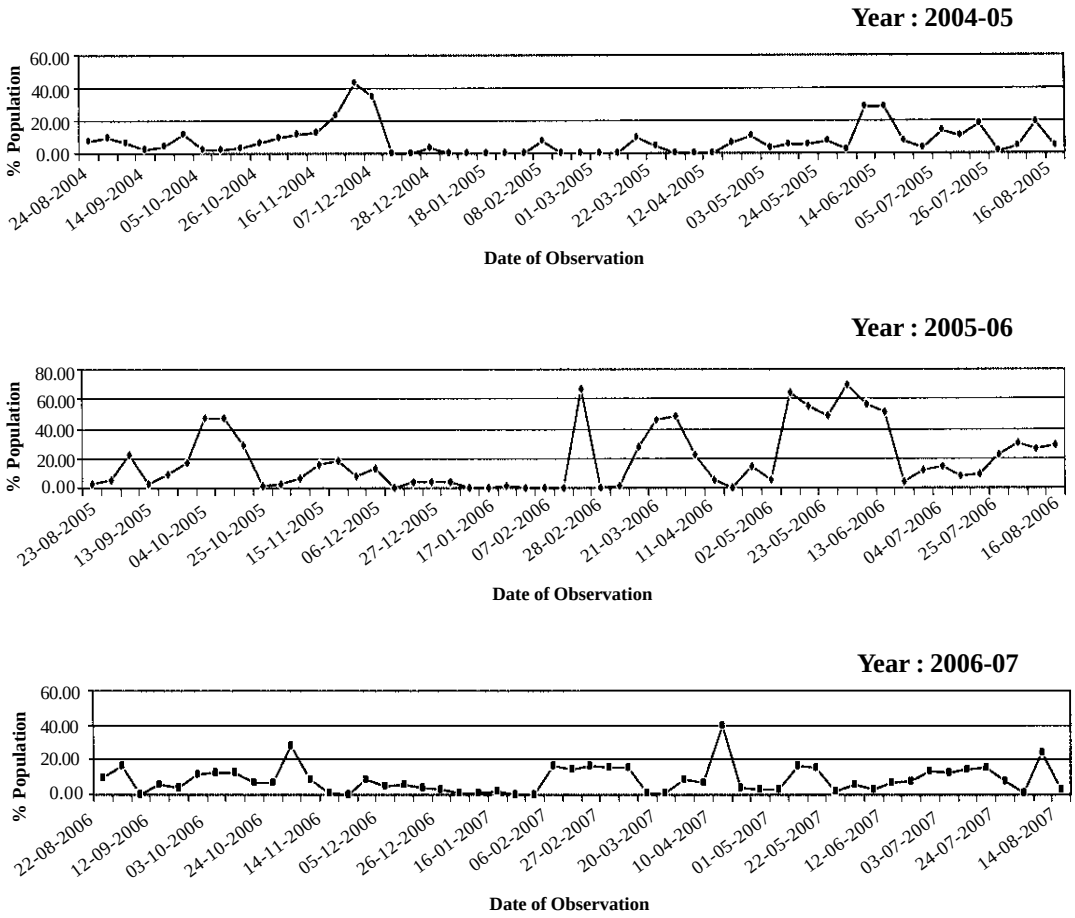

Fig. 2(a, b, c) : Incidence of *E. adustiscutum* on *A. pentatuberculata* in different years

During the final year of the study (2006-07) the observation was quite similar to that of the preceding year. The maximum population (122 per plant) was during the first week of September, 2006. The population recorded below the ETL from third week of November, 2006 till the end of June, 2007 (Fig. 2c). From July, 2007 the population slowly started to build up. The lowest population during the year of the study was recorded as 0.37 per plant in the fourth week of February, 2007.

During 2004-2005, higher percent parasitism of *A. pentatuberculata* on *D. decempuncta* was found in November and December while in August, September, October and beyond December till May the percentage remained low (Fig. 2a). The parasitism percentage started to increase with fluctuations in it after May. During 2005-2006, the percent parasitism was more than that of the previous year with high percentage noticed in February, May and June and lower from September to February (Fig. 2b). In the final year of the study (2006-2007) highest peak was noted in April (Fig. 8c). Very low percent parasitism was found in November to January and during the rest of the year the percentage was average.

Discussions

It can be observed from the data as well as the figures that the population of *A. pentatuberculata* showed almost a similar trend of increase in their number in the month of October during the first two years of the study. Further, it was found that the population size was good till the second week of November for three consecutive years. The population was very low

from the month of December to June during the entire study period (Fig. 1 a, b, c). This was in agreement with the observation of Sewify *et al*. (1996), who observed maximum population of *Bemisia tabaci* (Gennadius) (Homoptera : Aleyrodidae) during August and September on cotton. Further maximum incidence of *B.tabaci* was also reported during November (Rao *et al.*, 1989a and Venugopal *et al*., 1989). This observation was in variance with that of spiralling whitefly, *Aleurodicus dispersus* (Russel) (Homoptera : Aleyrodidae), which has recorded the peak incidence in summer (March – June) and low in winter (October – January) (Mani and Krishnamurthy, 2000). Severe infestation of *A.disperses* was observed in March in Guava (Ramani, 2000) and Sunhemp (Pruthi and Samuel, 1942). The peak infestation of sweet potato whitefly, *Bemisia argentifolli* (Bellows and Perring) was also noticed in spring (April – May) (Feidler and Sosnowska, 2002).

While correlating the percent parasitism on *A. pentatuberculata* with that of the abiotic factors, it was found that it is positively correlated with maximum and minimum temperatures. The relation was found to be significant for both maximum and minimum temperature. The significance level for maximum temperature was at 1% level whereas that of minimum temperature, it was at 5% level. The r values found were 0.272 and 0.199 respectively for maximum and minimum temperatures. The correlations of percent parasitism with that of maximum relative humidity, minimum relative humidity and rainfall were observed as negatively significant with maximum relative humidity and non significant with that of minimum relative humidity and rainfall. The r values for maximum relative humidity, minimum relative humidity and rainfall were found to be -0.295, -0.043 and -0.030 respectively. The correlation of the host population i.e. *A. pentatuberculata* with the percent parasitism, was noted to be negative and non significant with r value as -0.080.

Further, the data was analyzed through Linear multiple regression anlalysis, which has shown that abiotic factors of previous 27-33 days were found to influence the whitefly population. The derived regression equation was :

$Y= 46.792 + 0.611X_1 – 0.172X_2 – 0.664X_3 + 0.139X4 – 0.082X_5$

Where Y = Percent parasitism on *A. pentatuberculata*,

X_1 = Maximum temperature,

X_2 = Minimum temperature,

X_3 = Maximum Relative Humidity,

X_4 = Minimum Relative Humidity,

X_5 = Rainfall.

With coefficient of determination $R^2 = 0.104161$ (P = 0.00568901).

The host population of the same day was found to influence the percent parasitism on *A.pentatuberculata*.

$Y = 13.146 – 0.059\ X_1$

Where Y = Percent parasitism on *A. pentatuberculata*,

X_1 = Population of *A. pentatuberculata* .

With coefficient of determination $R^2 = 0.008593$ (P = 0.51673432).

The regression equation showing the combined effect of the abiotic factors (previous 27-33 days) and host population on the percent parasitism was derived as:

$Y = 39.221 + 0.784X_1 – 0.254X_2 – 0.674X_3 + 0.248X_4 – 0.052X_5 – 0.132X_6$

Where Y = Percent parasitism on *A. pentatuberculata*,

X_1 = Maximum temperature,

X_2 = Minimum temperature,

X_3 = Maximum Relative Humidity,

X_4 = Minimum Relative Humidity,

X_5 = Rainfall,

X_6 = Population of *A. pentatuberculata* (Fig. 3)

With coefficient of determination $R^2 = 0.12071$ (P = 0.007885857).

Year : 2005-06

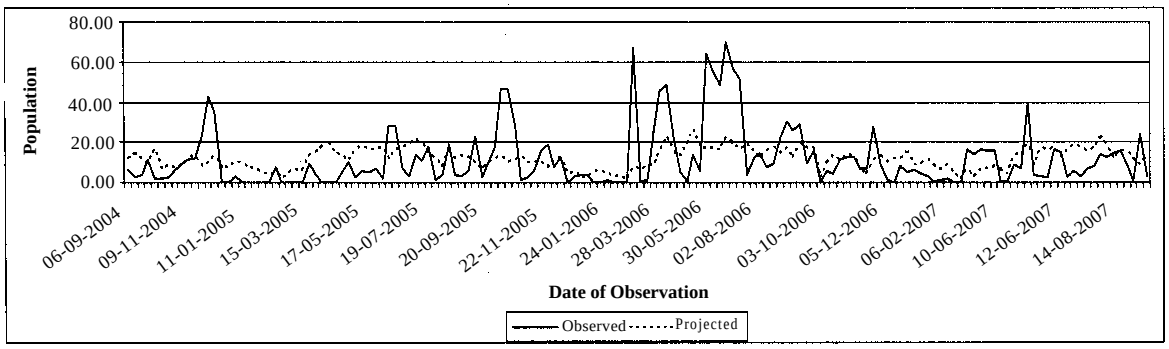

Fig. 3 : Correlation between seasonal incidence of *E. adustiscutum*, *A. pentatuberculata* and abiotic factors

It was evident from the correlation studies that the whitefly population was positively and significantly correlated with minimum temperature, minimum relative humidity and rainfall. While with other meteorological factors there is a difference in the correlationship. The correlation with the parasitoid, *E. adusticutum* shown in the form of percent parasitism was observed to be positive and non significant. This study will be helpful in formulating computerized forecasting models, that facilitates prediction of the incidence of bio-control agent, E. *adusticutum* in the mulberry fields of West Bengal. In case of low incidence, of this parasitoid, farmers need to be advised for inundative releases to manage whitefly infestations in mulberry eco-system.

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Occurrence of *Eustrongylides* larvae sp. in *Xenentodon cancila* (Ham.) from lower lake, Bhopal and its effect on ovaries

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Abstract : The present investigation was carried out to study the prevalence and effect of Dioctophymotidae nematode (*Eustrongylides* sp.) in freshwater fish, *Xenentodon cancila.* A bright red colored nematode of length 450 – 680 µm were found encapsulated or free in the body muscles, swim bladder, liver, intestine and ovaries of host fish. The overall prevalence of 56.63±36.99 and maximum prevalence of 100% was observed in the month of March and November, 2008 i.e. during the pre–spawning and post-spawning period. Female hosts were only found infected as compared to males. Ovaries showed 5.08± 0.83 prevalence and 0.125±0.0095 relative intensity of *Eustrongylides* sp. The calculated value of GSI (6.52±0.87) and fecundity (146.36±29.58) in infected fishes were observed to be low as compared to the GSI (11.84±1.53) and fecundity (209.6±23.68) of uninfected ones. The histopathological damage caused by parasites in ovaries is characterized by necrosis, reduction in the size and number of oocytes, atresia and degeneration of developing oocytes. The present observation attributes towards the reduction in fecundity and further decline in the population of the infected fish.

Keywords **:** *Eustrongylides, fecundity, GSI, lower lake, Xenentodon*

Introduction

Parasites can act as one of the factors regulating the host populations by affecting host survival and reproduction which may directly or indirectly influencing their behavior. The seasonal pattern of host reproduction is an important factor influencing population dynamics of host-parasite interactions (White *et al*., 1996). Parasitic infestations also tend to decrease the growth rate resulting in stunting of the fish. A heavy worm burden may reduce the host's reproductive potential (as in philometrid nematode infections) or may delay sexual maturity in the fish. The present investigation was carried out to study the prevalence of *Eustrongylides* sp. larva (Dioctophymotidae nematode) and their effect in the ovaries of freshwater fish, *Xenentodon cancila*. *Eustrongylides* have been reported to utilize aquatic annelids (oligochaetes) as the first intermediate host and fish as second intermediate host (Ibiwoye *et al*., 2004) and its larvae have been reported from 17 orders of fish worldwide (Spalding *et al*., 1993). As this represents the first record of *Eustrongylides* species from Bhopal region and their effect on the histology of ovaries.

Materials and methods

1. Collection of fish specimens and parasites : Living specimens of this fish were collected from the Lower Lake of Bhopal. They were brought to the laboratory and examined morphologically and internally for the occurrence of helminth parasites. The host fish, *X. cancila* was collected continuously for one year at regular intervals. Fish specimens were dissected out in physiological saline (0.75% NaCl solution) for collecting helminth parasites. Nematodes collected were washed thoroughly in normal saline. Then killed and fixed in hot 70% alcohol, stored in glycerine alcohol (1:3) and studied as wet mounts or temporary mounts in glycerine.

2. Histopathological study : The infected gonads of *X. cancila* was taken out and fixed in alcoholic Bouin's fluid for 72 hours. After the complete removal of picric acid, the tissue was processed for preparation of paraffin wax blocks. The tissue was then cut at 6 µm thick sections which after proper processing prepared in permanent slides.

3. Statistical analysis : Ecological analysis of parasites was done according to the method of Margolis *et al.* (1982).

Results

Out of 103 specimens of *Xenentodon cancila* examined, 43 were found infected with *Eustrongylides* sp. larva. A bright red coloured larva measuring 450 – 680 µm in length, were found encapsulated or free in the body muscles, swim bladder, liver, intestine and ovaries of host fish. The maximum prevalence (100%) was recorded in the months of March and November, 2008. In *X. cancila*, the maximum intensity (3.33) of the parasites was recorded in October, 2008 and the minimum (1.0) was recorded in January and September, 2008. The recorded value of abundance ranged from 0.142 to 2.8. The highest abundance (2.8) was recorded in November, 2008 and minimum (0.142) in September, 2008. The monthly variations in prevalence, intensity and abundance of parasite in *X. cancila* are given in Figs. 1 and 2. The infected specimens of *X. cancila* showing the larva extruding from anus (Fig. 3).

Histo-pathological effects – The *Eustrongylides* infected ovaries of *X. cancila* revealed irregular shaped and damaged oocytes and showing necrosis and atresia. The lumen of the ovary is filled with the sections of parasite which resulted in strong inflammatory reaction. Liquification of yolk globules was the dominant pathological response which shows the reduction in the yolk formation in ovary and indirectly attributes toward the reduction in fecundity (Figs. 4, 5 and 6).

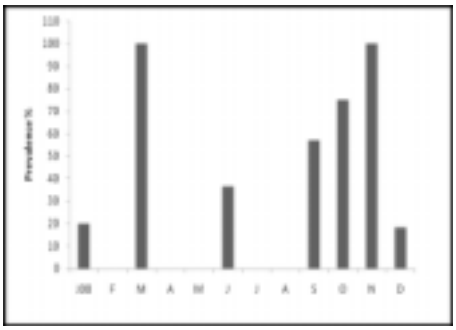

Fig. 1. Monthly variations in the percentage of prevalence of parasites in *Xenentodon cancila*

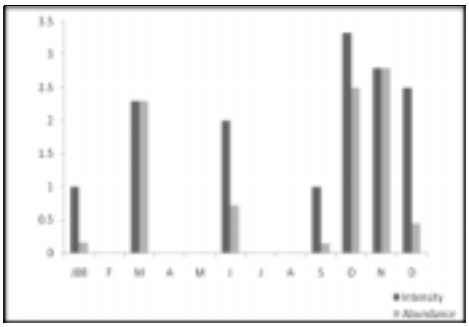

Fig. 2. Monthly variations in the rate of intensity and abundance of parasites in *Xenentodon cancila*

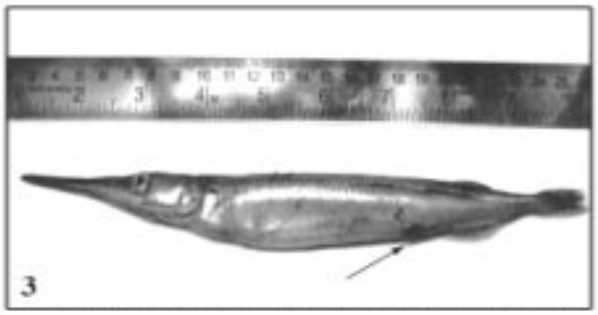

Fig. 3. Photograph of *X. cancila* Infected by the larvae of *Eustrongylides* sp. showing the larva extrading from anus (arrow)

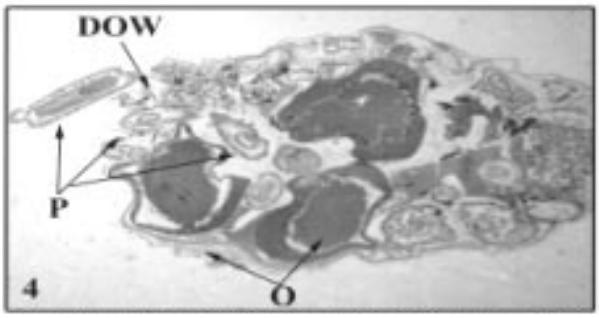

Fig. 4. Microphotograph of a cross section of infected ovary of *X. cancila* showing parasites (P), ovary (O) and damaged ovarian wall (DOW) X12

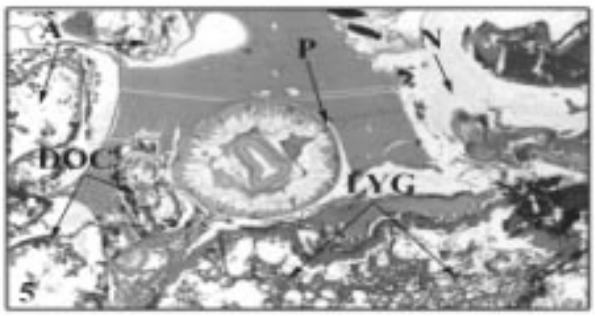

Fig. 5. Microphotograph of a cross section of infected ovary of *X. cancila* showing atresia (A), liquifaction of yolk globules (LYG), necrosis (N), deshaped oocytes (DOC) and parasite (P) X100.

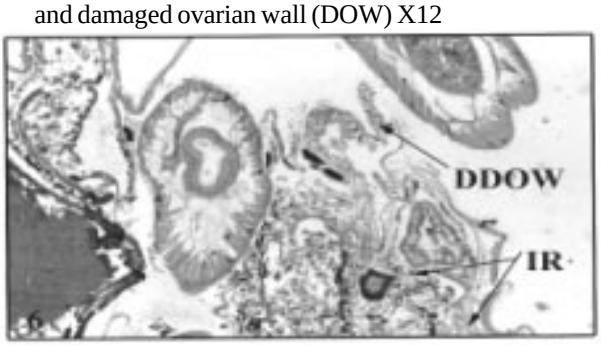

Fig. 6. Microphotograph of a cross section of infected ovary of *X. cancila* showing damaged and disrupted ovary wall (DDOW) and inflammatory reaction X100.

Fig. 1. Photograph of X. *cancila* infected by the larvae of E
Monthly variations in the percentage of prevalence of parasites in *Xenentodon cancila*

Quantitative analysis showed a significant change in the gonado-somatic index (6.52±0.87) and fecundity (146.36±29.58) values of infected ovaries of *X. cancila* as compared to gonado-somatic index (11.84±1.53) and fecundity (209.6±23.68) of non-infected ovaries (Tab. I).

Table-I : Mean values (±SD) and significance of the GSI and fecundity of *X. cancila* in infected and non-infected specimens

S. No.	Parameters (mean±SD)	Non-infected (mean±SD)	Infected	Significance
1.	Gonado-somatic index	11.84±1.53	6.52±0.87	P<0.05
2.	Fecundity	209.6±23.68	146.36±29.58	P<0.05

Discussion

Larvae of this genus were reported by Fernando and Furtado (1963) from three species of fishes, viz. *Heteropneustes fossilis*, *Ompok bimaculatus* and *Wallago attu* from Sri Lanka. Kalyankar (1974) reported *Eustrongylides* from *Mystus seenghala* from Maharashtra.

Chen (1973) and Wang *et al*. (1997) reported the larvae of *Eustrongylides* from the fish species of the families Engraulidae, Cyprinidae, Siluridae, Bagridae, Channidae and Percichthyidae. Ibiwoye *et al*. (2004) studied the density of infection cause by the larvae of *Eustrongylides africanus* in *Clarias* from Bida floodplain. They emphasized that the female fishes are more frequently infected with the parasite than the males. The same is observed under present investigation. The infection was maximum in winter or post winter season and minimum in rainy season. This was probable due to the decrease in water volume during dry season which caused nutritional imbalance results in less production of fish food organisms while during winter season the temperature of water falls which reduces the immune response of fish and increases its vulnerability to parasitic infection.

Under present investigation, in ovaries of infected *Xenentodon cancila* exhibited, necrosis and decrease in the yolk formation due to disappearance of vitellogenic oocytes. It has also been observed that the gonado-somatic index and fecundity of infected *X. cancila* decreased significantly as compared to non-infected specimens. The present observation gets support from the work of Heins and Baker (2003) who found that mean egg size in three spined stickleback (*Gasterosteus aculeatus*) was smaller in fish infected by the cestode, *Schistocephalus solidus* than in non-infected fish. Oliva *et al*. (1992) suggested that philometrid infection resulted in reduced fecundity in Chilean sea bass (*Paralabrax humeralis*) because the effective volume of the ovaries was reduced. Necrosis was described in the ovaries of striped mullet (*Mugil cephalus*) due to infection by *Philometra cephalus* (Ramachandran 1975).

Atrophy in the ovaries of New Zealand snapper (*Chrysophrys auratus*) due to infection by *Philometra* sp. was described by Hine and Anderson (1981). Similarly Moravec *et al*. (1997), Hesp *et al*. (2002) and Moravec *et al*. (2002) reported *Philometra* induced ovary damage in various reef-fishes. Present study on the prevalence and histo-pathological effect of parasite in ovaries of *X. cancila* is first of its kind and *Eustrongylides* sp. larvae is reported for the first time from Bhopal region.

Acknowledgment

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Abbreviations : O- ovary, P- parasites, DOW- damaged ovarian wall, A- atresia, LYG- liquification of yolk globules, N- necrosis, DOC- deshaped oocytes, DDOW- damaged and disrupted ovary wall, IR- inflammatory reaction.

Characterization of haemocyte types, their counts in different breeds of silkworm, *Bombyx mori* L. and their progressive changes following bacterial inoculation

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Abstract : Breed specific response against bacterial inoculation is observed in silkworm, *Bombyx mori* L. In insects cellular defense response to bacteria is well studied in which haemocytes respond to the infection through phaogocytosis, nodulation and encapsulation etc. Therefore, the difference in response pertaining to the total haemocyte count (THC) and differential count (DHC) has been studied. It was found that in circulation there was increase in THC in tolerant breeds than the susceptible breeds depending upon the pathogenecity of the bacteria invaded. In multivoltines the haemocyte counts ranged from 1.5 x 10^6 to 3.3 x 10^6 / mm^3 whereas in bivoltine the counts varied between 3.0 x 10^6 to 4.4 x 10^6 / mm^3 in mature 5^{th} instar larvae. Likewise DHC was observed increased with regard to plasmatocytes and granulocytes in case of tolerant breeds under normal and progressive bacterial infection. Plasmatocytes comprise 35 - 40% of the total haemocytes.

Running title : Characterization of haemocytes in *Bombyx mori* L.

***Key words* :** *Bombyx mori L, Bacteria, Differential Haemocyte Count (DHC), Haemocytes, Nodulation, Total Haemocytes Count (THC)*

Introduction

Insects depict two broad categories of defense responses to bacterial infection: hemocytic and humoral (Gupta, 1991 and Bulet et al, 1991). Hemocytic defense responses feature direct interactions between circulating haemocytes and invading pathogens and this typically occurs within minutes through phagocytosis, nodulation and encapsulation rather than hours. Humoral responses require several hours for their full expression and involve induced synthesis of antibacterial proteins such as cecropins, attacins, diptericins and defensins. Classification of blood cells of the silkworm *Bombyx mori* L. has been done by Nittono (1960). Lea (1964) reported that cell counts increase continuously during cocoon spinning, but decrease at the prepupal stage in *Hyalophora cecropia.* Prohaemocytes are reduced during the last larval instar up to prepupation in *Prodenia* (Yeager, 1945), *Galleria* (Shapiro, 1966) and *Euxoa* (Arnold and Hinks, 1976) and later the number increases with larval development. This study was conducted to have a first hand knowledge of the hemogram pattern in *B. mori* during different larval stages.

So, the present study was taken up with a view to characterize the haemocyte types of silkworm, *Bombyx mori* in order to further elucidate the role of the haemocytes during the defensive response. Besides, efforts have been made to understand the variation in total and differential haemocyte counts in different breeds of the mulberry silkworm, *Bombyx mori* L. against bacterial inoculation.

Materials and Methods

Insect

Larvae of the silkworm, *Bombyx mori*, L. were reared at room temperature (27 ± 2°C), relative humidity of 85 ± 5 % and natural photoperiod. Larvae were fed on mulberry (*Morus alba*) leaves. Healthy larvae were used in the experiment.

Collection of hemolymph

The fifth day fifth instars larvae weighing 1.52 ± 0.05 g on an average was chilled for 3 to 5 minutes at 4°C and was surface sterilized with 70% ethyl alcohol before bleeding. The larvae were bleeding by cutting a pro leg and letting the haemolymph

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drip freely into an Anticoagulant (AC) saline solution [0.098M NaOH, 0.146 M NaCl, 0.017M EDTA (Ethylene Diamine Tetraacetic Acid) and 0.041 M Citric acid at pH 4.5] capacity 500 µl in 1.5 ml sterile polypropylene tubes cooled with crushed ice. About fifty larvae were bleeding in this manner. The AC saline and haemolymph were mixed well using transfer pipettes (a fresh pipette was used for each haemolymph sample and discarded after use) and finally pooled and transferred to fresh tubes. Initially the cells were gently pelleted at 500g at 2°C in a swing-out rotor (SHMT-12) of a Sorvall RC5C centrifuge. The supernatant was gently discarded and the pellet was again diluted with AC saline. The process was repeated twice ensuring removal of breeds of plasma without cell lyses. Finally, the diluted haemocyte suspension (1:1) in AC saline (capacity 1 ml) was layered onto 50% continuous gradient of Percoll (Colloidal PVP coated silica particles) (7 ml of 50% gradient was composed of 0.7 ml anticoagulant and 3.15 ml pure Percoll and 3.15 ml Endotoxin free water). A continuous gradient was formed by centrifugation at 2°C at 22,000 g in an angle head rotor (SS-34) in a Sorvall RC5C centrifuge. The gradient was stored below 10°C and used within a few hours to avoid precipitation of the Percoll in the AC saline and centrifuged at 650g at 2°C in a swing out rotor (HB- 4) of a Sorvall RC5C centrifuge for 15 min. The granular cells formed a distinct band at the top with the plasmatocytes 1 to 1.2 cm below in two distinct bands. Pasteur pipettes were used to remove from top down the two layers of cells. For cell viability, dye exclusion test was carried out on some samples after 2 m incubation at 25°C with an equal volume of 0.2% trypan blue dye in 0.1 x AC saline.

Harvesting of haemocytes

The percentage recovery of prohaemocytes, spherule cells and oenocytoids is far less compared to that of the defensive cells like plasmatocytes and granular cells by using Percoll density gradients following Mead et al (1986) and Anggraeni and Ratcliffe (1991).

Collection of haemolymph for total and differential counts

Larvae of 2^{nd}, 3^{rd}, 4^{th}, 5^{th} instars, spinning day 1, pupa of day 3 and adult male and female were selected for elucidating the haemogram pattern. Twenty-five larvae were considered for study of total and differential haemocyte counts. The same procedure was followed for amputation and collection of haemolymph from silkworm larvae. Total and differential haemocyte counts were estimated following the method of Christensen *et al*. (1989). The haemolymph was diluted in ice cold Dulbecco's phosphate buffered saline (DPBS). 1µl samples of freshly collected haemolymph were diluted in 19 µl of ice cold DPBS (1.5mM K_2HPO_4, 8mM Na_2HPO_4-H_2O, 0.9 mM $CaCl_2$, 2.7 mM KCl, 0.5mM $MgCl_2$, 0.3mM NaCl, pH7.2, (Miranpuri *et al.,* 1991) and 5 µl aliquots placed on slides for 2 to 3 m to allow the haemocytes to settle.

Total (THC) and differential (DHC) haemocyte counts were determined from 200 cells using Neubauer hemocytometer in a Leitz Diaplan Clinical microscope (Cantwell, 1973) following identification key of Nittono (1960) and Gupta (1979).

The cellular defense response of the silkworm breeds (multivoltine and bivoltine) was evaluated against bacteria (highly pathogenic: *Bacillus thuringiensis* var.sotto. medium pathogenic: *Bacillus subtilis* and non-pathogenic: *Escherichia coli)*. Cultures of the bacteria were grown on 1.5 % Solid Nutrient Agar medium at 30°C in a bacteriological incubator following the standard procedure (Aneja, 2003). The bacteria were used in mid logarithmic phase at a titre of 3.2 x 10^4 c.f.u. / ml.

Three promising bivoltine breeds (BHR-2,SK6 and SK7) and five promising multivoltine breeds [Debra, Nistari (M), Nistari (DN)P, M6DP(c) and M12 (W)] were injected intrahemocoelically (2 to 4 µl @ 1.25 x 10^5 c.f.u /ml) at mature 5^{th} instar with three live bacteria of varied pathogenecity as mentioned earlier separately. Following inoculation the THC was recorded at various hours post inoculation (h.p.i.) to elucidate the cellular defense response of the breeds to the bacteria (30 m to 6 h.p.i.).

Results

Characterization of haemocytes

Plasmatocytes (PLs) (Plate-1)

The plasmatocytes were usually ovoid or spindle shaped structures, which had few to many pseudopodia. The plasma membrane contained many micropapillae, filopodia or other irregular processes, as well as pinocytotic or vesicular invaginations. The nucleus was round or elongate and was generally centrally located. The PLs were generally 3.5 to 15 µm long and 3 to 4 µm wide. The nucleus on the other hand was 3.0 µm long and 1.5 µm wide. The cytoplasm was granular or agranular. These cells comprised the bulk of the haemocytes ca. 35 to 40%. The most important character of the plasmatocyte was its spreading behavior with substrates or with glass surfaces.

Granulocytes (GRs) (Plate-2)

These cells were polymorphous with numerous digitations and pinocytic vesicles. The main characteristic of these cells was the presence of numerous granules or inclusions. These cells were small to large, spherical or oval cells with size ranging from 10 to 14 µm in diameter when spherical. The nucleus was relatively small compared to the PLs, round or elongate and was centrally located. The size varied about 1.2 to 2 µm. The granules were structured or structure less resembling globules. These cells also had the capacity to spread, but they degranulate before they were fully spread, thus making them easily distinguishable from the PLs.

Prohaemocytes (PRs) (Plate-3)

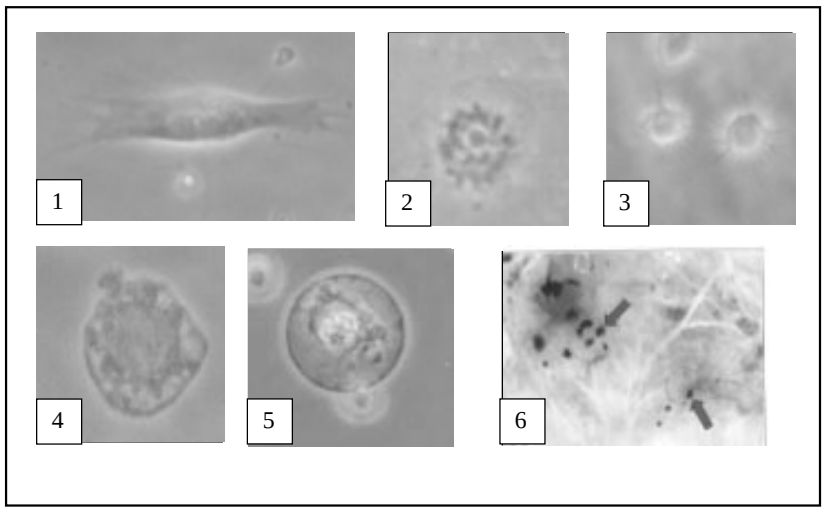
These cells had a regular rounded shape, deprived of pseudopods. These were small cells observed infrequently in circulation with a high nucleo-cytoplasmic ratio. The size varied between 7 to 9 µm, with the centrally located nucleus measuring about 2.8 µm. The plasma membrane was generally smooth but sometimes vesiculations were noticed. Sometimes the cytoplasm contained granules, droplets or vacuoles. The PRs were generally in groups.

Spherulocytes (SPs) (Plate-4)

When viewed under phase contrast optics, SP granules were highly refractile. This morphology was sufficiently distinctive. The cytoplasm of these cells was filled with large inclusions known as "spherules". These characteristic inclusions were membrane bound. In *Bombyx mori*, they consist of an internal structure of membrane arranged in concentric layers. These cells were observed to be smaller than the GRs, though, in certain instances they were larger than the GRs. The cells were 7 to 12 µm in diameter with small nucleus, which was central or eccentric. In phase contrast microscopy, the SPs appeared irregular in shape exhibiting an aspect of a 'morula' which facilitates their recognition.

Oenocytoids (OEs) (Plate-5)

These were large cells (15 to 20 µm) and regular in shape, homogeneous cytoplasm and eccentric nucleus. These never represent more than 1 to 2% of all the haemocytes present in *B. mori* L. The problem with recovery or obtaining pure populations of this cell type was that they were very labile in nature and lyse quickly, ejecting material into the haemolymph.



Different types of Haemocytes in *B.mori*

Plate : 1) Plasmatocytes (PLs) 2) Granulocytes (GRs) 3) Prohaemocytes (PRs) 4) Spherulocytes (SPs) 5) Oenocytoids (OEs) 6) Darkly melanized nodules (arrow) seen *in situ* against the background of larval fatbody

Total haemocyte counts during larval development

The total number of unfixed haemocytes increases significantly with the development of the larval stages and is the maximum in mature 5th instar day 5 larvae. With the commencement of spinning there was a fall in the THC, which drastically reduced in the pupal stage ($0.34 \times 10^{6} \pm 0.303$ / mm^{3}). THC was higher in adults than the pupae; however, no significant

difference was recorded among the sexes hence an average THC value irrespective of sex has been depicted in hemogram of silkworm, *B. mori* (Fig. 1).

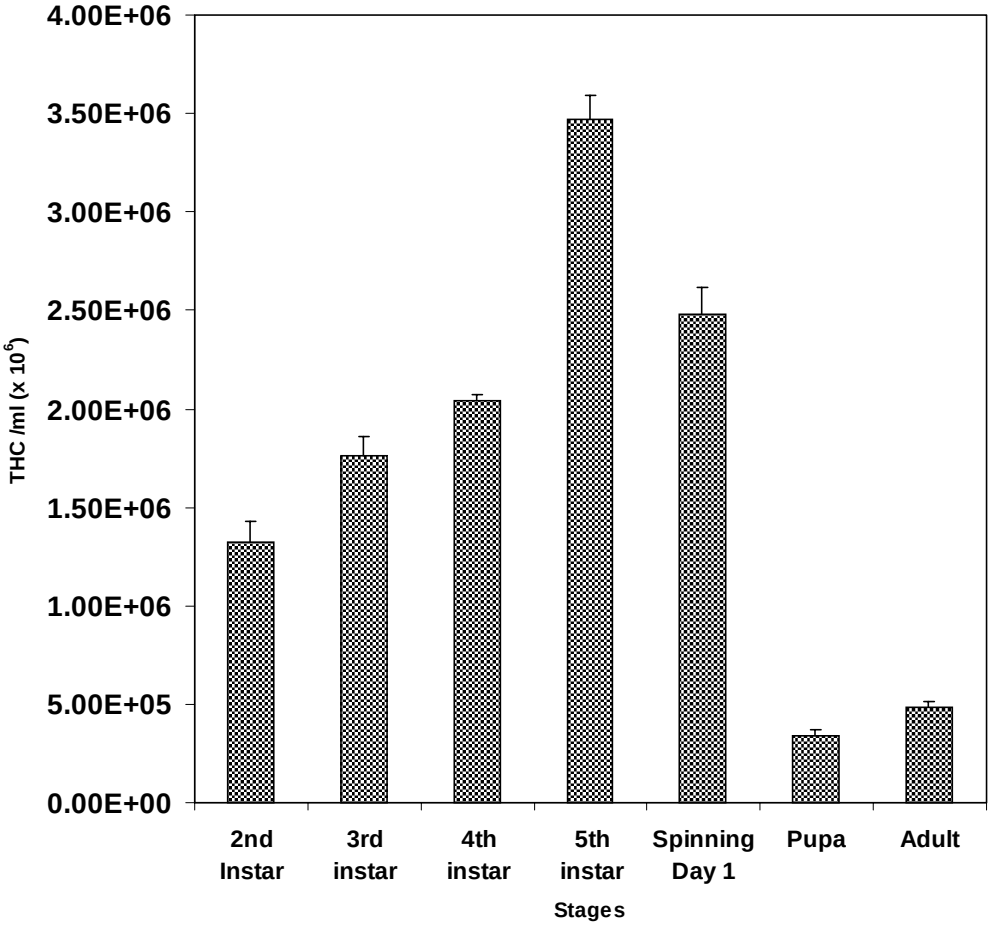


Fig. 1 : Hemogram of silkworm, *Bombyx mori* L.

Differential haemocyte counts during larval development

It was evident that the largest population of cells in all the stages was the plasmatocytes, followed by the prohaemocytes, the granular cells, the spherule cells and then lastly the oenocytoids. There was a gradual increase in plasmatocyte counts and maximum during the mature 5th instar and the granular cells also followed a similar pattern. The prohaemocytes, however, varied among various stages. The spherule cells appeared in the 5th instar and maximum during the spinning stage, but absent in adult haemolymph (Table 1).

Table 1 : Differential haemocyte counts (expressed as % of absolute values) during larval (2nd instar - 5th instar and spinning day 1), pupal and adult stages in *B. mori*

Haem ocyte type	2nd instar	3rd instar	4th instar	5th instar (mature)	Spinning (Day 1)	Pupa (3-4 Day old)	Adult (irrespective of sex)
PR	41	28	30	25	22	25	34
PL	36	38	40	42	35	36	38
GR	22	28	26	26	21	24	28
SP	0	2	2	3	20	12	0
OE	1	2	2	4	2	3	0

(PR = Prohaemocytes, PL = Plasmatocytes, GR = Granular cells, SP = Sperulocytes, OE = Oenocytoids)

The THC and DHC of five multivoltine [Nistari, Nistari (DN) p, M12 (w), M6DP(C), and Debra] and three bivoltine (BHR2, SK6 and SK7) breeds were enumerated in healthy condition of 5^{th} day 5^{th} larval instar. In multivoltines the haemocyte counts ranged from 1.5 x 10^6 to 3.3 x10^6 /mm^3 whereas in bivoltine the counts varied between 3.0 x 10^6 to 4.4 x 10^6 / mm^3 in mature 5^{th} instar larvae (Table 2a and 2b).

It is evident that largest population of cells among all the multivoltine breeds is plasmatocytes followed by prohaemocytes, granulocytes, oenocytoids and lastly spherulocytes. But there was no significant differeneces in DHC counts within the breeds (Table 3a). But in case of differential haemocyte counts in bivoltine breeds the largest population of cells among the breeds is the prohaemocytes followed by granulocytes, sphererulocytes, oenocytoids and then lastly plasmatocytes. But there was no significant difference in DHC counts within the breeds (Table 3b).

Table 2a : Total unfixed circulating haemocytes in multivoltine breeds. Data represent Range ± SD of mean (n= 5).

Breeds	**THC Range /mm^3 (Means)**	**SD of Means**
Nistari	3.3 x 10^6 - 3.75 x 10^6	± 0.04
Nistari DN(p)	1.8 x 10^6 - 3.58 x 10^6	± 0.16
Debra	1.9 x 10^6 - 3.65 x 10^6	± 0.08
M6DP(C)	3.0 x 10^6 - 3.15 x 10^6	± 0.07
M12 (w)	1.5 x 10^6 - 2.32 x 10^6	± 0.04

Table 2b : Total unfixed circulating haemocytes in bivoltine breeds

Breeds	**THC Range /mm^3 (Means)**	**SD of Means**
SK6	4.4 x 10^6 - 4.65 x 10^6	± 0.05
SK7	4.0 x 10^6 - 4.35 x 10^6	± 0.04
BHR2	4.2 x 10^6 - 4.55 x 10^6	± 0.04

Table 3a : Differential haemocyte counts of multivoltine breeds. Data represent Mean ± SE (n= 5).

Breeds	**Differential Haemocyte Counts (x 10^6 / ml)**				
	Prohemo cytes	Plasmato cytes	Granulo cytes	Spherulo cytes	Oenocytoids
Nistari	0.99±0.01	1.16±0.01	0.49±0.01	0.16±0.01	0.49±0.01
Nistari DN(P)	0.54±0.01	0.61±0.01	0.27±0.01	0.09±0.01	0.27±0.02
Debra	0.57±0.02	0.68±0.01	0.28±0.01	0.09±0.01	0.28±0.01
M6DP(C)	0.90±0.01	1.06±0.02	0.45±0.02	0.15±0.01	0.45±0.01
M12(W)	0.45±0.01	0.53±0.04	0.22±0.02	0.07±0.01	0.22±0.01

Table 3b : Differential haemocyte counts of bivoltine breeds

Breeds	**Differential Haemocyte Counts (x 10^6/ ml)**				
	Prohemo-cytes	Plasmato-cytes	Granulo-cytes	Spherulo-cytes	Oenocytoids
SK6	1.54±0.02	0.42±0.01	1.32±0.01	0.44±0.02	0.28±0.01
SK7	1.40±0.02	0.45±0.03	1.20±0.02	0.48±0.01	0.35±0.01
BHR2	1.47±0.02	0.45±0.02	1.26±0.01	0.50±0.02	0.50±0.02

It was noted that initially there is a sharp fall in the unfixed circulating haemocytes at 3 to 6 h.p.i. and then THC levels reaches (and in some cases exceeds) the normal levels. At 12 h.p.i. the THC was recorded to fall indicating the deployment of defense cells (mainly plasmatocytes and granular cells) to combat the multiplying bacteria. The initial decrease in the THC's is indicative of the prompt deployment of cells to the injury site to combat the invading pathogens. Thereafter hemopoesis occurs to replenish the depleted circulating cells. However, once the live bacteria start multiplication in the logarithmic phase the THC again decreases with further deployment of cells. In this 'physiological breed' since the quantum of inoculated bacteria was large, the defense process of the silkworm is slowly overcome by the pathogen.

A differential response based on THC and DHC was recorded in both bivoltine and multivoltine breeds of silkworm to the three kinds of bacteria (Table 4a, 4b, 5a, 5b).

Discussion

Information on the haemocyte population within an insect is essential for many types of physiological studies. The haemocyte picture of an insect at a particular time, or from one time to another, is called the hemogram and consists of measuring the total haemocyte count (THC), differential haemocyte count (DHC) along with the absolute haemocyte count (AHC), and the haemolymph or blood volume (BV) where necessary.

The haemolymph of *B. mori* showed the classic haemocyte types viz., Plasmatocytes, Prohaemocytes, Granulocytes, Oenocytoids and Spherulocytes described by other authors for Lepidopteran species and results are also in agreement with the work on the ultrastructure of larval haemocytes of *B. mori* (Nittono, 1960; Akai and Sato, 1973; Beaulaton, 1979; Wago, 1991; Yamashita and Iwabuchi, 2001). But the present are not in concurrent with the observations of Lea (1964), Yeager (1945), Shapiro (1966) and Arnold and Hinks (1976). Oenocytoids exhibited phenoloxidase activity, which is in agreement with the observations of Rebeiro *et al*., (1996). Spherule cells may be related to silk synthesis, which is also in consistent with the work of Nittono (1960).

Table 4a : Total Haemocyte Count (THC) (x 10^3) / mm^3 of bivoltine breeds in response to bacteria over various h.p.i.

Breeds		1 hour	3 hour	6 hour	9 hour	12 hour
BHR 2	A	3.79 ± 0.25	3.76 ± 0.21	3.80 ± 0.30	3.76 ± 0.35	3.79 ± 0.13
	B	2.88 ± 0.41	3.84 ± 0.45	3.72 ± 0.15	3.96 ± 0.42	2.64 ± 0.49
	C	1.93 ± 0.22	1.87 ± 0.33	3.32 ± 0.27	3.20 ± 0.23	2.20 ± 0.27
	D	3.32 ± 0.15	3.18 ± 0.34	3.45 ± 0.15	3.28 ± 0.35	3.32 ± 0.15
SK7	A	2.76 ± 0.64	3.12 ± 0.38	2.97 ± 0.32	3.16 ± 0.27	3.30 ± 0.13
	B	1.68 ± 0.20	1.98 ± 0.11	3.08 ± 0.35	2.48 ± 0.33	1.74 ± 0.13
	C	1.72 ± 0.57	1.83 ± 0.35	3.32 ± 0.20	3.12 ± 0.38	1.84 ± 0.31
	D	2.54 ± 0.13	2.45 ± 0.20	3.56 ± 0.33	2.28 ± 0.34	2.16 ± 0.25
SK6	A	3,48 ± 0.27	3.48 ± 0.27	3.62 ± 0.21	3.50 ± 0.24	3.57 ± 0.16
	B	2.51 ± 0.13	2.49 ± 0.18	4.26 ± 0.29	2.92 ± 0.15	2.02 ± 0.34
	C	2.05 ± 0.14	1.84 ± 0.32	3.60 ± 0.27	3.08 ± 0.23	2.08 ± 0.15
	D	3.32 ± 0.15	3.18 ± 0.34	3.45 ± 0.15	3.28 ± 0.35	3.32 ± 0.15

Table 4b : Total Haemocyte Count (THC) (x 10^3) / mm^3 of multivoltine breeds in response to bacteria over various h.p.i.

Breeds		1 hour	3 hour	6 hour	9 hour	12 hour
Debra (p)	A	1.88 ± 0.27	1.89 ± 0.44	2.12 ± 0.20	2.14 ± 0.18	2.09 ± 0.19
	B	1.39 ± 0.21	1.76 ± 0.22	2.56 ± 0.29	2.35 ± 0.07	1.69 ± 0.18
	C	1.18 ± 0.13	2.20 ± 0.20	2.40 ± 0.29	2.00 ± 0.21	1.56 ± 0.18
	D	1.40 ± 0.33	1.66 ± 0.21	2.55 ± 0.30	2.92 ± 0.40	1.84 ± 0.09
Nistari	A	3.44 ± 0.20	3.40 ± 0.14	3.45 ± 0.24	3.57 ± 0.17	3.70 ± 0.12
	B	1.96 ± 0.35	2.25 ± 0.18	3.21 ± 0.37	3.64 ± 0.27	2.23 ± 0.12
	C	1.82 ± 0.18	2.20 ± 0.16	2.76 ± 0.15	3.05 ± 0.26	2.04 ± 0.24
	D	3.32 ± 0.15	3.18 ± 0.34	3.45 ± 0.15	3.28 ± 0.35	3.32 ± 0.15
Nistari DN (p)	A	2.76 ± 0.27	2.75 ± 0.23	2.74 ± 0.31	2.65 ± 0.27	2.69 ± 0.21
	B	1.94 ± 0.09	2.13 ± 0.14	2.16 ± 0.29	2.36 ± 0.15	1.75 ± 0.06
	C	1.40 ± 0.33	1.66 ± 0.21	2.55 ± 0.30	2.92 ± 0.40	1.84 ± 0.09
	D	2.00 ± 0.21	2.71 ± 0.13	3.56 ± 0.15	3.39 ± 0.14	2.68 ± 0.20
M6DP	A	3.48 ± 0.42	3.69 ± 0.13	3.52 ± 0.50	3.36 ± 0.22	3.44 ± 0.08
	B	2.36 ± 0.15	2.72 ± 0.26	3.69 ± 0.13	3.77 ± 0.07	2.61 ± 0.25
	C	2.00 ± 0.21	2.71 ± 0.13	3.56 ± 0.15	3.39 ± 0.14	2.68 ± 0.20
	D	3.32 ± 0.15	3.18 ± 0.34	3.45 ± 0.15	3.28 ± 0.35	3.32 ± 0.15
M12(W)	A	2.92 ± 0.24	2.33 ± 0.07	2.34 ± 0.06	2.35 ± 0.09	2.32 ± 0.27
	B	1.52 ± 0.42	1.85 ± 0.15	2.64 ± 0.20	3.48 ± 0.30	1.96 ± 0.33
	C	1.28 ± 0.13	1.96 ± 0.27	2.40 ± 0.26	2.24 ± 0.22	1.80 ± 0.14
	D	2.32 ± 0.07	2.52 ± 0.07	2.88 ± 0.37	3.07 ± 0.19	2.19 ± 0.14

A=Healthy, B=Medium Pathogenic bacteria, C=Highly Pathogenic bacteria D=Non-pathogenic bacteria

Table 5a : Approximate Differential Haemocyte Count (DHC) (x10^3 /cu.mm) of plasmatocytes and granulocytes upon infection with bacteria at 12 h.p.i. (converted to absolute values from percentage) among multivoltine races.

RACE		Cell counts (x 10^3 /cu.mm)		Approx Cells Deployed (x 10^3 /cu.mm)		Nodulation response against bacterial infection (No.)
		Plasmatocytes	Granulocytes	Plasmatocytes	Granulocytes	
Nistari	A	1.11	0.59	0.87	0.43	57.45± 0.15
	B	0.24	0.16			
Nistari DN(p)	A	0.75	0.43	0.60	0.34	36.23 ± 0.12
	B	0.15	0.09			
M12(w)	A	0.65	0.37	0.47	0.26	28.35 ± 0.05
	B	0.18	0.11			
M6DP	A	0.96	0.52	0.70	0.36	32.05 ± 0.15
	B	0.26	0.16			
Debra	A	0.75	0.42	0.58	0.34	48.52 ± 0.34
	B	0.17	0.08			

A = Healthy, B = Infected

Table 5b : Approximate Differential Haemocyte Count (DHC) (x 10^3 / cu.mm) of plasmatocytes and granulocytes upon infection with bacteria at 12 h.p.i. (Converted to absolute values from percentage) among bivoltine races.

RACE		Cell counts (x 10^3 /cu.mm)		Approx Cells Deployed (x 10^3 /cu.mm)		Nodulation response against bacterial infection (No.)
		Plasmatocytes	Granulocytes	Plasmatocytes	Granulocytes	
BHR-2	A	1.16	0.73	0.22	0.17	18.34 + 0.14
	B	0.94	0.56			
SK6	A	1.26	0.70	0.34	0.15	24.67 ± 0.45
	B	0.92	0.55			
SK7	A	1.23	0.62	0.20	0.06	12.68 ± 0.14
	B	1.03	0.56			

A = Healthy, B = Infected

In insects, morphologically distinct haemocyte types (Price and Ratcliffe, 1974) have been shown to play different roles in the host defense reactions. In the silkworm *B. mori*, classification of haemocytes has historically been based on their morphology at the light microscope level (Iwasaki, 1930; Nittono, 1960). In *B.mori* there is a steady increase in the total haemocyte counts during larval development with the peak counts being reached at the mature 5^{th} instar larvae. The observation is in agreement with the work of Jones (1967). Further, the increment in count is in concurrence with an increase in haemolymph volume during various stages of development of the 5^{th} instar larvae. A fall in THC at the spinning day is also reflected by a fall in haemolymph volume on that day and simultaneously hemopoesis starts which is more in agreement with the observations of Rebeiro et al (1996) in the Lepidopteran, *Mythimna unipuncta*.

Only the granulocytes and plasmatocytes are apparently involved in nodule formation which is in agreement with the study of Krishnan *et al*. (2000). Thus it is apparent that after attaching to the target, granular cells initiate capsule / nodule formation by releasing factors that induce plasmatocytes to adhere to the target. This is supported by the experimental studies conducted by Krishnan et al (2000), which indicate that neither granular cells nor plasmatocytes are capable of forming a capsule independently.

It was observed that *B. mori* larvae are able to calibrate nodulation reactions to the sizes of the bacterial infection. It is in agreement with the observation made by Rahmet-Alla and Rowley (1989). The findings of this study are consistent with the general idea that the nature of infecting bacteria influences the nodulation response. High level of nodulation was noted in response to *Bacillus thuringensis* var. Sotto infection followed by significantly lower levels of nodules in response to *Bacillus subtilis*. Finally, *Escherichia coli* induced the least number of nodules. It is in consistent with the results reported by Howard et al (1998) in *Manduca sexta* and *Zophobas atratus*.

Nodulation is the first and quantitatively predominant cellular defense reaction to bacterial infection in most insects (Horohov and Dunn, 1983) and depends upon eicosanoid biosynthesis (Jurenka et al 1997). The findings of the work of Miller et al (1994, 1996) suggested that the ability to form nodules did not completely ablate due to inhibition of eicosanoid biosynthesis. Other signalling moieties are also involved in nodule formation in the event of inhibition of eicosanoid biosynthesis (Howard, *et al*., 1998). Microaggregates in *B.mori* chiefly consists of granulocytes, which release flocculent materials after lysis. Presumably through chemotaxis the microaggregates continue to grow attracting haemocytes and attached bacteria. In this process, plasmatocytes spread around the microaggregates (Krishnan et al 2000). The overall process culminates with the phase of melanization, which leaves darken nodules containing haemocytes, bacteria and some haemocytes, which have phagocytosed bacterial cells deposited in fat bodies and other internal tissues of the silkworm. This is in consistence with the results obtained by Rahmet-Alla and Rowley (1989) in their work with the Madeira Cockroach, *Leucophaea maderae*. Nodule formation is a rapid process, taking place within a few minutes of encounter with the foreign material (Ratcliffe and Gagen, 1977). It is, however, likely that cellular reactivity is enhanced or depressed depending on the insect species and size (Ratcliffe and Walters, 1983) and mode of pathogenicity of the bacterial strains.

The most important components of the bacterial wall, in terms of induction of nodule formation, are peptidoglycan, specially the lysozyme soluble fraction and the capsular polysaccharide. This capsular polysaccharide acts as an elicitor of nodule formation (Brookman et al 1989). They also established the participation of the pPO cascade in a cellular defense reaction in insects in nodule formation process. The identification of the nodules was normally relatively easy due to both their size and melanized appearance. They were loosely embedded in the fat body and on the surface of the alimentary canal. The extent of nodule formation in response to bacteria has been linked to the number of bacteria present in the challenge and to the pathogenicity of the species for the host (Walters and Ratcliffe, 1983).The principal defense reaction against bacteria was demonstrated to be the nodulation reaction in *B. mori*. The nodulation reaction is a temporal and can easily be quantified.

Finally, it is clear from the study that multivoltines in general with more circulating defense cells were capable of forming more nodules and hence better defending themselves against bacterial invasion. The difference in the differential response *per se* lies in the differences in the circulating cells.

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On the relation of meteorological parameters and malaria transmission

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Abstract : Malaria incidences are increased day by day particularly in Kolkata. The malaria incidence as per records is found maximum in the year 2004. The incidence of *vivax* malaria is found highest during the months August to October, whereas malignant malaria is highest in the month of October and November. The cases of *falciparum* malaria are found to increase from April to September. The average relative humidity of Kolkata (~70%) throughout the year is suitable for the production and transmission of malaria parasite. The transmission of malaria parasite is highest during July to September when the atmosphere is always humid. From the survey it is noted that the average malaria incidences per year at Bagbazar (borough 1) is 119.41. Statistical analysis shows that the correlation of *vivax* malaria with temperature is highly significant (r=0.84). Further investigations are required to infer a direct relationship of malaria transmission with climate.

Key words **:** *Malaria, Climate, Environment, Transmission*

Introduction

The World Health Organization ranked malaria in the eighth position among the global burden diseases in 2004. Malaria caused mortality and morbidity is high in tropics. In India this disease imposes enormous human and economic costs on the country (Mahapatra *et al*, 1999; Dev *et al*, 2001; Bhattacharya *et al*, 2007). Malaria parasite was identified by a military doctor Charles Louis Alphonse Laveran of Algeria in 1880. After that another researcher Dr. Ronald Ross took several years to bridge the life cycle of malaria parasite in humans and mosquito (Tren, 2002). The host of the malaria epidemiology is man and the vectors are protozoan parasite *Plasmodium* and anopheline mosquitoes. Transmission dynamics of vector borne diseases are very sensitive to climate. The climatic variables that have significant influence on transmission dynamics of malaria are ambient temperature, relative humidity and precipitation (Martens *et al*, 1995; Akhtar and McMichael, 1996; Singh and Sharma, 2002; Prakash *et al*, 2003a). Therefore public concern is raised over the risk of the disease due to climate change. The official number of malaria incidences is found about 2 million per year in India. This number is almost stabilized after 1989 as depicted from Fig. 1. There are severe outbreaks or epidemics of the disease on every five to seven years in India (Sharma, 1999; Teklehaimanot *et al*, 2004; Bhattacharya *et al*, 2006; Ramesh *et al*, 2008).

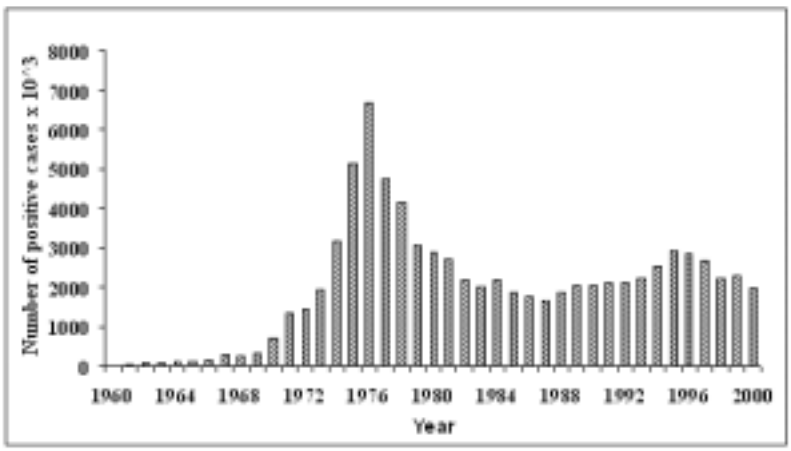

Fig. 1 : Positive malaria cases during the period 1960 – 2000 in India

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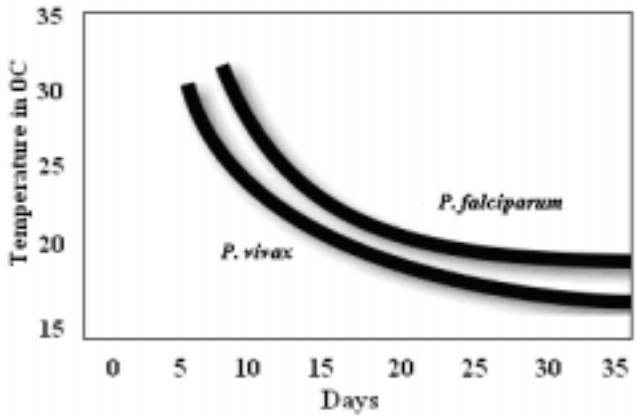

Fig. 2 : Minimum Temperature for development of *Plasmodium falciparum* and *Plasmodium vivax* parasite

There are six major species of malaria vectors *viz Anopheles culicifacies* (rural), *Anopheles stephensi (*urban) *Anopheles fluviatilis* (foothills) *Anopheles sundaicus* (Andaman and Nicobar Islands) and *Anopheles minimus* and *Anopheles dirus* (NE India). The two entomological variables, duration of gonotrophic cycle and extrinsic incubation period are very sensitive to environmental temperature (Gubler *et al*, 2001; Reiter, 2001; Pemola and Jauhari, 2006). Minimum temperature for development of *Plasmodium falciparum* and *Plasmodium vivax* parasite (Fig. 2) in anopheline mosquitoes are respectively 18ºC and 15ºC. Both *Plasmodium falciparum* and *Plasmodium vivax* occur in abundance in tropical countries but *Plasmodium falciparum* is the killer parasite which accounts to 65% of the incidences. 15°C to 40°C is assumed to be the broad malaria transmission window in terms of temperature. It has been observed that the *Plasmodium vivax* requires 15 to 25 days to complete its life cycle if the temperature remains in between 15°C to 20°C whereas life cycle may be completed in 6 to 10 days for the temperature >25°C provided the relative humidity lies from 55% to 80%. The best transmission window of malaria in India as reported by Bhattacharya *et al* (2006) is illustrated in Table I. The number of days decreases with temperature as we move from class I to class III.

Table I : Transmission window of malaria

Parasite	Class	Temperature (°C)	Complete life cycle (days)
Plasmodium vivax	I	15 - 20	15 - 25
	II	20 - 25	10 - 20
	III	25 - 30	06 - 10
Plasmodium falciparum	I	20 - 25	20 -30
	II	25 - 30	15 - 25
	III	30 - 35	08 -12

Table II : Climatic conditions and potential of malaria

	Cause	Effect
I	Heavy rain over preceding season	Larger reservoir of parasites /vectors
II	Warm preceding summer	
III	Prolonged high rainfall	
IV	Prolonged high temperature	
I	High water table	Transmission starts early
II	Preceding winter wet	
III	Preceding winter warm	
III	Early rain/temperature rise in spring	
I	Wet summer	Extremely high transmission
II	Warm summer	
I	Prolonged autumn rainfall	Transmission ends late
II	Prolonged high autumn temperature	

The climate risk factors depend on the degree of dryness and wetness, duration of warm days and also time of heavy rainfall (Tonnang *et al*, 2010). The Climatic conditions and the probability of malaria transmission are presented in Table II (Craig *et al*, 2004). It is observed that 0.5°C increase in temperature may cause 30% - 100% increase in mosquito abundance *i.e* an extremely high biological amplification (Pascual *et al*, 2006). High resolution climate model HadRM2 which integrate the atmosphere, surface variables, cloud covers, insolation *etc*. are used by Bhattacharya *et al* (2006) to identify the endemic regions of India during the period 1980 to 2000 and the regions to be affected by 2050 as shown in Fig. 3. This analysis will be helpful to take preventive measures to control the outbreak well in advance in addition to the conventional methods (Prakash *et al*, 2003b; Dev *et al*, 2006).

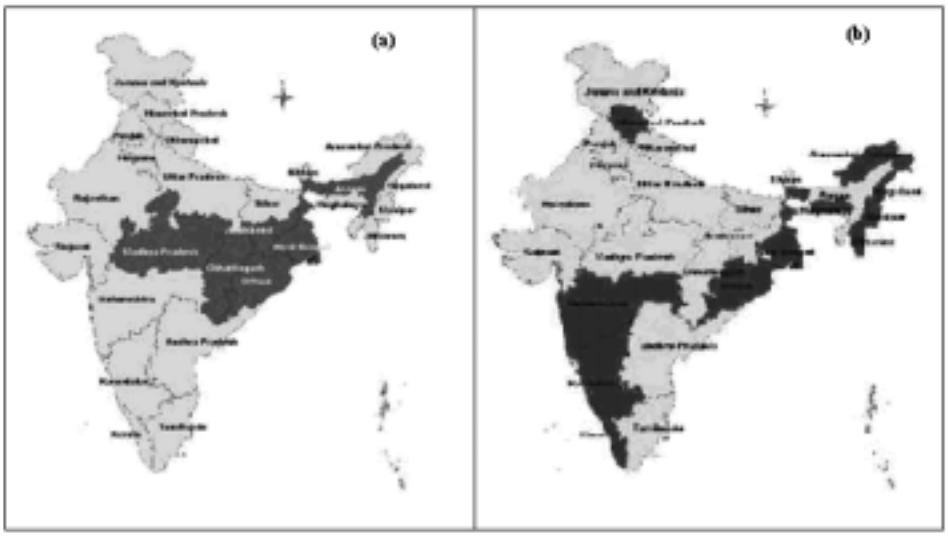

Fig. 3 : (a) Malaria prone regions and (b) likely to be affected by 2050 shown by dark shade

Increasing trend of surface temperature, change of rainfall pattern with respect to normal and occurrence of extreme events are the signals of climate change. As a result there must be growing concern about the climate sensitive diseases like malaria. The survival of malaria parasite also depends on the time of the year. The average temperature in West Bengal is around 34°C particularly in summer months and hence experience reduction of transmission window. However, there is still a growing need to identify the influencing factors, malaria prone zones and early warning system to take protective measures. The objective of the present work is to identify the causes behind the malaria transmission in Kolkata, West Bengal.

Materials and methods

Study Area : The study area extends from $22^{0}62^{/}$ N to $22^{0}68^{/}$ N and $88^{0}42^{/}$ to $88^{0}48^{/}$ E covering the Kolkata Municipal Corporation (KMC) area as shown in the Fig. 4. The population of the city is about fifty two lakhs. The region is highly prone

Fig. 4 : Map showing study area (source: www.mapsofindia.com)

to malaria transmission due to excessive rainfall particularly in the month of September promoting vector breeding and longevity due to humid and warmer climates. The climate normals of Kolkata as obtained from India Meteorological Department are given in the Table III.

Table III : Climate normals of Kolkata

Months	Daily Mean Temperature (ºC)		Rain fall	Rainy Days
	Maximum	Minimum	(mm)	
January	26.6	13.9	16.8	0.9
February	29.7	16.9	22.9	1.5
March	34.0	21.7	32.8	2.3
April	36.3	25.1	47.7	3.0
May	36.0	26.4	101.7	5.9
June	34.1	26.5	259.9	12.3
July	32.2	26.1	331.8	16.8
August	32.0	26.1	328.8	17.2
September	32.2	25.8	295.9	13.4
October	31.9	24.0	151.3	7.4
November	29.8	18.9	17.2	1.1
December	27.0	14.3	7.4	0.4

Data Collection : Data of malaria incidences in Kolkata are collected from Kolkata Municipal Corporation and Calcutta School of Tropical Medicine for the period 2000 to 2007. In addition a questionnaire including personal details, living environment, health conditions, literacy *etc* is prepared for face to face interview from the affected locality. Some sample environmental conditions of the malaria prone area under Kolkata Municipal Corporation are sited in Fig. 5.

Fig. 5 : Environmental conditions of the survey area (a) resting, (b)) adult and (c) & (d) breeding sites of mosquitoes

Daily records of temperature and relative humidity are obtained from National Data Centre, Pune. Silva ADC Pro (Resolution: Temperature 0.1°C, Relative humidity 0.1%) is used to record the environmental temperature of the locality during survey.

Analysis : MATLAB 7.0.4.365 (R14) Service Pack 2 is used to compute the data.

Results and discussion

The comparative epidemiological data on malaria incidences during the period 2000 – 07 as registered by the State Health Department are presented in Fig. 6. The malaria incidences as obtained from the record are found to be maximum

in the year 2004. The probability of mixed malaria is found less in comparison with *vivax* and *falciparum*. The month wise variation of malaria incidences during 2000 – 2007 is shown in Fig. 7. Variation of climatic parameters and associated malaria cases are represented in Fig. 8.

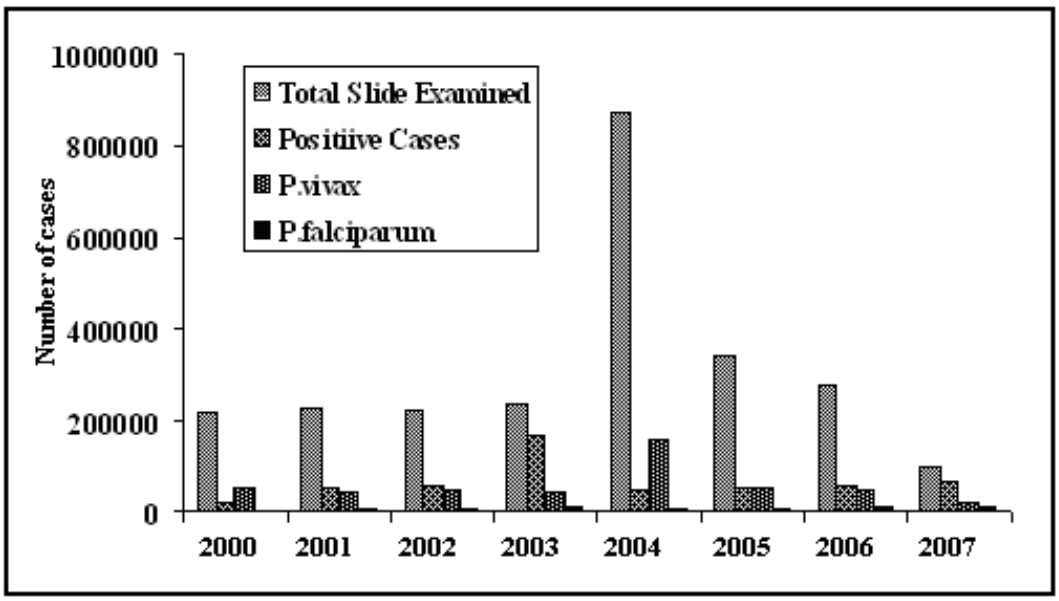

Fig. 6 : Comparative data of year wise malaria incidences

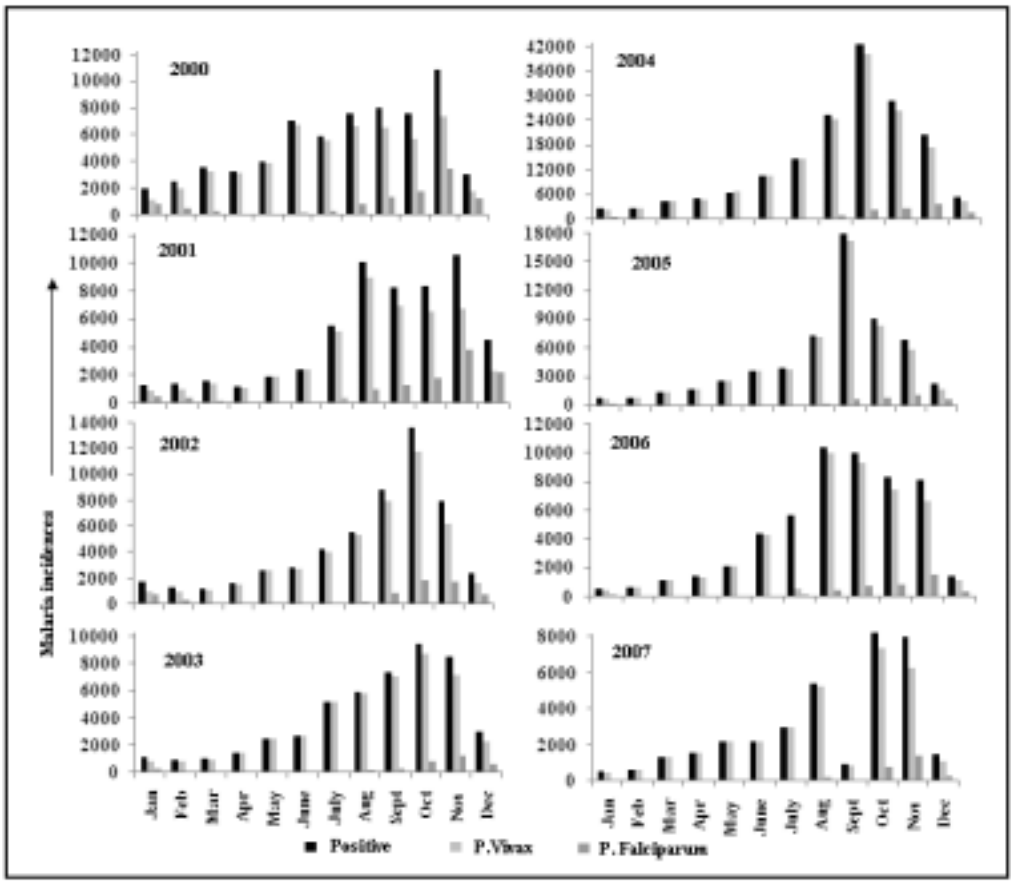

Fig. 7 : Variation of malaria incidences on different months of the year

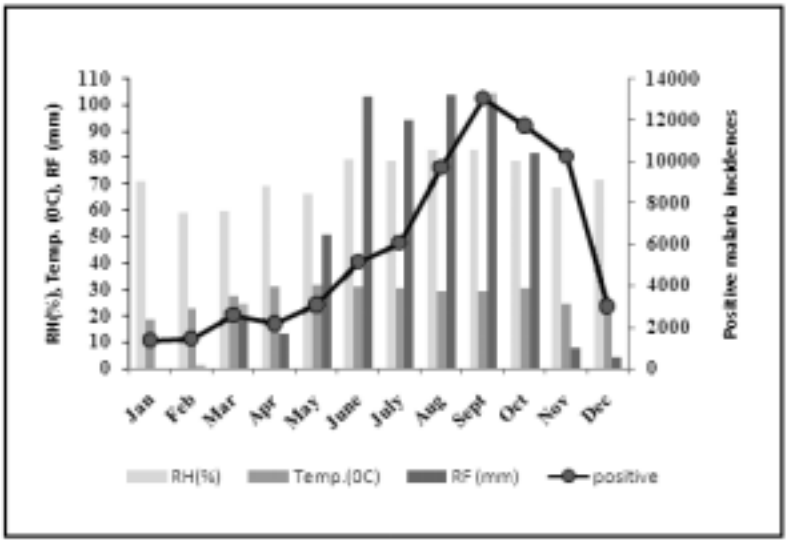

Fig. 8 : Variation of meteorological parameters and associated malaria incidences

The correlation of malaria incidences caused by *vivax*, *falciparum* and mixed malaria with the climatic parameters are given in Table IV. The correlation of *vivax* malaria with temperature is significant at $p < 0.01$.

Table IV : Correlation coefficient between malaria parasites and surface parameters

Parameter	Vivax	Falciparum	Mixed
Rainfall	0.49	-0.45	-0.49
Relative humidity	0.05	-0.05	-0.15
Temperature	0.84	-0.48	-0.79

From the survey it is noted that the locality of the effected persons may be one of the causes of malaria transmission of that area. It is also noted that majority of the affected persons never use mosquito net and they have lack of awareness about the precautions needed to prevent malaria transmission

Conclusions

It is observed from the analysis that the malaria incidence increases in dry season. Meteorological parameters are not sufficient to explain malaria transmission. Environmental conditions of a particular locality may also play key role (Guerra *et al*, 2008). Changing landscape pattern may have great influence on local climate compare to long term effect of climate change. Temperature, evaporation, surface runoff, PET, water logging etc are affected by the change of land cover and hence have significant influences in determining the mosquito abundance of that particular locality (Patz *et al*, 2005; Foley *et al*, 2005; Patz and Olsen, 2006). Presence of malaria vectors does not always translate into disease. There are many places in the globe where this vector borne disease disappeared in spite of having Anopheles species. Improved structure of house, water management, adequate health service, life pattern, living environment etc is the determinants for malaria transmission.

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Nematode infection in the fishes of Dolu Lake, Silchar, Assam, during different seasons of the year

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Abstract : A total of 749 fishes of different species of Dolu Lake were examined for parasitic infection. Out of 749 fishes examined, 53(7.07%) fishes were found to be parasitized by nematodes. The highest intensity was found 62 in *Mastacembelus armatus.* The highest percentage of infection was found 81.81% in *Notopterus notopterus* in Autumn and lowest was found 5.9% in *Channa punctatus* in Winter. The total highest percentage of infection was 108.3 in Summer and lowest was 53.33 in Spring.

Key Words **:** Fishes, Helminth, Sex, Length, Dolu Lake.

Introduction

Nematode parasite causes infection and diseases of fish both in freshwater and marine environments. Small numbers of nematodes often occur in healthy fish, but high numbers cause illness or even death. Juvenile fish infected by small numbers of nematodes are more likely to show signs of illness and also have reduced growth rates. Therefore it is an essential area for proper attention to be given by the scientists for sustainable aquaculture production.

The first comprehensive report on seasonal occurrence fish parasites was given by Chubb (1977b) who concentrated on the monoseneans of freshwater fish and this was followed by annually extensive studies of the same topic for digeneans by Chubb (1979), larval cestode and nematode by Chubb, (1980b) and cestoda, nematode and Acanthocephala by Chubb (1982).

Seasonal diversity of worms in the flesh and other organ systems of various marine food fishes investigated by Linton (1914). Sinderman (1966) showed that the effect of worm larvae on the host fish included growth retardation, tissue disruption, metabolic disturbances and even death in heavy infections. Rumpus (1974) discussed the seasonal fluctuation of parasites and the effect of the size maturity and sex of fishes on the incidence and intensity of their parasites.

The influence of parasitic infection in relation to seasons has been studied by many workers like Shomorendra *et.al*., (2005,2007), Chubb (1982), Pennycuick (1971), Jha and Sinha (1990), Tavares and Luque (2004).

In Assam, particularly in Barak Valley studies on the frequency of nematode infection and its variation due to seasons is limited. This study mainly fulfills the gaps in this direction.

Study Area : Fishes examined in this study were collected from Dolu Lake which is situated $24^0$55'23.4''N and $92^0$47'21.5''E. The lake water ranges from 20^0C - 23^0C during December- January and maximum is 31^0C - 32^0C during June-July. The pH value fluctuates between 6.38 to 7.08. The maximum value was recorded during April.

Materials and Methods

Different species of fish from Dolu lake were collected during different months of the year. The identification of each fish were determined after following Jayaram (2010). The external body surface and internal organs were thoroughly examined for parasites. After being relaxed the collected parasites were fixed in the fixatives prescribed for different parasite groups. Trematodes were fixed in AFA (alcohol-formalin-acetic acid) solution, stored in 70% alcohol. Acanthocephala were fixed and preserved in AFA , cestodes in 5% formalin and nematode after immersing in hot 70% alcohol, were finally stored in 70% alcohol after following Bylund et.al (1980).

Results

A total of 749 fishes of different species of Dolu Lake were examined for parasitic infection. Out of 749 fishes examined, 53(7.07%) fishes were found to be parasitized by nematodes. The highest intensity was found 62 in *Mastacembelus armatus* (Table-I. A & B). The highest percentage of infection was found 81.81% in *Notopterus notopterus* in Autumn and lowest was found 5.9% in *Channa punctatus* in Winter. The total highest percentage of infection was 108.3 in Summer and lowest was 53.33 in Spring.(Table-II).

Table-I.(A) Nematode parasite burden in the fishes of Dolu Lake.

Fish species	No. of fishes Examined	No. of fishes Infected	Percentage of infection	Intensity of infection
Channa punctatus	126	3	2.38	1.33
Notopterus notopterus	59	38	64.40	13.57
Heteropneustes fossilis	55	1	1.81	1
Mystus tangara	58	2	3.44	1
Clarias batrachus	8	2	25	1
Colisa fasciatus	21	2	9.52	3.5
Mastacembelus armatus	3	1	33.33	62
Channa striata	8	2	25	5
Lepidocephalus guntea	14	1	7.14	1
Channa orientalis	8	1	12.5	1

Table-I.(B). Prevalence and intensity of nematode infection in the fishes.

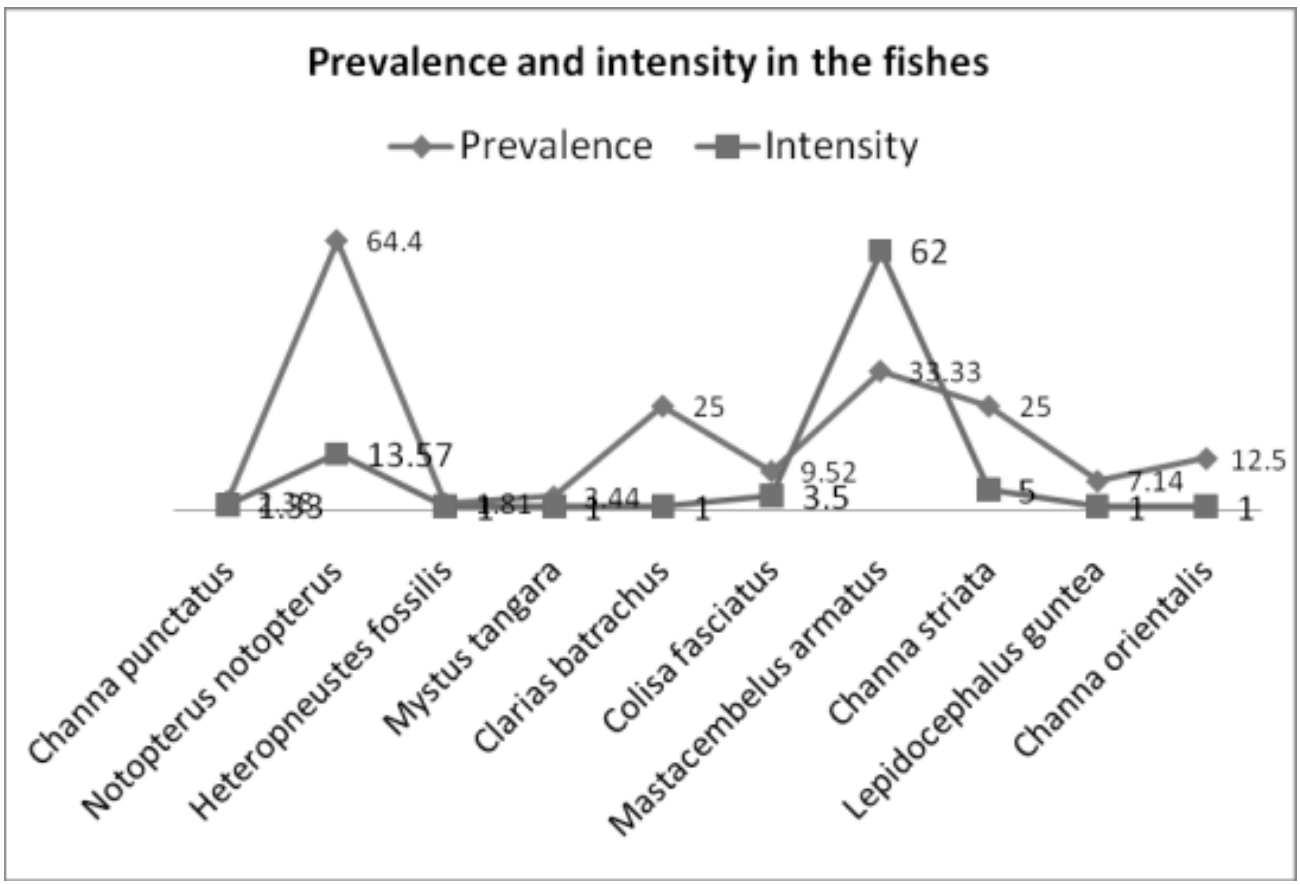

Table- II. Percentage of infection in the four different fish species during different seasons at different water temperature and pH.

Fish species	Autumn		Winter	Spring	Summer
	Temp (^{0}C)	28^0 C – 32^{0}C	20^{0}C – 25^{0}C	24^{0}C - 27^{0}C	29^{0}C -32^{0}C
	pH	5.5 - 7.7	6.4 - 6.8	6.4 - 7.0	6.3 - 6.5
Channa punctatus		9.0	5.9		
Notopterus notopterus		81.81	66.66	33.33	75
Heteropneustes fossilis				20	
Mystus tangara			9.0		33.3
Total		90.81	81.56	53.33	108.3

The parasite species collected from the fishes of the study area are :

Nematode

1) Species : ***Pseudoproleptus notopteri***
   Host : *Notopterus notopterus*
   Location : Mouth, stomach, Intestine.
2) Species : ***Pseudoproleptus vestibulus***
   Host : *Notopterus notopterus*
   Location : Mouth, stomach, Intestine
3) Species : ***Paragendria* sp**
   Host : *Channa striata*
   Location : Intestine
4) Species : ***Parascarophis* sp**
   Host : *Lepidocephalus guntea, Channa punctatus*
   Location : Intestine
5) Species : ***Camallanus* sp**
   Host : *Colisa fasciatus, Clarias batrachus*
   Location: Intestine
6) Species : ***Paracamallanus* sp**
   Host : *Colisa fasciatus*
   Location : Intestine
7) Species : ***Spinitectus* sp**
   Host : *Notopterus notopterus*
   Location: Intestine
8) Species : ***Contracaecum* sp**
   Host : *Lepidocephalus guntea*
   Location : Intestine

Discussion

The highest percentage of infection by nematodes was found in *Notopterus notopterus*. This findings supports earlier work of Mgbemena (1983) who discovered higher number of nematode infection on *C.tilapiae*. Llcwellyn proved that there was an exacting topographical relationship between a parasite and its host and this was probably an important factor in the

mechanism of host specificity. Highest infestation rate among nematodes have been observed by the *Pseudoproleptus vestibulus* and *Pseudoproleptus notopteri*, adult recovered from stomach and intestine.

It can be noted from the data that the percentage of infection in different fish species is more or less variable with seasons of the year. The total value of infection for all four fish species is found to be highest (108.3%) in Summer and lowest is (53.33%) in Spring. But in Autumn the highest percentage of infection was found 81.81% in *Notopterus notopterus* and in Winter lowest was found 5.9% in *Channa punctatus*. This findings agreed in case of Autumn only with Moharram (1980) who said,' Autumn and Winter are the two seasons in which we meet the biggest number of infected fish with cope pods, as well as the greatest number of parasites per fish. The present observations showed that the effect of temperature on the fishes is an important factor for the infestation rate of parasites. During the hot season, the infestation rate was high. It has also been reported by Kelle, 1977; Bussmann and Ehrich, 1979; Fatima and Bilqees, 1989.

One of the most important factors to emerge is environmental temperature, which affects the life cycles of intermediate and definitive hosts and parasites, temperature also affects the immune response of the fish hosts, which has itself emerged as an important factor in seasonal variations, producing seasonal change in resistance to infection. Many of these aspects were discussed by Polyanski (1958) in reviews of the ecology of parasites of marine fishes. Biotic factors such as overcrowding or interaction with other parasites and abiotic factors such as temperature, they all contribute to the pattern of seasonal variation.

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Nature of parasitic infestations in three economically important *Labeo* spp. inhabiting natural and cultured water bodies of Tripura

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Abstract : A detailed investigation was conducted on the parasitic fauna harbored by three species *Labeo calbasu*, *L. gonius* and *L. bata* of genus *Labeo* Cuvier, 1816 collected from the four different districts of Tripura. The survey was done for a period of two years from April 2007 to March 2009. The host fishes were collected in live condition for carrying out the pathological examinations. In all the host species, external infections especially on the gills, fins, scales and skin was found most prevalent than infections on the visceral organs. Majority of the infected fishes including fishes carrying a heavy parasitic load did not exhibit any notable external disease symptoms and appeared healthy. Parasitic fauna was represented by both protozoan and metazoan parasites. Six species of protozoan parasites and twelve species of metazoan parasites were obtained. Of the protozoan parasites there were five species of the myxozoan group and a single representative of the ciliophoran group. Metazoans were represented by five monogenean species, two digenean species, one species each of *Ergasilus*, *Neoergasilus* and *Catlaphilla* and two species of *Argulus*. With regard to seasonal analytic studies, the prevalence of infection of the protozoan and the helminth parasites showed no remarkable variations. Among the copepod parasites, *Neoergasilus longispinosus*, exhibited pronounced seasonal changes. The infection by *N. longispinosus* was intense during winter seasons with maximum load of parasites on the fins of the infected hosts. *Labeo calbasu* exhibited the maximum prevalence of infection (88.30%) and *L. bata* was the least infected with a prevalence of infection (29.50%) among the host species examined. The mean intensity of parasites was maximum in *Labeo gonius* (5.88). Myxozoan and monogenean infection was highest in *Labeo calbasu* and *Labeo bata* had the highest copepod infection. The fry and fingerling stages of the host species also showed instances of infection. Pronounced parasitic infestations in host fishes collected from the culture ponds indicate that overstocking and stunting leads to large scale multiplication of parasites in aquaculture systems.

Key words **:** *Labeo, metazoan, parasite, prevalence, protozoan*

Introduction

Tripura one among the eight north-eastern states occupies a total geographical area of 10,491 sq.km and lies in the sub-Himalayan region. Among the native minor carps of the state *Labeo bata, L. calbasu* and *L. gonius* of the genus *Labeo* Cuvier, 1816 are very popular nutritionally and commercially throughout the state. Though there is ample information on the biological and culture aspects of these *Labeo* spp. a comprehensive knowledge regarding the parasitic infestation of this group is very limited. Majority of the earlier studies done on the *Labeo* spp. of the north eastern region and of Tripura concentrated on the taxonomic classification and distribution (Ghosh and Lipton, 1982; Lipton, 1983; Barman, 1988; Govt. of Tripura, 2006) Moreover in spite of the great consumer demand for these fishes, the parasitic investigations done on them are very scarce compared to the Indian Major carp, *Labeo* rohita. Kabata (1985) reinstated the need of a thorough and comprehensive research on the fish parasites of the tropical waters which are inadequately explored in comparison with the temperate regions. The present work represents the first endeavor from the north-eastern part of the country as well as from the state of Tripura to collect an inclusive knowledge on the total parasitic fauna harbored by the three important *Labeo* spp. other than *Labeo rohita*.

Materials and methods

The survey was done for a period of two years from April 2007 to March 2009. The host fishes selected were *Labeo calbasu*, *Labeo bata* and *Labeo gonius* belonging to family- Cyprinidae and genus- *Labeo* Cuvier, 1816. They were collected from the natural collection sites like Rudrasagar Lake, the principal rivers of the state, River Gumti, River Manu, River Dhalai and a major reservoir called Dumbur reservoir and culture ponds spanning over the four districts; the North, South and West

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Tripura and the Dhalai district. The fishes were brought in live or moribund condition to the laboratory. Prior to dissection, the body weight and the standard length of the specimens were recorded. A general examination of the fish was done and abnormalities like red colorations, lesions or swellings were recorded. The specimens were then subjected to thorough examination for protozoan and metazoan parasites according to the methods proposed by Kennedy (1979), Meyers (2000) and Woodland (2006).

Pattern of infection, parasite density and monthly and seasonal variations of the parasite faunas were studied using the statistical analysis measures like prevalence of infection (P) or percentage of fish infected calculated as total fishes infected/ total fishes examined x 100; mean intensity of infection (MI) or the mean number of parasites per infected fish calculated as mean no. of parasites collected/ infected fish; simple dominance index (DI), calculated as abundance of a given parasite species/ taxa ÷ total abundance of all species/taxa; richness index (RI), calculated as (S-1) / loge N, where, S = number of species/taxa of parasites and N = total number of parasites; evenness index (EI), calculated as H / loge S, where H = Shannon index of diversity and S = number of species/taxa of parasites; shannon index of diversity (H), calculated as H = (n loge n) – (fi loge fi) / n, where n = fi; fi = abundance of parasite species/taxa expressed as percentage.

Results

1. Taxonomical Studies

Out of the thousand one hundred and twenty seven fishes examined, six hundred and seventy-four were infected with protozoan and metazoan parasites (Table1). Eighteen species of parasites comprising six protozoan species and twelve metazoan species were obtained with a total parasite count of three thousand nine hundred and nineteen.

The protozoan parasites obtained from the present study were from genera *Myxobolus* Butschli, 1882, *Thelohanellus* Kudo, 1933 and *Tripartiella* Lom, 1959. The parasite species were *Myxobolus rohitae* from the scales of all three host species; *Myxobolus* sp.I.n.sp. from gills of all three hosts; *Myxobolus* sp.II.n.sp. from gills of *Labeo calbasu* and *L. bata*. *Thelohanellus* sp.I.n.sp. from fins of all three host species ; *Thelohanellus* sp.II.n.sp. from gills of *Labeo bata* and *Tripartiella copiosa* from gills of *Labeo gonius*.

The metazoan parasites encountered in the present survey were monogenetic trematodes, *Dactylogyrus labei* from the gills of all the three hosts species; *Dactylogyrus catlaius, Dactylogyrus spinitubus* and *Dactylogyrus vicinus* from gills of *Labeo calbasu* and a new sp. of genus *Dactylogyrus* Diesing, 1850 from gills of *Labeo bata.*

The digenetic trematodes found were metacercaria of *Centrocestus formosanus* from gills of all three host species, and metacercaria of *Clinostomum* sp. from gills of *Labeo bata*.

The crustacean parasites obtained from the study were a new sp. of genus *Ergasilus* Nordmann, 1832 from the gills and a described species, *Neoergasilus longispinosus* of the genus *Neoergasilus* Yin, 1956 from the fins and gills of all the three host species; *Argulus foliaceus* and *Argulus japonicus* of genus *Argulus* Muller, 1785 were found from the scales and skin of the host fishes; *A. foliaceus* from *Labeo calbasu* and *L. bata* and *A. japonicus* from *L. gonius* and *L. bata*. A new species of genus *Catlaphilla* Tripathi, 1960 was encountered on the gills of *Labeo calbasu*.

Discussion

In all the host species external infections was found most prevalent than infection on the visceral organs. The infected fishes which carried a heavy parasitic load were also apparently healthy. From among all the three host species, *Labeo calbasu* exhibited the maximum prevalence of infection, 88.30% and *L. bata* was the least infected with a prevalence of 29.50%. The mean intensity of parasites was maximum in *Labeo gonius*, 5.88. Protozoan and trematode infection was highest in *Labeo calbasu*, 19.68% and 70.48% respectively. *Labeo bata* exhibited the highest infection prevalence, 15.93% with crustacean parasites. Among the crustacean parasites, the infection by *Neoergasilus longispinosus* was intense during winter seasons with maximum load of parasites on the fins of the infected hosts.

With regard to the seasonal analytical studies, dominance indices (DI) which represents the dominance of any one particular parasite species upon all the others were very similar throughout the year except for the winter months in *Labeo calbasu* and *L. gonius*, whereas it was higher in case of *Labeo bata* (Table 2, Figs. 1,2 and 3). *Labeo bata* harbored the richest parasite fauna with Richness Index (RI) 2.58 followed by *Labeo calbasu*, 2.24 and for *Labeo gonius* the richness index was low, 1.81. The Shannon index of diversity (H) shows similar peak values almost throughout the season for all species which signifies that all the three species harbored the same number of parasite species. The Evenness index (EI) indicates the

Table I. Summary of Total parasitic infestation by the protozoan and metazoan parasites on three *Labeo* spp. of Tripura.

Name of host sp.		Monogenean infection				Digenean infection				Crustacean infection				Protozoan parasites infection				Total infection			
	No. of fishes examined	No. of fishes infected	No. of parasites collected	Prevalence(P)	Mean intensity (MI)	No. of fishes infected	No. of parasites collected	Prevalence(P)	Mean intensity (MI)	No. of fishes infected	No. of parasites collected	Prevalence(P)	Mean intensity (MI)	No. of fishes infected	No. of parasites collected	Prevalence(P)	Mean intensity (MI)	No. of fishes infected	No. of parasites collected	Prevalence(P)	Mean intensity (MI)
Labeo calbas u	376	218	1357	57.9	6.2	4	6	1.0	1.5	43	108	11.4	2.5	74	459	19.6	6.2	332	1930	88.3	5.9
Labeo gonius	368	161	1000	43.7	6.2	5	7	1.3	1.4	38	104	10.3	2.7	35	235	9.5	6.7	229	1346	62.2	5.9
Labeo bata	383	34	171	8.8	5.0	5	6	1.3	1.2	61	210	15.9	3.4	33	256	8.6	7.8	113	643	29.5	5.6
Total	1127	413	2528	36.6	6.1	14	19	1.2	1.4	142	422	12.6	3.0	142	950	12.6	6.7	674	3919	59.8	5.8

Table II. Monthly and seasonal variations of different ecological indices for parasitic infections in three *Labeo* spp.

Labeo calbasu

Ecological Indices	February	March	April	May	June	July	August	September	October	November	December	January	Summer	Monsoon	Winter	Annual
Shannon Index (S)	0.85	1.87	1.77	1.80	1.71	1.53	1.78	1.59	1.83	1.30	1.35	1.56	2.03	1.98	2.06	2.16
Richness Index (RI)	0.73	1.92	1.48	1.36	1.46	1.31	1.27	1.34	1.51	0.90	0.86	1.25	1.94	1.82	1.93	2.24
Dominance Index (DI)	0.57	0.19	0.20	0.20	0.24	0.29	0.20	0.27	0.20	0.32	0.30	0.29	0.16	0.17	0.16	0.14
Evenness Index (EI)	0.61	0.85	0.80	0.86	0.78	0.74	0.85	0.77	0.83	0.81	0.84	0.80	0.85	0.83	0.86	0.84

Labeo gonius

Ecological Indices	February	March	April	May	June	July	August	September	October	November	December	January	Summer	Monsoon	Winter	Annual
Shannon Index (S)	0.62	1.18	1.47	1.02	1.17	1.33	1.04	1.31	1.59	1.47	1.09	1.12	1.51	1.46	1.86	1.78
Richness Index (RI)	0.66	0.89	1.46	0.67	1.00	1.17	1.19	1.16	1.47	1.08	0.67	0.76	1.51	1.55	1.65	1.81
Dominance Index (DI)	0.69	0.36	0.32	0.43	0.38	0.35	0.42	0.31	0.26	0.28	0.39	0.37	0.31	0.31	0.19	0.22
Evenness Index (EI)	0.45	0.85	0.71	0.73	0.65	0.68	0.54	0.67	0.76	0.82	0.79	0.81	0.73	0.66	0.84	0.77

Labeo bata

Ecological Indices	February	March	April	May	June	July	August	September	October	November	December	January	Summer	Monsoon	Winter	Annual
Shannon Index (S)	1.44	1.35	1.631	1.76	1.48	1.46	1.48	1.31	1.47	0.65	0.5	0.61	2.04	1.93	1.79	2.22
Richness Index (RI)	1.09	0.79	1.51	1.4	1.39	0.94	1.01	0.76	1.57	0.38	0.43	0.43	1.89	1.79	2.07	2.59
Dominance Index (DI)	0.25	0.27	0.236	0.21	0.28	0.25	0.25	0.29	0.26	0.54	0.68	0.58	0.14	0.16	0.2	0.13
Evenness Index (EI)	0.9	0.97	0.784	0.85	0.76	0.9	0.92	0.95	0.82	0.94	0.72	0.88	0.89	0.88	0.86	0.86

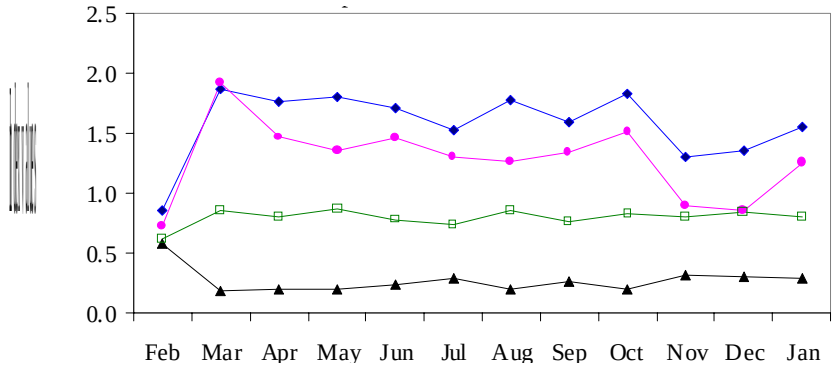

Fig.I Monthly variation of different species level diversity indices for parasitic fauna in *Labeo calbasu*

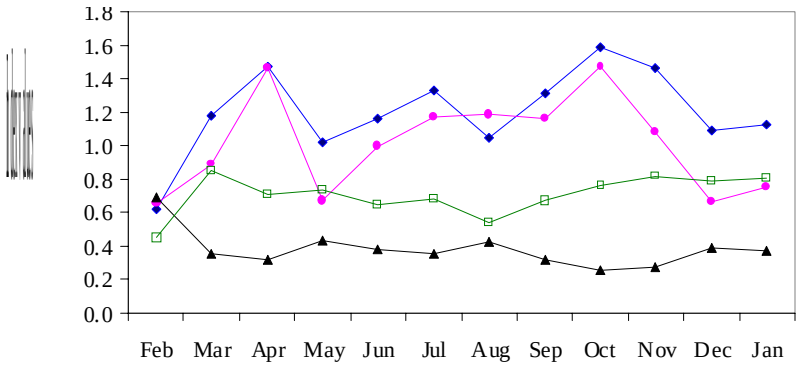

Fig.II Monthly variation of different species level diversity indices for parasitic fauna in *Labeo gonius*

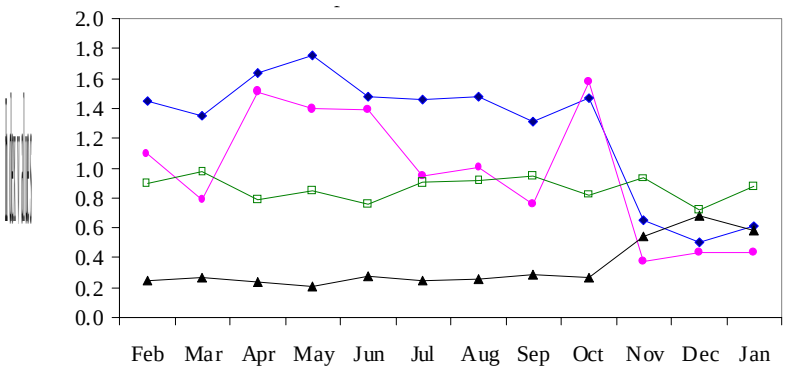

Fig.III Monthly variation of different species level diversity indices for parasitic fauna in *Labeo bata*

uniformity of the number of parasites of the different parasite species present at a particular time within a given host. In the present study, the parasites were almost evenly distributed throughout the year represented by similar values. In the course of the survey it was observed that the fry and the fingerling stages of the host fishes also showed instances of infection.

The present results indicate that *Labeo calbasu*, *L. bata* and *L. gonius* which are the most preferred minor carps in Tripura harbour a rich and diverse parasitic fauna represented by four major taxa- Protista, Monogenea, Digenea and Crustacea. This result contradicts the view of Thoney (1991, 1993) that the parasite diversity of freshwater or estuarine fishes are less than the off shore region. Manter (1947) and Dogiel *et al.* (1958) observed that the phylogenetic closeness of the hosts is the major reason for the similarity in their parasite fauna thus it can be suggested that the three *Labeo* spp. have remarkable phylogenetic closeness.

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The effect of parasitic infestation on tissues and organs of *Mystus aor* (hamilton) and *Mystus bleekeri* (day)

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Abstract : A total of 1011 *Mystus aor* and 1039 *Mystus bleekeri* were examined during January 2004 to December 2005. The juvenile trematode *Isoparorchis hypselobagri* was recovered from body muscles, swim bladder and visceral organs of the fishes having prevalence 80.02 %, intensity 6.83 ± 3.79 in *M. aor* and 58.90% , intensity 5.23 ± 2.89 in *M. bleekeri*.

In both the hosts, encysted and free *I. hypselobagri* were frequently found in the body cavity, viscera and more rarely in the mouth, urinary system, biliary system, ovaries etc. The main effect of the parasite was found on the skin surface, body musculature and visceral organs. *I. hypselobagri* were found attached to the body muscles causing extensive tissue damages including inflammation, necrosis, and empty spaces with fragmented blood capillaries, tissue debris's, lymphocytes and fluids.

Infected liver, swimbladder and kidney showed vacuolation and massive melanization. Helminth parasite specially *I. hypselobagri* caused tissue damages through formation of tunnels by lysing and ingestion of tissues. Fluid accumulation, tissue destruction, hemorrhage and massive melanization in different parts and organs of the hosts were observed.. . Due to the presence of some gut helminth, the intestinal mucosa and villi were disrupted, ruptured blood vessels and necrosis of tissues were also observed.

Key words **:** *Histopathology, Isoparorchis hypselobagri, Mystus aor, Mystus bleekeri, Parasitic infestation.*

Introduction

Parasite causes damage to their host and they occupy a definite position or site in suitable environment on their hosts. Fishes are one of the most common hosts of helminth parasites. The influence of the parasite may result in extensive change in individual organs or tissue or it can exert a general effect on host. Helminth parasites generally affect the internal organs of the host fish, particularly the gut and inhibit host's growth. The normal growth of fishes is interrupted and inhibited if they are heavily infested with endoparasites viz., trematode, nematode, cestode and acanthocephalan and microscopic lesions in their host's tissues which become the site for the secondary infection by bacteria . Helminths are very common in freshwater fishes. Very few lesions have been attributed to intestinal forms. Histozoic helminths, particularly migrating forms, cause greater damage in fishes. In severe cases hypermia, hemorrhage, cellular infiltration, lesion, narcosis, fibrosis etc. After encystment and fibrotic encapsulation, many larval helminths produce no further obvious damage except pressure on adjacent host tissue. Migrating larva cercariae, plerocercoides and nematode produce the most serious reactions: leukocytes, fibrosis, hemorrhage and necrosis. Continual migrations, such as larvae of *Contracaecum* spp. produce peritonitis which results in fibrosis and extensive adhesions. Rapid invasion by large number of cercariae produce extensive hemorrhage, hypermia, necrosis and even death if present in sufficient numbers (Ribelin and Migaki, 1975).

Among the workers who have undertaken histological research on tissues of various freshwater fishes are Mackiewicz (1972); Hine and Kennedy (1974); Jain *et al.* (1976); Ahmed and Sanaullah (1979); Mitchell, *et al.* (1982); Khanum (1994); Khanum and Farhana, (2002.) etc. Helminth in fishes is also recognized as causing serious effect on their hosts [1]. Changes brought on by nematodes have been noted by Yeh (1960).

Very few studies have been done on parasite infestation, host parasite relationship and histopathology of *I. hypselobagri*. (Siddique and Nizami, 1978) reported incidence of this trematode from *W. attu*.], studied the incidence of this trematode and its destructive effects on air bladder of *W. attu*, viscera and body musculature of *Callichrous bimaculatus* and ovary of *Mystus aor.*. Mahajan *et al*. (1978) reported the effect of parasitization of juvenile *I. hypselobagri* in *Channa punctatus.* Bhalerao (1932) gave a note on the probability of infection of man and domestic carnivores by this trematode. Verma and Ahluwalia (1980) reported similar unusual records and high survival index of *I. hypselobagri*, but none of them explained the pathology of these

infections. Khanum (1994) observed severe pathogenic lesions done by juvenile *I. hypselobagri* on the skin surface, body musculature, liver, intestine, kidney and other visceral organs in two species of *Ompok spp.*

Parasite -induced histopathology in *M. aor* and *M. bleekeri* have never adequately described. According to Bashirullah (1972) the occurrence of young fluke in the muscle and body cavities of fishes, suggested, that the parasite bored their way in the tissue. Sometimes the juvenile parasites penetrate the intestinal wall and migrate into the swimbladder.

Materials and methods

A total of 1011 *Mystus aor* and 1039 *Mystus bleekeri* were autopsied during January 2004 to December 2005 from the rivers of Kuliarchar upazila and brought to the Parasitology laboratory, Department of Zoology University of Dhaka for the investigations of helminth infestation.

The affected parts and organs of the fish, e.g. skin, liver, muscles, kidney, swim bladder and alimentary canal were separated and treated according to the methods instructed by for histological studies. After detection, the affected tissues were carefully fixed by a gradual addition of 10% Buffered neutral formalin solution.

The methods of [17] and [18] were followed for the preparation of the permanent histological slides. For preparation of histological slides, the tissue materials were kept in Buen's fluid for 24-48 hr for fixation, dehydrated in ascending grades of ethanol (50%, 70%, 10% and 100%), impregnated and embedded in paraffin and sectioned at 5µ. Sections were mounted on slides, deparafined by low grading and stained with haematoxylin and counter stained with eosin, dehydrated and finally mounted in Canada balsom..

Results

In the present study, some pathological changes were observed on the skin, body musculature, swimbladder and visceral organs of *M. aor* and *M. bleekeri*. Trematode, Histopathological studies showed that skin, muscle layer, swimbladder, intestine, lower part of intestine, liver and kidney were damaged by the infestation of helminth parasites. Skin, muscles, intestine, liver, kidney tissues were found to be more infected. *I. hypselobagri* was found to be the most pathogenic and damaging one. In *M. bleekeri* it was mainly found in the swimbladder and body cavity but in *M. aor* predominantly found almost every organ such as swim bladder, liver, kidney (over the surface) and also in cavity, musculature (Plate- VIII), skin surface etc.

Due to the structural construction and the ability of the immature *I. hypselobagri*, they are normally capable of dissolving and penetrating the skin and muscle layers when they are living in the regular habitat and corresponding microenvironment. But along with the onset of decomposition of viscera or any unusual change of host body's microenvironment, the trematode become stimulated or compelled to utilize the dissolving and penetrating capacity for their survival. It can be noted as a homeostatic adjustment or adaptation of the juvenile trematode *I. hypselobagri*. The juvenile parasites dissolve the skin with the help of the penetration glands, located in the oral sucker situated at the anterior region of the body and in the acetabulum (Plate- I & VII). Many fishes were observed with marks of perforation, lacerations and scars on their surface and abdomen. It is assumed that many metacercarial forms might be lost through these laceration processes. Any kind of internal changes or due to the cause of host's death, metacercarial forms need to leave the host immediately. At that time they bored their way in to the external environment for their survival through laceration processes. Sometimes, it was also observed during the study period that, the trematode reaches the buccal cavity or gill and sometimes came out through the anus or genital opening of lower abdomen.

In the present observation, due to the presence of juvenile *I. hypselobagri* in the swimbladder, liver and intestine massive melanization was observed. Sometimes, small vacuoles were formed in the liver due to the presence of cysts. Melanin-macrophage centers along with heavy melanin deposition were observed in the infected liver. The melanin-macrophage centers should possibly be considered as a component of the reticuloendothelial system and hence, part of the defensive system of the fish against any infection. Presence of massive melanization again confirms this. Sometimes hepatic blood vessels were ruptured. The affected liver showed mild hepatic or hemopoietic degenerative changes with hemorrhages (Plate- II & III).

A number of observable histopathological changes occurred in the intestinal tissue of infected fishes. The gut helminths damaged the walls at the sites of their attachment. This disruption was mainly due to the action of sucker of the trematode parasites. As a result the intestinal wall was heavily destroyed. Intestinal tissues were observed with deposited melanin. Perforation in the gut wall was found where the host tissue reacted vigorously. Large vacuoles and fluid filled empty space along with debris and lymphocytes were present. The intestinal mucosa and villi tissue was disrupted, the blood vessels were ruptured and necrosis of intestinal tissue was also found (Plate-V).

In infected kidneys, some vacuolar renal tissue degeneration was observed. The important changes included chronic inflammation, degeneration of blood vessels, and degeneration of kidney tubules as well as interstitial cells. Due to parasitic infestation, vacuolar empty spaces and tunnels filled with fluid. Sometimes the associated haemopoietic tissue became loose and in severe cases renal tubular necrotic lesions were observed. Large macrophages containing erythrocytic debris in the hematic sinusoids were found (Plate- IV).

During the migration of immature *I. hypselobagri* from visceral cavity to the swimbladder, massive disruption and dislocation of the visceral organs occurred. Irregular black pigmentation was scattered throughout the swimbladder, due to the infection by juvenile *I. hypselobagri*. In severe cases, the alveolar sacs and capillary plexuses were disrupted causing necrosis (Plate-VI).

Discussion

Of the low level animal protein consumption, 80% is contributed by the fisheries with proper utilization of these rich resources, the demand of animal protein for the entire population of the country can be resolved to a fairy extent. It is also a high quality food containing high percentage of protein and variable quantities of vitamins, fats, calcium, phosphorus and other nutrients essential for human health. To achieve healthy fish stock, we have to implement program like fish parasitological research, control of fish diseases, some biological aspects and maintenance of healthy relationship between fish and their environments.

Now a days, the need and importance of study of Parasitology cannot be denied. The study of parasites and its function is a long felt demand in a developing country like Bangladesh which could promote the Nation's agricultural economy, particularly the economy of fish conservations. Parasites require some vital factors which they can obtain only from their hosts often inflicting some degree of injury affecting its welfare. Recent physiological studies of nutritional requirements of parasites especially endoparasitic forms, have indicted that, depletion of host's nutrients by parasites may have serious consequences. The organ which are heavily infested by parasites may cause malnutrition, destroy tissues, reduce growth rate and immunity against virus, bacteria produce toxin etc.. Even man and piscivorous wild and domestic animals may become affected from the consumption of parasitized fishes. All of these influences may lead to disease. These parasites play an important harmful role on aquatic and terrestrial food chain.

In the body musculature, intestine and kidney fluid filled empty spaces along with debris's and lymphocytes were present. Similar observation also reported by Sanaullah *et al.* (1997). This observation also showed positive agreement with the findings of Kinkelin *et al*. (1968). The melanin-macrophage centers should possibly be considered as a component of the reticuloendothelial system and hence, part of the defensive system of the fish against any infection (Ribelin and Migaki, 1975). They also theorized that the black pigment of the interfacicular connective tissue of the host was mediated by an enzyme reaction, which caused mechanical obstruction due to the occurrence of parasite in clusters. The reported that the internal organ of infected fish showed only mild histopathological changes which may be the result of background pathology. In 1966, larvae of *Bucephalus polymorphus* caused high mortality of cyprinid fish in the Seine river near Paris, France (Kinkelin *et al*. 1068). They found cysts in many parts of the fish and the mouth and bases of caudal and anal fins with marked inflammation and hemorrhage. Migrating cercariae cause muscle necrosis. In some cases metacercariae were numerous in the cornea and retina with retinal hemorrhages.

The metacercaria of certain digenean parasites, found encysted in the skin and muscle of teleost fish, stimulated production of black spots which are visible in naked eye, (Mcqueen *et al*. 1973). stated that, one of the means by which a host combats internal parasites is by production of melanin pigments from the surrounding tissues. Presence of cysts with increasing juvenile *I. hypselobagri* again confirmed the purpose for which melanin pigments were produced. Edelstein, (1971) suggested a number of potential biological functions for melanin compounds apart from direct pigmentation. They added that, melanin has a capacity for binding aromatic and cyclic compounds, and also it may selectively take up harmful compounds. The number of empty cysts tended to increase slightly with increase in size of the host. The degree of pigmentation depends on the species, age and state of health of the fish.

A number of observable histopathological changes occurred in the kidney tissue of infected fishes. The important changes included chronic inflammation, degeneration of blood vessels, fluid accumulation and degeneration of renal tubules as well as interstitial cells. Kidney samples have shown tubular, vacuolar degeneration with granular occlusion and haemopoietic tissue degeneration and focal proliferation. Damage to kidney of teleosts occurs when cercariae of various

species enter the fish and migrate through the tissues and most cercariae encysted in the tissues following the migratory period, which cause tissue destruction due to pressure necrosis as the organism continues a short period of growth and stimulates fibroplasias (Hoffman, 1967). They also noticed that, the organism was surrounded by a fibrous tissue capsule which contains a few lymphocytes and melanophore that have migrated into the area, and the metacercariae remain in the encysted state until ingested by the final host.

The present study provides an account of parasitological aspects of taxonomic studies, distribution, association and pathogenecity of the helminth parasites of the host fishes as well as nutritional aspects of infected and uninfected host fishes. It has been established that, the catfish *M. aor* of all ages are not highly susceptible to all groups of metazoan parasites and some of those parasites have zoonotic importance (e.g. *I. hypselobagri* and *G. spinigerum*) which are potentially transferable to man only through freshwater fishes. Bhalerao (1932) wrote a note on the probability of infection of *Isoparorchis hypselobagri* in man and domestic domestic carnivores. He listed the occurrence of *I. hypselobagri* from gas bladder of *Wallago attu* and also found these parasites in the stomach of a crocodile which had devoured some siluroid fishes. He also stated that any animal eating the infected siluroid fish was exposed to infection with *Isoparorchis*. Among the internal organ, intestine was heavily infected by a large no of gut helminth specially trematode. In conclusion, controlling measures should be taken to interrupt the steps of parasitic transmission from one host to another. Therefore, extensive study should be carried out on the parasites of *M. aor* and *M. bleekeri*; otherwise they will lower the productivity of these catfishes.

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On the seasonal occurrence of clinostomatid metacercariae infections in an Indian mudskipper *Channa punctatus* Bl. from the fresh water swamps near Darbhanga town, north Bihar

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Abstract : The seasonal occurrence of clinostomatid metacercariae in the mudskipper *Channa punctatus* from the freshwater swamps near Darbhanga town, North Bihar was studied from June, 2009 to May, 2010.

The matacercariae belong to two genera - *Clinostomum* and *Euclinostomum*. These showed incidence during February to September with peak periods in June (44.44-54.17%). Average worm burden too was found higher during peak periods of July-August (4.0-7.2). The findings showed clearly that the warmer months favoured the incidence as well as average worm burden of these metacererariae in this fish host indicating a significant and positive relationship between the incidence and worm burden ($r = + 0.828$ at $P>0.001$, $df = 11$) whether male or female fish host.

Therefore, as to the Indian record of the seasonal occurrence of the clinostomatid metaceracariae the present report is worth mentioning.

Key Words **:** Seasonal, occurrence, Clinostomum, metacercaria, Channa, fish host

Introduction

Cultivation of edible fish species has now-a-days been promoted, rather on commercial scale, with a view to enhancing not only economic profit but also the supply of protein in our diet at a relatively cheaper rate. So, the fish cultivation has now come to share in uplifting the agricultural or rural economy. Our country, India, is a vast tropical country with varied climatic conditions, say climatic instability, which are certain to affect the fish living in the freshwater habitats, particularly the wetland or swampy waters so much so that there is even high mortality rate of fish particularly living in shallow ponds or swampy areas. Perhaps this may be due to one or other kind of environmental stress. Helminthic infections, concomitantly with microbial infections, prove to be adding to the miseries as being much fatal to the fish cultivation. Stocked ponds or swampy areas provide a congestion that definitely favours for rapid spread of infections among the fishes living therein and thus infestations become more liable to occur and reoccur. It becomes more imperative to know the period of heavy occurrence so as to plan out prophylactic measures. As such seasonal studies warrant attention. Due to the scarcity of seasonal studies of helminths in freshwater fishes in tropical parts of the world, including India, very little is known about the seasonal pattern and occurrence of this group of metacercaria. Rather, as far as the authors are aware, no study on the seasonal changes of occurrence of this group of parasites has yet been attempted. Therefore , the present study carried out from June 2009 to May 2010 provides the first report on the seasonal occurrence of clinostomatid metacercariae from the mudskipper *Channa punctatus* syn. *Ophiocephalus punctatus* from Bihar, India.

Materials & methods

The live specimens of *Channa* were collected at regular monthly intervals with the help of local fishermen from the freshwater swamps with water temperature ranging from 11 to 40^{o}C at the outskirts of Darbhanga town, North Bihar. Immediately after collection the live fresh samples were brought to the laboratory and are examined for the helminth parasites. A total of 319 fish were examined during the period from June 2009 to May 2010.

The metacercariae recovered from the body cavity and even attached to mesentery were removed out and fixed in 4% alcohol and stained in Gower's carmine for morphological study.

Results (Table-1&2; Fig-1)

Out of 319 fish (86 males and 233 females) only 71 (=23.19%) were found to be infected with these metacercariae including 15 (16.28%) males and 56 (24.88%) females. These metacercariae belong to the family Clinostomatidae including two genera-

Clinostomum & *Enclinostomum.* Although these two genera have been reported by other workers from *Channa* (*syn. Ophiocephalus*) p*unctatus,* only a few from other fish hosts viz.

Table-1. Prevalence of clinostomatid metacercaricae in *Channa punctatus* from freshwater swamps near Darbhanga town.

Months	Mean Temp (°C)	Fish Examined		Fish Infected		Prevalence (%)		Average Wormburden	
		M	F	M	F	M	F	M	F
Jan	16·2	8	12	0	0	0	0	0	0
Feb	22·4	8	16	0	2	0	12.50	0	2.0
Mar	30·6	6	16	1	3	16.66	18.75	2.1	3.4
Apr	33·2	9	17	1	4	11.00	23.50	1.5	3.5
May	35·4	9	19	2	7	22.22	36.80	2.5	5.5
Jun	38·6	9	24	4	13	44.44	54.17	3.2	5.8
Jul	33·2	8	24	3	11	37.50	45.83	4.0	6.2
Aug	32·1	7	21	3	9	42.57	42.86	5.2	7.2
Sep	28·2	8	22	1	5	12.50	27.77	3.8	4.8
Oct	25·6	5	21	0	2	0.0	9.00	0.0	4.0
Nov	18·2	5	23	0	0	0.0	0.0	0.0	0.0
Dec	15·3	4	18	0	0	0.0	0.0	0.0	0.0
		86	223	15	56	16.28	24.88		
Total =		=319		=56.71		= 23.19			

M = Male, F = Female

Table-2. Seasonal variation in prevalence of metacercariae infection in **Channa punctatus** from freshwater swamps.

Particular	MALE FISH			FEMALE FISH		
	W	S	R	W	S	R
Incidence (%)	**0**	**24**	**25**	**3**	**36**	**31**
Av. Worm burden	**0**	**2**	**3**	**1**	**5**	**6**

Trichogaster and *Mastacembellus* (Jaiswal, 1957) which too live in the similar habitats. Very remarkably these metacercariae were **neotenic** i.e. premature adults marked by conspicuous and prominent genitalia but without uterus and eggs, as if these were just to become adults in suitable hosts such as fish–eating birds or mammals. It means that this fish host serves as **paratenic** host. Data presented in Table (1 & 2) clearly show that metacercariae not recovered during October-February but only during March to September show the marked and statistically significant seasonal difference ($F=25.19$, $P>0.001$ for $df= 2,7$).

Discussion

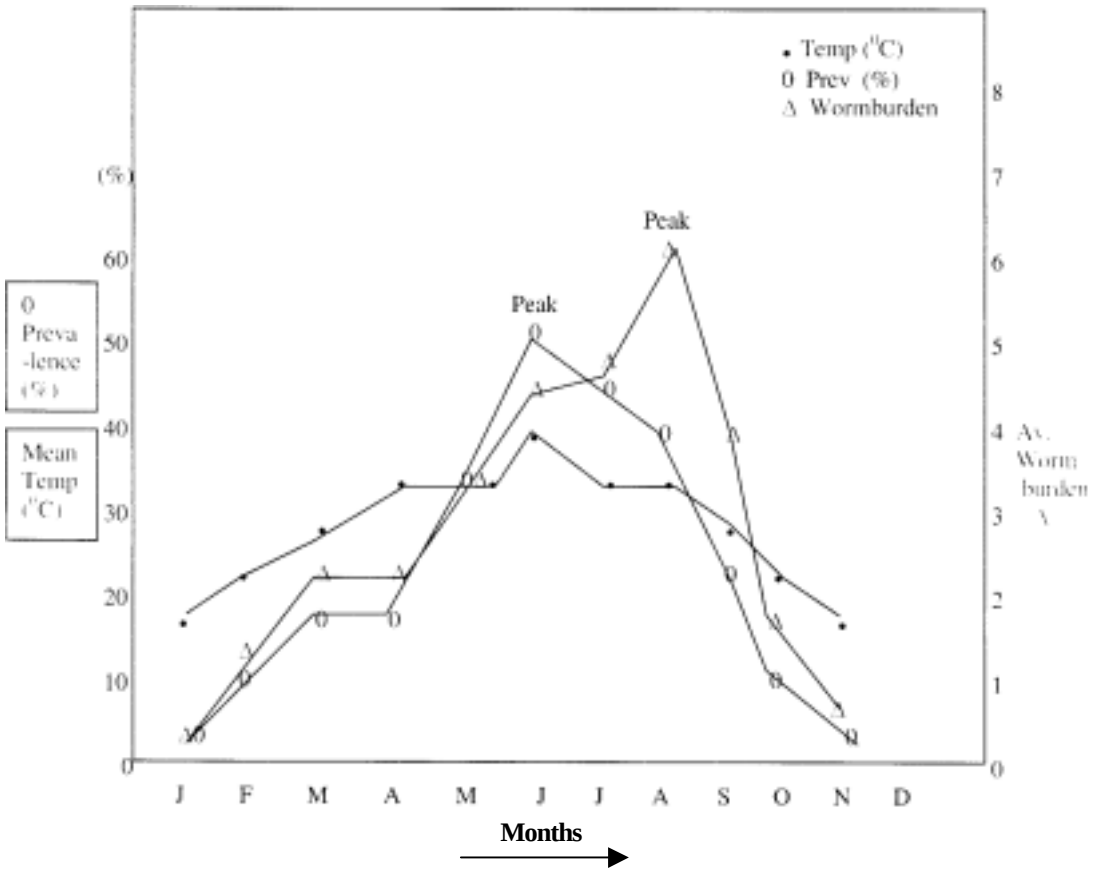
So far the authors are aware no reports on the seasonal occurrence of the clinostomatid metacercariae have been made from the tropics including India except those of Sinha (1991) on that of *Diplostomulum* from a catfish *H. fossilis* Bl. Therefore, the present study provides the first report on the seasonal occurrence of the clinostomatid metacercariae from an Indian mud skipper *Channa punctatus* .As evident from the works of Grabda-Kazubska (1974) on the occurrence of the adult C*linostomum*

Figures rounded off to the nearest whole number

W = NOV-FEB

S = MAR-JUNE

R = JULY-OCTOBER



Graph showing relationship between the mean temp (^{0}C), prevalence (%) and Av. Worm Burden of clinostomatid metacercariae infection in Channa punctatus Bl.

complanatum from herons and also of Pojmanska (1976) in Poland an association between the habitat of the lakes and occurrence of the metacercariae has been suggested. Rather in Poland, in the months of February and July the water has average monthly temperature of 6.87^{0}c and 29.16^{0}c respectively and it has been speculated that the increased water temperatures would have favoured hatching of eggs .The findings of the present study show increasing incidence as well as average worm burden during the months of February to September with peak periods during June to August. The water temperature too shows increasing trend from February to a maximum of 38.6^{0}c in June and then decreasing due to rainfall during July-October .Hence the findings of the present study do support the above speculation that the warm water of these months would have favoured not only hatching of eggs but also the emergence of cercaria from the snail host into water and their entry into the fish host where develop further into metacercariae. Further, the warmer months-Summer and Rainy-favoured not only the occurrence but also to a greater extent maturity, hence neotenic or precocious, as if only waiting for the definitive host, whether aquatic birds/mammals, to eat upon such metacercariae infested fish and these becoming adult ones. In other words, the season plays an important role, along with temperature as a factor, in influencing the occurrence of these metacercariae. In conformity with the hypothesis of Chubb (1979,1982) and also De (1993) that several factors, but no less important, including water temperature have a significant influence on the seasonal changes in occurrence of helminths in freshwater fish in the tropical areas, and presumably the host interactions play the dominant role. However, the seasonal studies on the other helminth parasites should be carried out in the rainy climatic zone of tropical areas to find the determining factors.

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Acid tolerance as a vehicle for food-borne shigellosis

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Abstract : Shigellosis still remains an important cause of mortality and morbidity in India and other South Asian countries where people live in densely populated, poor sanitation areas. The problem is aggravated with an increasing incidence of multiple drug resistance among shigellae. Incidences of food-borne shigellosis are reported, mainly associated with consumption of fresh produce. The growth of global food trade makes food from around the world available, the increase in fresh produce consumption and the growing popularity of minimally processed fruits and vegetables may play a role in the introduction of *Shigella* spp. in these types of foods. Shigellosis is characterized by a low infective dose and *Shigella* spp. are pathogens of faecal origin can be carried by food handlers and transmitted through fruit-products The safety of fruit and fruit products relies mainly on their low pH and storage under refrigeration which are considered as barriers for growth and survival of microbes. However, a few closely related species of *Shigella* showed extraordinary tolerance to low pH. So the rationale of the present study was – the application of a combination of suboptimal intrinsic and extrinsic factors such as reduced pH and reduced temperature can indeed inhibit the outgrowth but may induce prolonged survival of highly virulent pathogens such as *Shigella*. With this objective, the growth and survival efficiency of shigellae in acidified media was monitored at different time intervals and viable counts were determined. At 37ºC shigellae die rapidly at pH 4.0 or lower, but these pathogens could survive for an extended period under similar adverse pH conditions when kept at refrigeration temperatures. A die-off of the shigellae was observed at pH 3.5 -4.0 and pH 3.0-3.5, respectively after 10 days at 4ºC and 6 days at 20ºC. Thus the recovery of shigellae was affected by exposure to reduced pH but increases with refrigeration. So it can be concluded that acid foods such as tomato juice, apple juice, orange juice, acidic cheese, freshly cut papaya etc, usually kept at refrigeration temperatures, may support survival of *Shigella* and cause food poisoning. Once contaminated during cutting, juicing or in transit, these pathogens can survive for an extended period even under adverse pH conditions and these foods may serve as a vehicle for *Shigella* infections.

Key words **:** *Shigellosis; acid foods; refrigeration; survival efficiency; food poisoning*

Introduction

Shigellosis still remains an important cause of mortality and morbidity in India and other South Asian countries where people live in densely populated, poor sanitation areas. The problem is aggravated with an increasing incidence of multiple drug resistance among shigellae. The *Shigella* bacteria are found in human feces which often contaminate the soil and water. Infection is initiated by ingestion of shigellae (usually via fecal-oral contamination). For most other infections, it takes thousands of organisms before an illness becomes apparent but it only takes 10 shigellae to cause the disease (1).

Fruits and their products can serve as vehicles for pathogenic microorganisms (2). Often involved are fruit products that have undergone some kind of minimal non-thermal processing followed by time or temperature combinations that permit pathogens to survive and grow. Normally the peel of a fruit serves as a natural protectant, preventing bacteria from penetrating to the interior but on wounded fruit or fresh cut fruit slices, this external barrier is broken, thus creating an opportunity for bacterial colonization. Mechanical processing of fresh fruit such as transport, cutting, juicing and mild heat treatment makes fruit products more vulnerable to microbial penetration and growth (3). Enteric pathogens have been implicated in fruit-associated outbreaks. Outbreaks of *Salmonella* infections were traced to precut watermelons and fresh tomatoes (3). Outbreaks of *Escherichia coli* O157:H7 infection was associated with un-pasteurized fresh apple cider (4). Parish (1998) reported un-pasteurized orange juice contained *E. coli* (5) and outbreaks of enterotoxigenic *E. coli* infection associated with orange juice have been reported by Singh et al. (6).

Although the reported incidence of food-borne shigellosis, mainly caused by *Shigella sonnei* and *S. flexneri* is lower among enteric pathogens, each year there are a significant number of outbreaks, mainly associated with consumption of fresh

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produce (7; 8). The chief contributing factor leading to *Shigella* food-borne outbreaks is poor personal hygiene of a food handler (7). The growth in global food trade that makes food from around the world available, the increase in fresh produce consumption and the growing popularity of minimally processed fruits and vegetables may play a role in the introduction of *Shigella* sp. in these types of foods (3).

The safety of fruit and fruit products relies mainly on their low pH and storage under refrigeration which are barriers for growth and survival (9). Growth of *Salmonella* spp. and *E. coli* O157:H7 on peeled orange was observed only at abusive storage temperature. Refrigeration effectively inhibited growth and caused a reduction in the population of *Salmonella* spp. (10). *E. coli* O157:H7 showed extraordinary tolerance to the low pH upon refrigeration (11). *Shigella* is closely related to *E. coli* in their DNA homology and closely resemble in pathology, biochemical activity and antigenicity.

Few data are available on the growth and survival of shigellae in acid conditions. *S. flexneri* grown to stationary phase has the ability to survive a few hours at pH 2.5. This acid resistance may contribute to the low infective dose associated with shigellosis (1). In laboratory conditions the minimum pH for growth was 4.9– 5.0 (9). All of the strains of *S. flexneri* and *S. sonnei* survived for 4 h in a nutrient medium at pH 4.0 or 4.5, however, at pH < 4.0 shigellae die rapidly. In this part of the globe the prevalence of *S. dysenteriae* is more than any other species of *Shigella* (1). Therefore, in the present study the behaviour of *S. dysenteriae* Type 1 strains under acid conditions was studied covering a pH interval which is relevant for the pH of acid foods. It was also monitored that these pathogens could survive for an extended period under similar adverse pH conditions when kept at refrigeration temperatures.

Materials and methods

Bacterial strains and preparation of inoculam

S. dysenteriae Type1 strains were obtained from the National Institute of Cholera and Enterc Diseases, Kolkata, India. Stock cultures of the strains were maintained on Tryptone Soy Agar (TSA;Hi-Media) at 4°C and revived by transferring a loop inoculum into 5 ml Tryptone Soy broth (TSB; pH 7.0) and incubating for 18–20 h at 37°C.

Acid tolerance of Shigella using acidified media

The behaviour of the *Shigella* strains under acidic conditions was determined by inoculating 0.1 ml of a 100-fold diluted 18–20 h TSB culture into 9.9 ml of TSB adjusted to pH 3.0–5.0 (intervals of 0.5) with a citric acid (Merck, India) solution (10%) in order to obtain an inoculum of 10^5 cfu /ml). The TSB was held at 37°C during subsequent incubation. Viable counts of *Shigella* were determined after 6, 24 and 30 h incubation.

Survival efficiency of Shigella in acidified media stored at refrigeration temperatures

The behaviour of the *Shigella* strains under acidic conditions was determined in the same way by storing the inoculated media at 4 ºC and 20 ºC, respectively up to 15days. The cultures were taken out at regular intervals to determine viability.

Determination of viability of Shigella

Viable counts of *Shigella* were determined by preparing 10-fold serial dilutions in sterile 0.1% peptone water and plating on TSA at each sampling time. If low numbers were expected, 0.1 ml of sample was directly spread out on the surface of TSA. All plates were incubated at 37°C for 24 h before colonies were counted. The difference in log cfu /ml) was determined on TSA plates were considered as an index of the number of injured cells.

Results

Acid tolerance of Shigella dysenteriae using acidified media

The results are shown in Table 1. At pH 5.0 *Shigella* strains grew to maximum numbers (10^8 cfu /ml) within 6 h. At pH 4.5 Shigella had multiplied in 6 h, however, variation in the maximum numbers obtained was noticed (Number of injured cells was 0.9). The minimum pH for growth of *Shigella* was thus determined as pH 4.5. They were able to multiply at pH 4.5 but the number of injured cells was much more than that obtained at pH 5.0. At pH 4.0 the bacterial strains showed a viable count within the range of the initial inoculum level after 24 h (10^5 cfu /ml). At pH 3.5 the tested *Shigella* strains could be recovered after 24 h incubation although they survived for 6 h at pH 3.5 with quite a remarkable reduction in the population as revealed by the number of injured cells obtained.

Table 1 : Indication of the number of injured cells of *Shigella* after 6h incubation at 37°C in acidified media (Difference in log cfu/ml was determined on TSA plates)

	pH 5.0	**pH 4.5**	**pH 4.0**	**pH 3.5**
Number of Injured Cells	0.5	0.9	1.8	3.8

Studies reported a 4 h survival time for a few strains of *S. flexneri and S. sonnei* at pH as low as 3.5. However, no survival was noted after 24 h for all the strains at pH 4.0. Though it is conceivable that different strains of microorganisms differ in their acid resistance, the results of the present study confirm that the *S.dysenteirae* Type1 strains found in this country are in general more stress resistant. Direct enumeration of *Shigella* in acid foods using a selective medium as TSA may lead to an underestimation of the pathogen's presence and prior enrichment of media with different supplements are recommended by FDA BAM (12) which may aid in the resuscitation and recovery of stressed cells.

Survival efficiency of Shigella in acidified media stored at refrigeration temperatures

Table 2 and table 3 show the enhanced survival efficiency of *Shigella* in acidified media after keeping them at refrigeration temperatures.

Table 2 : Indication of the number of injured cells of *Shigella* after incubation at 4°C in acidified media.

Number of Injured Cells				
Culture Time in Days	**At pH 5.0**	**At pH 4.5**	**At pH 4.0**	**At pH 3.5**
2	0.2	0.3	0.5	0.7
4	0.4	0.5	0.8	1.1
6	0.7	0.8	1.2	1.8
8	0.9	1.2	1.4	3.7
10	1.1	1.8	3.8	Not Detected

Table 3 : Indication of the number of injured cells of *Shigella* after incubation at 20°C in acidified media

Number of Injured Cells				
Culture Time in Days	**At pH 5.0**	**At pH 4.5**	**At pH 4.0**	**At pH 3.5**
2	0.4	0.4	1.6	4.0
4	0.6	0.7	1.8	5.1
6	0.9	1.1	2.7	Not Detected

From table 2 and table 3 a clear die-off of the *S.dysenteriae* Type1 was observed at pH 3.5 or below after keeping the cultures 10 days at 4 ºC and 6 days at 20 ºC. So it can be revealed that acid foods, especially if kept at refrigeration temperatures support survival of *Shigella*.

Discussion

Shigella dysenteriae, the causative agent of bacillary dysentery, is a major public health problem in developing countries were sanitation is poor. It is endemic in Eastern India, especially in Kolkata and adjoining districts of West Bengal, India (1).

The minimum pH (4.0) for growth under otherwise optimum conditions was established in the present study for *Shigella.* This was lower than the minimum pH for growth mentioned by ICSMF (9). Aerobic log-phase cultures grown at pH 5.0 were similarly acid resistant whereas survival in exponential phase cells grown at pH 8.0 survival decreased 10- to 100-fold (13).

Acid resistance depends on several factors including the test strains, the growth phase and the pH of the medium in which cells are grown prior to the acid challenge. In this study, it was observed that aerobic stationary-phase cultures of *S. dysenteriae* Type1 grown initially at external pH of 5–8 showed 100% survival after an acid challenge of pH 3.5 for 2 h (data not shown). The acid resistance of *Shigella* depends on the expression of the stationaryphase-specific sigma factor sigma S, a homologue of RpoS. The RpoS requirement can be overcome by anaerobic growth in moderate acid (13). In the present study, acid tolerance of non acid-adapted aerobic stationary-phase cultures was determined.

In foods, the interactions between such factors as temperature, pH and the presence of salt can effectively inhibit the growth of *Shigella* spp. Because of the acidity of fruits, fruit juices do not constitute particular hazards for growth of human pathogens but they can sustain survival during a restricted period of time depending upon strain and exact pH. Many fruit juices on the market are pasteurized. This keeps them free from spoilage micro-organisms (9). *Shigella* is heat-sensitive which means that common pasteurization temperatures are sufficiently high to kill the pathogen (9). However, problems with Shigella in fruit juices could occur by post-contamination after opening or by under-pasteurized or fresh-squeezed non-pasteurized juice kept in refrigeration, the latter ones gaining popularity both in the market and in bars because of increased nutritional health benefits. The safety of non- or under-pasteurized juices relies mainly on juice pH and proper sanitation during processing and distribution. Shigellosis is characterized by a low infective dose and *Shigella* as other foodborne pathogens of faecal origin are known to be carried by food handlers and have been transmitted and caused vegetable-fruit associated illness (7). Decreased temperature enhanced survival of *Shigella*. In a study on the survival characteristics of *Shigella flexneri*, Zaika (2001) also reported that in general survival increased as temperature decreased and as pH increased (14). The application of a combination of suboptimal intrinsic and extrinsic factors such as reduced pH and reduced temperature can indeed inhibit the outgrowth of pathogens in foods but may induce prolonged survival of highly virulent pathogens such as *Shigella*. In laboratory media shigellae die rapidly at pH 4.0, but in the present study on survival of *Shigella* it was observed that they can survive for an extended period under adverse pH conditions, if kept at refrigeration temperatures.

Fruit juices, such as tomato juice usually has pH 3.9-4.1, apple juice pH 3.3-3.4, orange juice pH 3.5, freshly cut papaya pH 5.6 and also these types of fresh produces are usually kept at low temperatures. Once contaminated during cutting, juicing or in transit, these pathogens can survive for an extended period at low temperatures even under adverse pH conditions and these foods may serve as a vehicle for *Shigella* infections.

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Immunohistopathological changes in the bursa of fabricius during the hypersensitivity reactions-IV induced by experimental ascaridiasis in white leg horn chicks

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Abstract : Bursa is a primary lymphoid organ develops as a dorsal diverticulumn of the proctadeal region of the cloaca. The luminal surface of the bursa in chicken is plicated with as many as 15 primary and 7 secondary plicae or folds. The B cell differentiates in the bursa or its equivalent and is the responsible for the synthesis of Ig and antibody. During present investigation chicks were hyperensitized with the high and low doses of the egg antigen of *ascaridia galli* after the 10 days of the primary sensitization with the same doses subsequently. Immunohistopathological changes have been observed at 25th, 35th, 45th days of the experiment which showed thickening of the muscle layer of the capsular wall of the bursa. At the 35th, 45th days lumen of bursa was found to be greatly reduced. In each follicle cortex and medulla revealed abundant concentration of B lymphocytes. Marked hypertrophy of follicles was also distinct. The arterioles were found to be dilated. Surface epithelium was observed to be ruptured at several places.

Key words **:** *Ascaridia galli, Bursa of fabricious, hypersensitivity type-IV, Hypersensitization, white leg horn chicks*

Introduction

Parasitic infection account for hundred of millions of dollars annual losses for livestock and poultry industry throughout the world. The most costly parasite in terms of production losses are the gastrointestinal nematodes in ruminants and poultry (Gamble and Zarlenga, 1986). Infections with the fowl nematode *Ascaridia galli* (Shrank 1788) are widespread among domesticated and wild birds throughout the world (Soulsby 1982). *Ascaridia galli* (gastrointestinal nematode) cause *ascaridiasis* in the white leg horn chicks. Parasites induce different immune responses in the host. Sometime these immune responses are severe .These inappropriate responses called hypersensitivity reactions. Type –IV immunity reaction involve immunopathological changes in the lymphoid organs. The lymphoid system of chicken consists of unique organs (bursa and thymus). Bursa is a primary lymphoid organ develops as a dorsal diverticulumn of the proctadeal region of the cloaca. lymphoid follicle associated epithelial cells possess micropinocytotic activity responsible for the uptake of antigen present in the bursal lumen and their transfer inside the medulla of the lymphoid follicle(Bockman&Cooper,1973; Schaffner et al. 1974; Sorvari et al.1975; Bockman &Stevens, 1977).These antigens causes severe changes in the immunohistological archeitecture of the tissue. Hypersensitivity reaction against experimental ascaridiasis has not been study so far hence the present studies were taken up.

Material and method

Experimental host- white leghorn chicks

Experimental parasite- *Ascaridia galli*

Dose of infection – low dose- 1000 embryonated eggs of the *A.gall i/chick*
High dose-2000 embryonated eggs of the *A.galli/chick*

Culturing of the egg-

Adult worms were isolated from the intestine of fowl from the local abattoir. *A .galli* eggs were cultured upto infective stage according to Reidel method (1947). The worms were gently squeezed with the help of a pair of blunt end forceps so that all extraneous material except the uteri were separated and removed from the petridish.The distal ends of uteri containing

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mature eggs were separated and gently squeezed for fertilized eggs. The eggs were kept in sterile solution at 32°c for embryonation for 2-3 weeks. The eggs of *A. galli* were washed regularly with normal saline. Two drops of 0.1% fromaline was added to prevent fungal infections. Now these embryonated eggs have been used for sensitization and hyper sensitization of the chicks.

Preparation of the inocula-

The number of embryonated eggs per dose was estimated by dilution technique. The eggs were suspended in known volume of normal saline. The embryonated and infective eggs were counted with the help of stereoscopic binocular microscope. Three values were taken by repeating the process. Mean of these three values was used for calculating the number of embryonated infective eggs of *A.galli*.

During present investigation chicks were divided into 3 groups-

Group-A having 6 nonsensitized (control) chicks,

Group-B having 10 chicks, sensitized with the low dose of the egg antigen

Group-C- having 10 chicks, sensitized with the high dose of the egg antigen.

15 days old white leg horn chicks were sensitized with the low dose and high dose of the egg antigens. Now both the group-B and Group-C were further divided into two group-Ba &group-Bb and group-Ca & group-Cb. Groups Ba &Ca were the sensitized groups with the low and high doses subsequently. Sensitized chicks with the low and high doses were autopcised at 25th (Ba1&Ca1), 35th (Ba2&Ca2), 45th day (Ba3&Ca3) of the experimental design. While the 4 chicks form each group (Bb&Cb) were hypersensitized after the 10 days of primary sensitization. The hypersensitized chicks were autopcised at 35th (Bb1&Cb1) and 45th days (Bb2&Cb2) day of the experimental design.

Preparation of the tissue used for screening-

Specimens of bursa of fabricious were obtained from freshly killed animals according to experimental design. Tissue samples have been fixed in 10% formalin. Regular washing has been done after fixation. Tissue has been processed for microtomy and histolpathoogical slides were prepared.

Results

Immunopathological changes in the bursa of fabricious of the white leg horn chicks have been observed during sensitization and hypersensitization of the chicks with the low and high doses of the embryonated eggs of the *A.galli* at 25th ,35th and 45th days of the experiment in the following groups-

Group (Aa1) - Nonsensitized group (Control) - fig-1a, fig-1b)

The transverse section of bursa from nonsensitized chicks showed the following structure. The bursa was broadly divided into two regions-

1. Capsule- : It was made up of outer thin serosa layer and inner muscularis.
2. Sub-capsule: It consisted of mucosa and surface epithelium. It is made up of two parts-

1. Plicae- Mucosa developed into a number of villus like projections invading the central lumen called plicae.(1a).

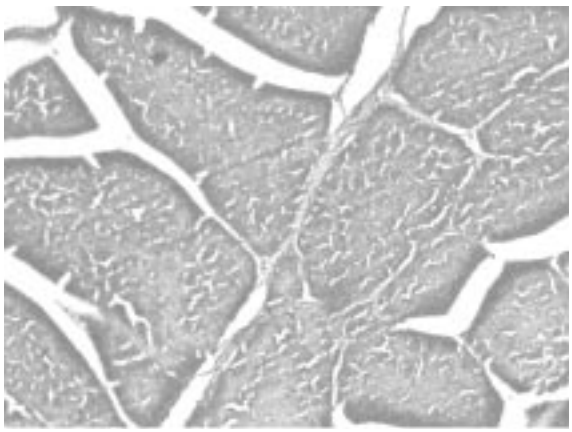

Figure 1(a) : Slide showing several plicae in the bursa of the nonsensitized (control) (10 x)

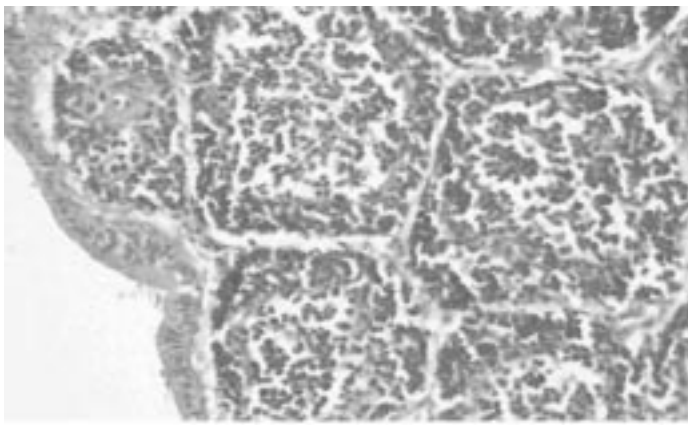

Figure 1(b) : Slide showing a plicae having polyhedral follicles in the bursa of the nonsensitized chicks. (40 x)

2. Follicles- Each plicae consisted of a number of polyhedral follicles, closely packed together with small amount of connective tissue separating them and blood vessels lying between them.(1b.)

Each follicle was divided into two regions :

a) Outer cortex and

b) Inner medulla. Both cortex and medulla possessed a supporting .network of stellate reticulo-endothelial cells whose meshes were filled with lymphoid cells or lymphocyte cells.

Group (Ba1) - Sensitized group with low dose - (fig.-2)

During sensitization with the low dose, Surface epithelium of plicae was found to be ruptured at several places. Interfollicular spaces were not so distinct. Moderate enlargements of follicles size were observed. Lymphocyte percentage was significantly increased in follicles.

Group (Ca1)-Sensitized group with high dose-(fig-3)

During sensitization with the high dose, surface epithelium was also ruptured at several places. Slightly atrophied follicles were observed and also revealed comparatively less lymphocytes in the cortex region of the follicles. Sensitized group showed some inflammatory responses.

B. Analysis of immunopathological changes at 35th day of the experimental design-At 35th day immunopathological study of the bursa was done in the following group-

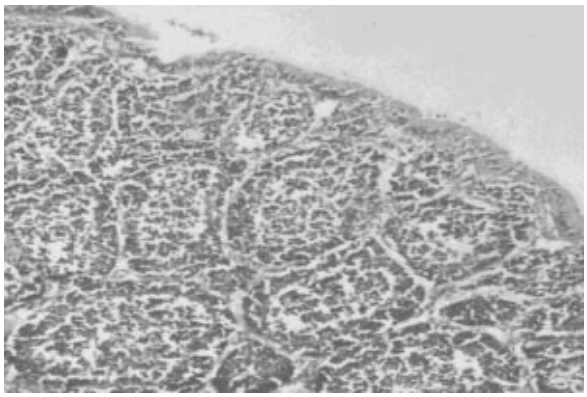

Figure 2 : Slide showing ruptured surface epithelium at several places, moderate enlargement of follicles size, and increased lymphocytes. (10x)

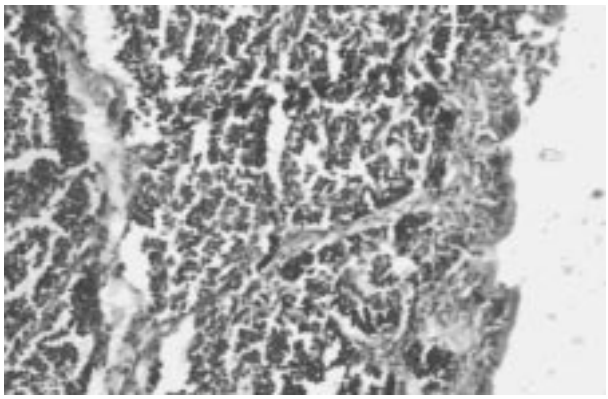

Figure 3 : Slide showing ruptured surface epithelium at several places, slightly atrophied follicles, less lymphocyte in the cortex region of the follicles. (40x)

Group (Ba2) - Sensitized group with low dose- (fig-4)

Bursa of this group revealed atrophied lymphoid follicles. Blood vessels get enlarged.interfollicular space showed hemorrhage. This group revealed severe retrogressive changes of lymphocyte cells in most of the follicles. The medulla of the follicles showed comparatively more depletion of lymphocytes than cortex.

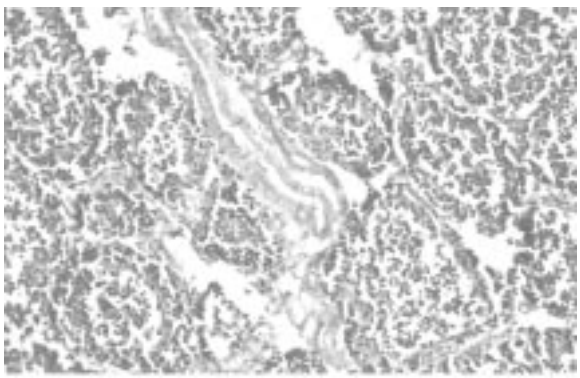

Figure 4 : Slide showing atrophied follicles, haemorrhage in interfollicular space, less depletion of lymphocyte in cortex region than medulla (40 x)

Group (Bb1) - Hypersensitized group with low dose – (fig-5)

In this group, hypertrophied follicles were found. The pathological disturbances were observed more in cortex revealing depletion of lymphocytes. The interfollicular spaces were filled with oedematous fluid.

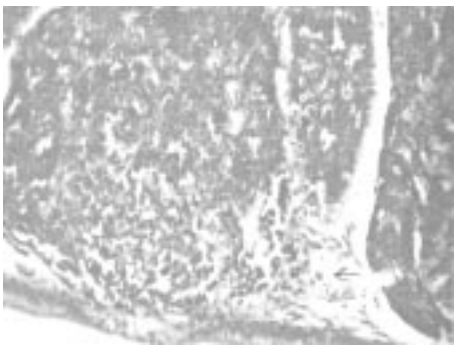

Figure 5 : Slide revealing depletion of lymphocytes in the cortex region, interfollicular spaces filled with oedematous fluid. (40x).

Group (Ca2) - Sensitized group with high dose– (fig-6a,6b)

In this group, bursa was characterized by hypertrophy of follicles in the plicae. Interfollicular spaces were filled with oedematous fluid. At certain places, plicae wall observed to be disappeared while at some places thickening was also observed in the plicae epithelium. Lymphocyte increased in the cortex region of the follicle.

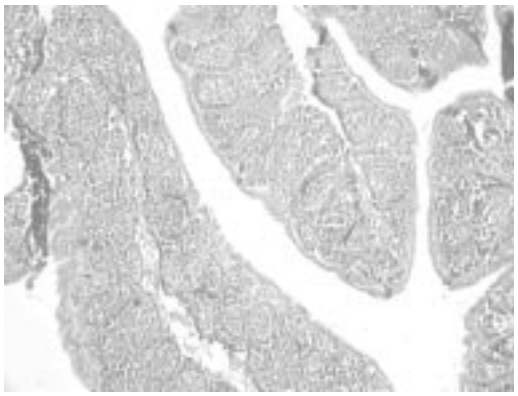

Figure 6(a) : Slide revealing hypertrophied follicles, thickening in plicae epithelium, increased lymphocyte in the cortex region(10x).

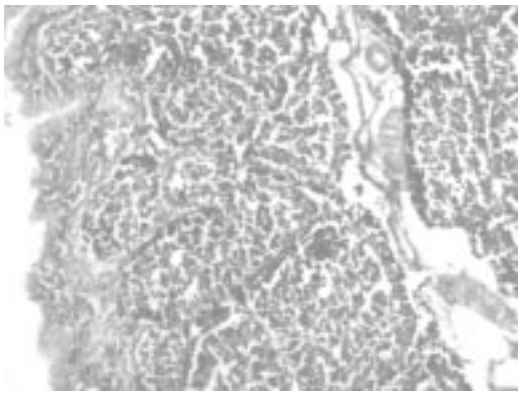

Figure 6(b) : Slide showing hypertrophied follicles, thickening in plicae epithelium, increased lymphocyte in the cortex region(40x).

Group (Cb1)-Hypersensitized group with high dose– (fig-7a,7b)

In this group, surface epithelium of the plicae was thickened. Atrophoid follicles showed depletion of lymphocytes in both cortex and medulla region. Interfollicular spaces were clearly observed.

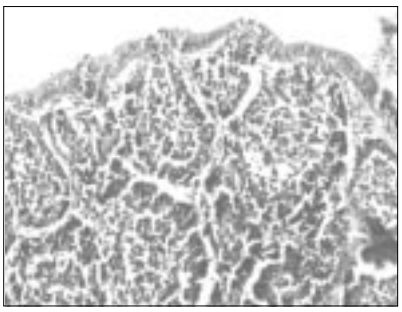

Figure 7(a) : Slide showing thickened plicae epithelium, atrophied follicles, depletion of lymphocyte in both cortex and medulla region(10x)

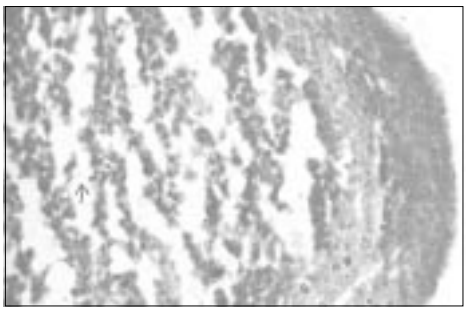

Figure 7(b) : Slide showing cloudy swelling in the plicae epithelium,atrophoid follicles, depletion of lymphocytes in both cortex and medulla region(40x)

C. Analysis of immunopathological changes at 45th day of the experimental design-At 45th day changes were observed in the following groups-

Group (Ba3) - Sensitized group with low dose–(fig-8a,8b)

In this group, at some places plicae wall ruptured. The bursal lumen became irregular. The follicles were observed to be decreased in size while some follicles showed hypertrophied condition.

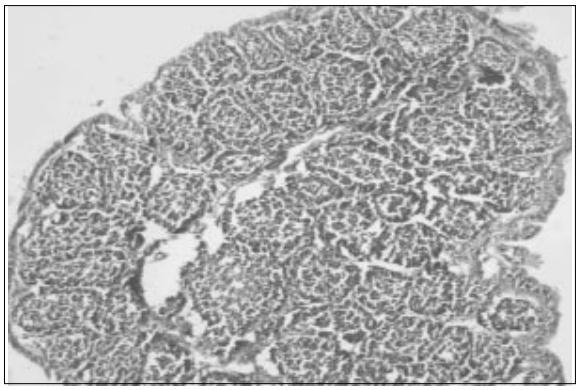

Figure 8(a) : Slide showing ruptured plicae wall, atrophoid follicles, as well as hypertrophoid follicles (10x)

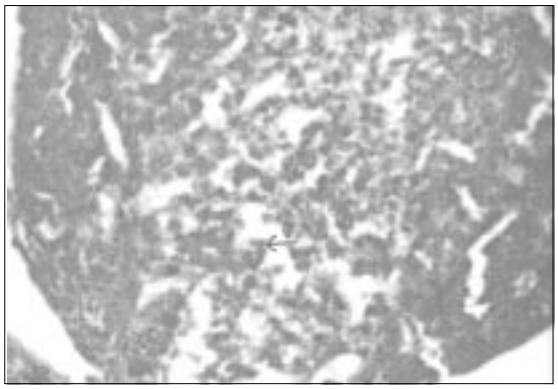

Figure 8(b) : Slide showing increased lymphocyte percentage (40 x)

Group (Bb2) - Hypersensitized group with the low dose–(fig-9)

Hypersensitized chicks of this group revealed that at certain places follicles became elongated in shape and interfollicular space of some plicae was increased while bursal lumen was noticeably wider. There was distinct vacuolization and degeneration of follicles, the follicles revealed rounded vacuoles numbering 2 to 4, increased in number and showed edema.

Group (Ca3) - Sensitized group with high dose– (fig-10)

Bursa of this group showed atrophoid follicles.Interfollicular spaces was clearly observed. There was depletion of the lymphocytes from both cortex and medulla region.

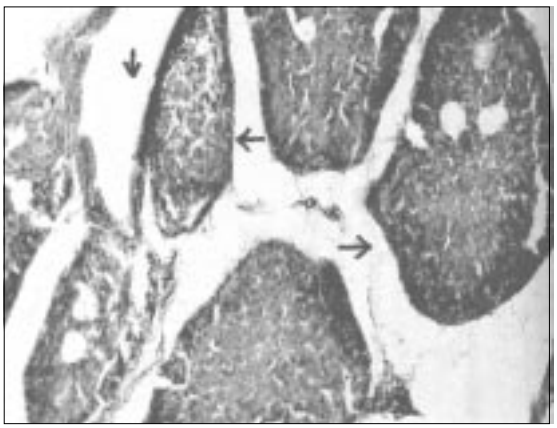

Figure 9 : Slide showing elongated follicles, wide bursal lumen, distinct vacuolization and degeneration of follicles. (40 x)

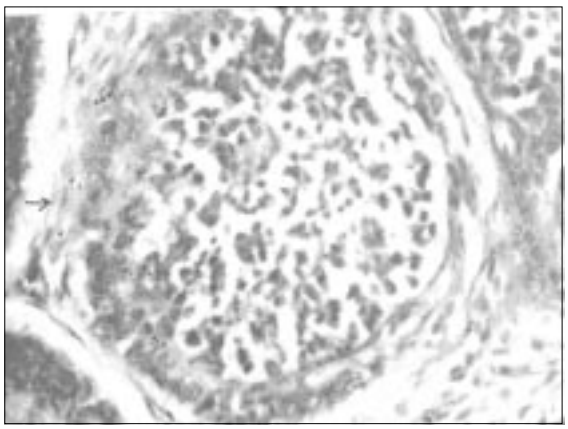

Figure 10 : Slide showing atrophied follicles, clear interfollicular spaces, depletion of lymphocytes in both cortex and medulla region. (40 x)

Group (Cb2) - Hypersensitized group with high dose– (fig-11)

At 45th day hypersensitized chicks with the high dose showed atrophy in the follicles. Follicles were appeared to be oval in shape. Some follicles were separated from epithelial layers. Cortex region showed marked depletion in lymphocyte concentration.

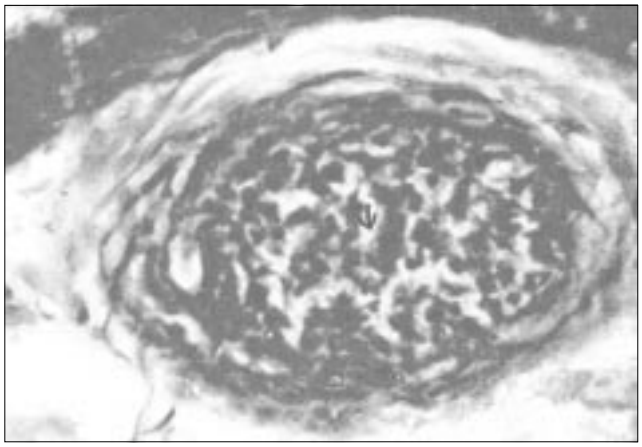

Figure 11 : Slide revealing atrophied oval follicles, separated from epithelial layer, marked depletion of lymphocyte. (40 x)

Discussion

During present investigation several degenerative changes have been found in the bursa of fabricious of sensitized and hypersensitized chicks with the low and high doses of the embryonated eggs. Slightly enlargement in the follicles in the sensitized group with low dose at 25th day showed the effect of the low dose. Hypertrophied condition in the sensitized and hypersensitized chicks show high rate of maturation of lymphocytes due to high amount of antigen interaction. Ruptured surface epithelium caused by enlargement of follicles. Similar finding have been showed by (Carpenter et al., 1992). Histopathologically the capsular wall was found to be more or less thickened. The follicles were found to be atrophied and depletion of lymphocytes was observed in present study. Lymphoid follicle atrophy was probably the result of the migration of the lymphocytes to the site of infection.Immunopathological lesions in infected chicken are formed resulting into hypoplasia and atrophied bursa of fabricius (Bagust et al. 1979; Taniguchi et al., 1977). Degenerative changes and depletion of lymphoid cells were observed in bursa of fabricius of vaccinated broiler chicks (Jeurissen et al.. 1998; Stoevetal.,2000).Present author find out the immunopathological changes were directly proportional to the virulence of the infection of the parasites. Hypersensitized group revealed more severe histopathological changes in the bursa of fabricious in comparison to sensitized chicks. Vacuolization and degeneration of the follicles were observed due to the leakage of endotoxins into the bursa, released during antigen-antibody interaction. It may be due to severity of infection. Bursal lesions have been observed by Okoyo (1984), Mohanty and Rao (1984) and Mishra (1984).Immunopathologically the bursa of fabricious revealed severe retrogressive changes of lymphocytes in most of the lymphoid follicle and many of the lymphocytes were replaced by reticuloendothelial cells. Diffused lymphoid tissue was noted in the bursal canal and the follicles became separated from epithelial layer with the medulla. The epithelial layer and lumen also disappeared leaving muscle, connective tissue, blood vessel and follicles with and without medulla. The main cause of these severe changes is that T cell play important role during hypersensitivity reaction-IV. T cell, sensitized to antigen, release lymphokines following secondary contact with the antigen. cytokines induce inflammatory responses; they also activate and attract macrophages, which release inflammatory mediators. These mediators affect lymphoid organs.

Acknowledgement

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Studies on immunological impact of some chemicals, botanicals, antibacterial proteins and live non-pathogenic bacteria in silkworm, *Bombyx mori* L. to control bacterial disease

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Abstract : *Bombyx mori* L., mulberry silkworm is susceptible to protozoan, bacterial, fungal and viral infections like other economic insects. Bacterial diseases affecting silkworms are collectively known as 'flacherie' due to the flaccid conditions of the diseased larvae. 40% crop losses are attributed due to diseases of silkworm in India including more than 15% crop loss is figured due to bacterial diseases under conditions of low humidity and extreme heat, particularly during pre-monsoon seasons (April - June). Poor disinfection, accumulation of pathogenic load in the rearing trays, feeding of contaminated mulberry leaves and improper use of bactericide leads to large scale crop loss due to bacterial diseases. Biological and chemical control measures as well as development of disease tolerant silkworm breeds are insufficient to control the disease in the present degraded environmental scenario. Therefore, silkworms are very often attacked by the pathogens. To overcome this problem, promising bivoltine and multivoltine breeds available in our Germplasm bank have been screened on the basis of haemocyte counts in response against lower to higher pathogenic bacterial challenge for future breeding programme. Highly pathogenic bacteria, *Streptococcus* sp. is known to play an active role in causing '*Bacteremia*' a bacterial disease of digestive organ of silkworm. This communication reports the impact of induced immunity by induction of some immunogens to control bacterial disease in silkworm. A comparative study based on economic parameters was undertaken among the treatments of distilled water sprayed healthy control; highly pathogenic bacteria sprayed infected control; immunogens sprayed immunized larvae challenged with highly pathogenic bacteria. This study could help to select the highly effective immunogens (s) to improve the humoral defense response to control the bacterial disease in silkworm at the farmer's field.

Key words **:** *Antibacterial protein, Bacterial disease, Bombyx mori* L., *Flacherie, Immunogen.*

Introduction

Bombyx mori L. is highly susceptible to various diseases like Pebrine (Microsporida), Flacherie (Bacteria), Grasserie (Virus) and Muscardine (Fungus) and the pathogens have a great role in deterioration of sericulture industry. 40% crop losses are attributed due to silkworm diseases in India (Sheeba Rajakumari et al, 2007). About 36 % crop loss is attributed due to Pebrine disease with occasional crop failure (Nataraju et al, 2005). Further, crop loss due to silkworm diseases ranged from 0.58 - 11.18 %, 2.03 - 12.44 % and 3.65 -15.58 % respectively, in three potential districts i.e., Murshidabad, Birbhum and Malda of West Bengal and the crop loss was high during unfavourable seasons (May-September, 1987) (Subba Rao et al, 1991). Grasserie, Flacherie and Muscardine are the major silkworm diseases and average crop loss ranged from 35.7 - 44.7 % during unfavourable seasons in West Bengal, when both temperature and humidity remain high, specially prevailing high humidity is the major constraint (Madana Mohanan, 2009). Among the silkworm diseases, Bacterial or 'Flacherie' disease is prevalent and about 33 - 44% crop loss was recorded out of total loss in West Bengal during unfavourable seasons (Subba Rao et al, 1991). The larvae die in 3-7 days and 100% mortality was obtained by 7^{th} day of post inoculation due to 'Bacteremia' (Nataraju et al, 2005). Several disease control options are available; these include biological and chemical control as well as introduction of disease tolerant silkworm breeds based on direct antibiotic principle. Most of the studies are conducted for evaluation of disease tolerant silkworm breeds on screening of silkworm from Germplasm bank and making hybrids from the tolerant silkworm breeds.

However, the main draw back of such studies is that the Lethal Dose (LD) levels of the pathogens vary with the environmental factors and the identified tolerant silkworm breeds are fail to cope up with day to day degraded environment and easily attacked by bacterial, viral and fungal infection. On the other hand total haemocyte counts responsible for a cellular

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defense of a breed remain almost same. So, there is no way to increase further disease tolerance through cellular defense response of the tolerant silkworm breeds, identified so far.

In insects two different types of immune systems exist - cellular and humoral. These two immunological responses are complementary and both may be seen in the same infection. The immunity in insects differs from higher vertebrates as insect produce no lymphocytes, no T cells and no helper cells. Further insect do not biosynthesize immunoglobulin. Haemocyte a complex circulating cell in haemolymph are involved in conferring a cellular immunity. Prohemocytes, Plasmatocytes, Granulocytes, Spherulocytes and Oenocytoids are well defined haemocyte are found in haemolymph of silkworm. Humoral immune system does not involve antigen-antibody reactions and has efficient self defense mechanism against bacterial infection through induction of antibacterial peptides (Dunn, 1986; Stanley-Samuelson et al, 1991)

Therefore, present attempt is restricted to immunize the silkworm breeds by the induction of immunogens through humoral immunity to give more resistance to the silkworm against bacterial disease. Many workers have also worked in different insects (Chadwick, 1970; Hultmark et al, 1980; Hoffman et al, 1981; Hughes et al, 1983, Stanley-Samuelson, 1991; Ischimori et al, 1992, Morishima et al, 1992; Sumida et al, 1992; Hara and Yamakawa, 1995; Kato et al, 1993; Abhram et al, 1995; Iketani et al, 1999; Sharma et al, 2005, Sheng et al, 2005; Choudhury et al, 2004; Cytraynska, 2006). But no retrospective work is available to immunize *B.mori* with some micronutrient supplements, botanicals, antibacterial proteins and live non-pathogenic bacteria to control bacterial disease caused by, *Streptococcus* sp. except the preliminary work done by Sheeba Rajakumari et al (2007) with feed supplements of some probiotic.

Materials and methods

I (a) : Screening of suitable micronutrient supplements for immunization

20 ml. of five different micronutrient supplements (Retinol - T1, Pyrodoxine hydrochloride - T2 , Ascorbic acid - T3, Cholecalcipherol – T4 and Tochopherol - T5 purchased from the market) were sprayed on the fresh mulberry leaves (*Morus alba*, var. S_{1635}) i.e., orally immunized separately with different selective doses chronologically, 1250 IU/ml, 2mg/ml, 25mg/ml, 1500 IU/ml and 20mg / ml along with suitable adjuvant (distilled water and 90 % ethyl alcohol) to the five different batches of 60 larvae of *B.mori* (Breed: Nistari, multivoltine maintained at the Institute) in each batch after brushing (1^{st} day '0' hr.) as five treatments. All the above treatments were challenged with *Streptococcus* sp. (stock prepared from the infected larvae, Choudhury et al, 2004) with selective dose (1×10^8 c.f.u. / ml) to the larvae after resumed from 2^{nd} moult (1^{st} day '0' hr.). Triplicate replications each of 60 larvae were maintained for each treatment. Triplicate replications each of 60 larvae were maintained as healthy control (HC) where neither any treatment for oral immunization nor any inoculation of pathogen was done. Triplicate replications each of 60 larvae were also maintained as infected control (IC) where only selected dose of pathogen was inoculated without oral immunization. Rearing was conducted under 26 - 33ºC and 68 - 86% relative humidity with 12L and 12D photoperiodic condition. After completion of rearing, mortality %, mature larval weight at fifth stage larvae before spinning (g) Effective Rearing Rate (No.), Single pupa weight (g), single cocoon weight (g) and single shell weight (g) were recorded and total pupal protein (mg/g tissue weight) was estimated following standard method (Lowry et al, 1951).

I (b) : Identification of larval stage suitable for immunization

For the 2^{nd} experiment, the same procedure was followed in case of oral immunization and inoculation of pathogens to silkworm except that the larvae resumed from 3^{rd} moult (1st day '0' hr.) were considered for the experiment and selective dose of pathogen inoculations was done after 48 hrs of oral immunization. Triplicate replications as healthy control and infected control were also maintained following the same procedure followed in previous experiment. Rearing was conducted under same climatic condition followed in previous experiment. Data were recorded, calculated and analyzed statistically as like as previous experiment.

I(c) : Short listing of immunogens for immunization

For the 3^{rd} experiment, the same procedure was followed in case of inoculation of pathogens to silkworm except that oral immunization was done with micronutrient supplements to the larvae resumed from 2^{nd} moult (1st day '0' hr.) and again immunization was done with live non-pathogenic bacteria, *Lacobacillus acidophilus* etc. in the dose of 1.5×10^8 c.f.u./ml to the larvae resumed from 3^{rd} moult (1^{st} day '0' hr.) and selective dose of pathogen inoculations was done after 48 hrs of second immunization. *L. acidophillus* were grown overnight at 30ºC in Luria broth (g / L : tryptone 10, yeast extract 5, NaCl 5) in a rotary shaker (200 rpm, Adolf Kuhner AG). Bacteria were washed by centrifugation in the next day (4°C, 5000 rpm, Sorvall RC

5B plus SS34) and the pellete was resuspended in sterile saline (Choudhury et al, 2004). Triplicate replications of healthy control and infected control were also maintained following the same procedure followed in case of previous experiments. Rearing was conducted under same climatic condition followed in previous experiment. Data were recorded and calculated as like as previous experiment.

All the data were analyzed statistically and Tochopherol in 90 % alcohol (T1) along with live non-pathogenic bacteria, *Lacobacillus acidophilus* etc. performed better than others and those were selected for further experiment as well as the larvae resumed from second moult (1st day '0' hr.) were considered for the experiment. Results of the first three experiments are presented in the Table-I.

II. Selection of immunogens during unfavourable seasons

For the 4th experiment, the same procedure was followed for oral immunization to the larvae resumed from second moult (1st day 1st hr.) and inoculations of pathogens was done 48 hrs of post imminuzation, where, Tochopherol in 90 % alcohol (T-1) selected as best from previous experiment, Nicotinic Acid in saline water (0.65% NaCl) (T-2) as micronutrient supplement, Proline in saline water (0.65% NaCl) (T-3), Proline in 90 % alcohol (T4) and Lysozyme in saline water (0.65% NaCl) (T-5) are as antibacterial proteins, *Lactobacillus acidophilus* etc, in saline water (0.65% NaCl) (T-6) as live non-pathogenic bacterium selected as best from previous experiment as well as *Spriluna* sp. in saline water (0.65% NaCl) (T-7) and 20 % *Helica chibulia* powder in distill water (T-8) as two botanicals were considered for the experiment. Triplicate replications of 60 larvae as healthy control (HC) and infected control (IC) were also maintained following the same procedure followed in previous experiments. Rearing was conducted during three unfavourable seasons during June – July, 2009 under 28 - 35ºC and 68 - 93 % relative humidity, July-Aug, 2009 under 26 - 32ºC and 74 - 93 % relative humidity and Aug - Sep, 2009, under 27 - 32ºC and 74 - 93 % relative humidity with 12L and 12D photoperiodic condition in three unfavourable seasons. After completion of rearings the data were analyzed statistically. Results of this experiment are presented in the Table-II.

III. Effect of selected immunogens in available breeds during favourable seasons

For the 5th experiment, the same procedure was followed for oral immunization to the larvae resumed from 3rd moult (1st day '0' hr.) and inoculation of pathogens was done 48 hrs of post immunization, where, Nicotinic Acid in saline water (0.65% NaCl) (T1) as micronutrient supplement, Proline in saline water (0.65% NaCl) (T2) and Lysozyme in saline water (0.65% NaCl) (T3) are two antibacterial proteins as well as *L.acidophilus* etc. in saline water (0.65% NaCl) (T4) as live non-pathogenic bacterium performed better than other immunogens in the previous experiments and were considered for the present experiment. Besides Nistari, two other popular multivoltine breeds in West Bengal , M 12(w) and M6DP(c) were considered for the experiment.

Triplicate replications of 60 larvae healthy control (HC) and infected control (IC) were also maintained following the same procedure followed in first experiment. Rearing was conducted during two favourable seasons during Nov. – Dec, 09 under 22 - 29ºC and 51- 85 % relative humidity and during Feb. – Mar,10 under 19 - 31ºC and 43 - 77 % relative humidity with 12L and 12D photoperiodic condition.

Mortality %, mature larval weight at fifth stage larvae before spinning (g) Effective Rearing Rate (No.), Single pupa weight (g), single cocoon weight (g) and single shell weight (g) were recorded. After completion of three rearing all the data were analyzed statistically. One micronutrient supplement, two antibacterial proteins and one non-pathogenic bacterium, which performed better than other immunogens, were selected for further experiment. Results of this experiment are presented in the Table-III.

Results

All the experiments, treatments as well as experiments and treatments were found highly significant ($P<0.01$) in respect of larval mortality%, Effective Rearing Rate (No.), weight of whole pupa, mature larval weight, single shell weight and fecundity (Table-1). All the treatments among the experiments were effective to reduce larval mortality%. However, T-5 was very much effective to reduce maximum larval mortality% in both the 2nd (3.33 %) and 3rd experiment (2.00%) whereas larval mortality % were recorded more than 99.0 % and 13.0 – 30.7% in infected control (IC) and healthy control (HC) batches respectively. Larvae resumed from 3rd moult (1st day '0' hr) were suitable for oral immunization than larvae resumed from 2nd moult (1st day '0' hr) as larval mortality % for all the treatments were found lower in 2nd and 3rd experiments than 1st experiment (Table-I).

Table-I. Screening, selection and short listing of suitable of immunogens against bacterial disease of silkworm, *Bombyx mori* L. [T1 = Retinol, T2 = Pyrodoxine hydrochloride,T3 = Ascorbic acid,T4 = Cholecalcipherol, T5 = Tochopherol in 90 % alcohol, HC = Healthy control in distill water, IC = Infected Control in distill water, Data in parentheses represents standard error of mean,*** = p > 0.001(significant at 0.1 % level) , ns = non significant, EXP = Experiment]

EXP	Treat	Mortality %	Mature larval wt (g)	ERR (No)	Single Pupa wt. (g)	Protein (mg/Gm pupa tissue wt.)	Single Cocoon wt (g)	Single Shell wt. (g)	Fecun-dity
1	T1	62.00	2.321	3600	1.805	91.2	1.021	0.114	220.7
		(2.00)	(0.14)	(100)	(0.035)	(0.643)	(0.21)	(0.03)	(6.0)
	T2	55.00	2.217	4722	1.932	73.3	1.009	0.122	199.3
		(2.00)	(0.18)	(48)	(0.019)	(1.94)	(0.31)	(0.03)	(4.0)
	T3	48.00	2.243	5433	1.865	71.4	1.067	0.121	291.7
		(2.00)	(0.16)	(58)	(0.310)	(1.70)	(0.17)	(0.20)	(5.7)
	T4	65.00	2.175	3500	1.582	73.4	1.035	0.115	192.7
		(2.65)	(0.17)	(100)	(0.044)	(2.76)	(0.21)	(0.02)	(5.1)
	T5	44.33	2.175	5655	1.549	88.8	0.961	0.124	219.7
		(1.667)	(0.17)	(51)	(0.016)	(1.51)	(0.29)	(0.02)	(2.5)
	HC	25.33	2.287	7552	1.812	88.4	1.040	0.136	207.3
		(1.53)	(0.22)	(63)	(0.039)	(1.00)	(0.21)	(0.03)	(2.5)
	IC	98.00	1.172	0333	1.032	21.20	0.732	0.098	84.7
		(2.00)	(0.22)	(12)	(0.010)	(0.98)	(0.11)	(0.01)	(2.5)
2	T1	40.00	2.875	7685	1.810	91.8	1.260	0.168	232.3
		(2.00)	(0.23)	(17)	(0.021)	(0.79)	(0.24)	(0.02)	(6.4)
	T2	8.667	3.197	8891	1.944	73.1	1.379	0.190	259.0
		(1.53)	(0.31)	(60)	(0.012)	(0.20)	(0.24)	(0.02)	(6.6)
	T3	11.667	3.176	9517	1.869	71.8	1.322	0.178	321.6
		(1.53)	(0.21)	(104)	(0.007)	(0.54)	(0.33)	(0.03)	(3.8)
	T4	16.00	2.818	7650	1.602	73.7	1.221	0.166	231.0
		(2.00)	(0.30)	(50)	(0.020)	(0.25)	(0.20)	(0.04)	(3.6)
	T5	3.333	3.147	9629	1.584	89.2	1.266	0.168	335.0
		(1.53)	(0.15)	(279)	(0.031)	(0.46)	(0.27)	(0.02)	(5.0)
	HC	30.667	2.867	8233	1.822	88.8	1.214	0162	321.3
		(2.58)	(0.21)	(100)	(0.011)	(0.42)	(0.27)	(0.03)	(1.5)
	IC	99.00	1.181	0500	1.002	21.4	0.985	0.087	89.7
		(2.00)	(0.12)	(21)	(0.010)	(0.10)	(0.10)	(0.01)	(2.5)
3	T1	14.000	2.888	9364	1.822	92.5	1.251	0.152	260.7
		(1.00)	(0.24)	(200)	(0.011)	(0.26)	(0.26)	(0.04)	(3.1)
	T2	4.000	2.997	9747	1.851	74.5	1.204	0.175	253.3
		(1.00)	(0.30)	(122)	(0.066)	(0.29)	(0.23)	(0.03)	(1.5)
	T3	7.333	3.185	9962	1.914	73.3	1.330	0.180	364.0
		(1.53)	(0.23)	(28)	(0.066)	(0.53)	(0.24)	(0.03)	(2.0)
	T4	6.000	2.874	9974	1.654	75.3	1.252	0.164	282.7
		(1.00)	(0.21)	(36)	(0.045)	(0.31)	(0.37)	(0.03)	(2.5)
	T5	2.000	2.995	9985	1.649	90.8	1.300	0.181	370.3
		(1.00)	(0.31)	(17)	(0.036)	(0.40)	(0.30)	(0.04)	(2.5)
	HC	13.000	2.914	8877	1.886	90.1	1.260	0.171	349.0
		(2.00)	(0.27)	(11)	(0.013)	(0.12)	(0.29)	(0.03)	(3.6)
	IC	99.10	1.182	0166	1.012	20.0	0.973	0.093	86.0
		(1.89)	(0.17)	(12)	(0.010)	(0.10)	(0.10)	(0.01)	(4.6)
	E	***	***	***	***	ns	***	***	***
	T	***	***	***	***	***	ns	***	***
	ExT	***	***	***	ns	ns	ns	***	***
	CD	1.06	0.058	61.32	0.016	ns	0.043	0.006	2.071
	at 5 %	1.49	0.082	86.72	0.022	3.898	ns	0.007	3.163
		2.59	0.142	150.22	ns	ns	ns	0.014	5.478

Table II : Selection of immunogens in silkworm *Bombyx mori* (Breed : Nistari) against bacterial disease during unfavourable seasons

Treat	3rd instar 10 larval wt before immuni-zation (g)	3rd instar 10 larval wt before moulting (g)	4th instar 10 larval wt before moulting (g)	5th instar 10 larval wt before pathogen innocula-tion (g)	5th instar 10 larval wt (g) before spinning	Spin-ning worms (No)	Total Coccon/ Pupa (%)	Good Co-coon (%)	Mortal-ity (%)	Single Cocoon Wt (g)	Single Shell wt (g)	Shell (%)	Rank (Mano Index)
T1	0.262	1.054	4.455	12.921	15.854	66	66	57	34	0.708	0.084	11.85	9
T2	0.249	1.137	4.602	12.884	16.027	74	71	61	29	0.767	0.088	11.53	6
T3	0.250	1.183	4.598	14.708	16.912	74	67	61	33	0.718	0.087	12.09	2
T4	0.251	1.106	4.430	14.291	16.139	69	66	58	34	0.794	0.095	12.01	4
T5	0.257	1.158	4.744	13.832	16.044	72	65	60	35	0.764	0.093	12.17	1
T6	0.251	1.079	4.620	13.519	16.129	80	73	67	27	0.748	0.085	11.39	3
T7	0.245	1.024	4.290	14.198	16.920	72	61	57	39	0.755	0.086	11.44	8
T8	0.251	1.100	4.307	13.903	17.447	83	71	61	29	0.750	0.084	11.25	5
HC	0.247	1.058	4.544	13.460	16.474	74	71	67	29	0.675	0.083	10.83	7
IC	0.250	1.087	4.470	13.404	15.167	48	39	35	61	0.655	0.069	10.49	10
CD value at 5%	ns	ns	ns	1.000*	1.160**	11.5**	ns	4.078**	4.439**	0.0628**	0.00114**	ns	

(ns = Non significant , * = Significant at 5% level , ** = significant at 1% level)

Treatment details :

T1 = Tochopherol in 90 % alcohol, T2 = Nicotinic Acid in saline water (0.65% NaCl), T3 = Proline in saline water (0.65% NaCl), T4 = Proline in 90 % alcohol, T5 = Lysozyme in saline water (0.65% NaCl), T6 = *Lactobacillus acidophilus* etc, in saline water (0.65% NaCl). T7 = *Spriluna* in saline water (0.65% NaCl), T8 = *Helica chibulia* in distill water, HC = Healthy control in distill water IC = Infected Control in distill water.

Higher mature larval weight was observed in the T-2, T-3 and T-5 in both the 2nd and 3rd experiments. Mature larval weights of all the treatments were observed more than healthy control (except T-4 in the 2nd and 3rd experiments and T-2 in 2nd experiment as well as T-1 in all the three experiments). Mature larval weight at 5th stage was found higher and at par in 2nd and 3rd experiments respectively. But it was recorded highest in T-3 than T-2 and T-5. Highest ERR (No.) were also observed in T-5 of Experiment - 2 and 3. ERR (No.) were recorded higher in all the treatments of the 3rd experiment and T-2, T-3 and T-5 of 2nd experiment than healthy control. Weight of whole pupa was recorded highest in 3rd experiment among the three treatments. But T-2 and T-3 were effective to increase pupal weight in Experiment-1 and 2 but Treatment-3 were effective in 3rd experiment compare to healthy control. All the treatments were found highly significant ($P<0.01$) in respect of pupal protein per gram tissue weight. Pupal protein per gram tissue weight was found maximum in T-1 and T-5 in all the 3rd experiment but highest protein was observed in T-1 in 3rd experiment. All the experiments were found highly significant ($P<0.01$) in respect single shell weight. Single cocoon weight and single shell weight was found highest in T-3 and T-5 of 3rd experiment. Mean fecundity was recorded higher in 3rd experiment than 1st and 2nd experiments. Fecundity was recorded highest T-5 of 3rd experiment. Average fecundity was recorded highest in T-5 in all the experiments (Table - I).

Mortality % was recorded significantly lowest ($P< 0.1$) in larvae immunized under T-6 (27.0 %) followed by T-2 and T-8 (29.0 %) which is at par with Healthy Control (HC) where the mortality % was recorded highest (61.0%) in Infected Control (IC) during three unfavourable seasons (pool data) in 4th experiment. Mature larval weight followed by inoculation of pathogen was found highest in larvae under T-8 (17.4 g) followed by T-3 and T-7 (16.9 g) in 2nd experiment. However, Shell % was found maximum (12.17%) in T-5 in 2nd experiment (Table - II).

No mortality was recorded in larvae of M6DP(c) and mortality % was recorded significantly lowest ($P < 0.1$) in larvae immunized in T-3 (1.03 %) of M12 (w) followed by T-2 (1.09 %) of Nistari. Mortality % was recorded highest (17.8 – 29.9 %)

Table III : Effect of selected immunogens in available breeds of silkworm, *Bombyx mori* during favourable seasons

Treat	Race	3rd instar 10 larval wt before immuniza-tion (g)	3rd instar 10 larval wt before moulting (g)	4th instar 10 larval wt before moulting (g)	5th instar 10 larval wt before pathogen innoculation (g)	5th instar 10 larval wt before spinning (g)	Spinning worms (No)	Good Cocoon (No)	Mortality (%)	Single Cocoon Wt (g)	Single Shell wt (g)	Shell (%)	Rank (Mano Index)
T1	Nistari	0.296	1.134	5.965	9.692	28.4	93	89	2.14	1.115	0.139	12.5	15
T2	Nistari	0.299	1.124	5.732	10.334	29.2	92	91	1.09	1.111	0.140	12.6	13
T3	Nistari	0.297	1.145	5.916	10.653	28.7	92	91	2.14	1.152	0.144	12.5	12
T4	Nistari	0.307	1.120	5.900	9.783	30.0	93	91	1.79	1.156	0.144	12.5	11
HC	Nistari	0.298	1.078	5.792	10.270	28.1	90	89	2.86	1.103	0.138	12.5	17
IC	Nistari	0.295	1.095	5.754	9.741	29.0	89	75	17.82	1.148	0.139	12.1	18
T1	M12 (w)	0.328	1.347	6.474	12.321	30.0	91	89	2.55	1.270	0.181	14.3	5
T2	M12 (w)	0.328	1.366	7.035	12.409	33.6	92	89	3.51	1.280	0.186	14.5	3
T3	M12 (w)	0.323	1.418	6.768	13.406	32.6	99	93	1.03	1.289	0.182	14.1	1
T4	M12 (w)	0.313	1.549	6.783	13.192	33.5	92	92	2.81	1.326	0.191	14.4	2
HC	M12 (w)	0.315	1.266	6.705	14.355	32.0	92	80	13.21	1.327	0.201	15.1	4
IC	M12 (w)	0.317	1.196	6.616	13.877	32.3	94	75	19.65	1.266	0.180	14.2	6
T1	M6DP (C)	0.311	1.273	5.822	10.284	30.4	89	86	0.73	1.186	0.161	13.5	10
T2	M6DP (C)	0.312	1.246	5.992	10.034	31.0	94	93	0.69	1.191	0.163	13.7	7
T3	M6DP (C)	0.311	1.219	5.978	10.603	31.0	93	90	1.79	1.203	0.159	13.2	8
T4	M6DP (C)	0.295	1.182	5.848	9.893	31.4	92	91	0.00	1.184	0.160	13.5	9
HC	M6DP (C)	0.288	1.096	5.843	9.689	30.1	87	84	11.21	1.191	0.164	13.8	16
IC	M6DP (C)	0.329	1.131	5.770	9.865	30.2	95	66	29.91	1.159	0.154	13.3	14
CD value		0.030**	0.217**	0.369**	1.603**	2.707**	ns	7.346**	6.873**	ns	0.020**	1.255**	

(ns = Non significant , * = Significant at 5% level , ** = significant at 1% level)

Treatment details

T1 = Nicotinic Acid in distill water , T2 = Proline in saline water (0.65% NaCl),T3 = Lysozyme in saline water (0.65% NaCl), T4 = *Lactobacillus acidophilus* etc, in saline water (0.65% NaCl), HC = Healthy control in distill water IC = Infected Control in distill water) . Nistari,M12(w) and M6DP(c) are the breeds of *B.mori*.

in infected control (IC) of all the breeds during two favourable seasons (pool data) in 5th experiment. Good cocoon % and percentage of spinning worms was also more than 86 - 93 % and 90 – 94 % respectively in case of all the treatments and breeds. Besides, there was no significant difference was observed in single cocoon weight. However, shell % was highly significant ($P < 0.1$) and at par with the healthy control in case of Nistari and M6DP(c) and more than infected control in M12 (w). Larvae treated with T-3 performed best in all respect followed by T-4 and T-2 in M12 (w) than other breeds during favourable seasons. All the treatments performed best in M12 (w) in all respect followed by M6DP(c) and Nistari (Table-III)

Discussions

The primary defense of insects against pathogens is the prevention of infection via structural barriers such as rigid cuticle and periotrophic membrane that protects the midgut (Ono and Kato, 1968). Even after this if the bacteria persist in the system and then initial hemolymph response is mediated by circulating hemocytes by the process of phagocytosis, encapsulation and nodulation. If this innate mechanism of wiping out the antigen fails, synthesis of several proteins occurs through *de novo* synthesis of RNA and specific proteins, including lysozymes (Sharma et al, 2005), cecropins, defensins, attacins and moricin (Chadwick and Aston, 1991; Hara and Yamakawa, 1995) which give rise to increasing antibacterial activity in haemolymph (Hughes et al, 1983). This study reports first time on the immunological effects of few chemicals, micronutrient supplements, antibacterial proteins and live non-pathogenic bacteria in silkworm, *Bombyx mori* L. to control the bacterial disease.

Two of the micronutrient supplements, nicotinic acid and tochopherol are found very effective and essential for mounting optical immune responses and recommended doses are sufficient to give the best immune responses in young, healthy and uninfected silkworms. Diets of selective doses of micronutrient supplements could protect against certain signs such as weight loss, larval mortality % and economic parameters and high dose of micronutrient supplements safely enhance in immune responses in healthy elderly. Micronurient supplements can really inverse infections disease resistance especially in young aged silkworm larvae (Sheeba -Rajakumari et al, 2007).

The biological defense against pathogens in insects includes the innate physical barriers viz., integument and intestinal wall and humoral responses such as activation of prophenol oxidase cascade and induction of immune proteins namely, lysozyme, lectins, antibacterial proteins and antifungal proteins primarily by the fat bodies. Intestine harbours a great diversity of native microbes that promote gut maturation and integrity, antagonism against pathogens by producing antibacterial proteins and immune modulations (Girishkumar et al, 2005). Two of the antibacterial proteins, Proline and Lysozyme are found very much active for immune response to the silkworm in the present study. Inducible antibacterial proteins are present in muga silkworm which is a complex multicomponent, inducible, haemolymph protein system involved in defense against bacterial infection (Sharma et al, 2005).

Today the microbes are widely used in probiotic therapy where live microbial feed supplements which beneficially affect the host improving the intestinal microbial balance. These non-pathogenic bacteria play a key role in enhancing resistance to colonization by exogenous potentially pathogenic organism (Orhange and Nord, 2000). A mixture of live microorganism (probiotic) and non-digestible oligosaccharides (prebiotic) have been demonstrated to modify the composition of the micro flora , restore the microbial balance and therefore have the potential to provide health benefits when normal intestinal flora is disturbed due to diarrhea, food intoxification etc. Probiotics prevent infections by means of exclusion of pathogens due to competition for binding sites and available substrates, lowering luminal PH, production of bactericins and production of other antibacterial substances enhancement of intestinal motility and up gradation of genes medialing innate immunity. Prebiotic promote the bifidobacterial growth (Roberfroid, 2000). As innate immunity is an important defense system in *B.mori* (Ponnuvel and Yamakawa, 2002) the non-pathogenic bacteria enhanced the immunity factors and reduced the susceptibility to bacterial pathogenic infections in *B.mori*. Mortality% was recorded lowest in all the treatments supplements with ascorbic acid, so oral immunization with *L. acidophillus* is giving satisfactory result than micronutrients. Total pupal protein per gram tissue of body weight were found are at par in the larvae immunized with micronutrient supplements and healthy control batches of larvae, it indicates that immunization is not interfering the protein level required for formation of silk in later stages. Protein profile of ‘vaccinated, larvae (muga) challenged with live (pathogenic) bacteria was similar to that of the control suggesting unhindered metabolism (Choudhury et al, 2004). It has been known that insects inoculated with live non-pathogenic bacteria can acquire resistance to subsequent challenge by bacterial pathogen (Boman and Hultmark, 1987).

Hence, it is concluded that supplementation of non-pathogenic bacteria *L. acidophillus* etc., antibacterial proteins and induction with micronutrient supplements had enhanced the immunity in *B.mori* and hence the bacterial infection was decreased.

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On the Studies of Parasites Infestation in *Clarias batrachus* (Linnaeus) and *Clarias gariepinus* (Burchell)

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Abstract : *Clarias batrachus* (Linnaeus) and *Clarias gariepinus* (Burchell) were examined in this present investigation (June 2004 to May 2006).

Out of *1000 C. batrachus*, 16 species of parasites were recovered (ten were *cestodes*, three trematodes and three nematodes). The parasites recovered from *C. gariepinus* (500) two were two cestodes, one trematode, and one nematode. The prevalence was 80.60% in *C. batrachus* and *mean* intensity *was* 13.74 (SD ± 12.67) while, in *C. gariepinus prevalence was* 37.20% and intensity was 1.60 (SD ± 0.958). Most of the parasites preferred their habitat at anterior and posterior intestine, except *Paraquimperia tenerrima* (larvae) which was harbored the body cavity.

The parasite community was dominated by cestodes, comprising 65.79% of the total number of parasites recovered from *C. batrachus* and 58.39% of those in *C. gariepinus*. The most numerically dominant cestode was *Djombangia penetrans*, 20.5% in *C. batrachus* and 18.6% in *C. gariepinus*. *Allocreadium isoporum* was more prevalent in *C. batrachus* (9.5%) than in *C. gariepinus* (7.4%). The larvae of *Paraquimperia tenerrima* having prevalence 15.3% and mean intensity of 8.37 ± 3.07 in *C. batrachus*, while, *Procamallanus bengalensis with* 7% *prevalence and intensity* 1.14 ± 0.03.

Key words **:** *Clarias batrachus, Clarias gariepinus, Helminth parasites, organal distribution.*

Introduction

Bangladesh is by its water resources are blessed with various species of fishes, both marine and freshwater. A number of exotic fish are also recently introduced to our water bodies for higher production, which make further change in the nature of parasitic infestations of exotic origins [1].

Parasite is an important factor in fisheries and aquaculture as it causes diseases and in many cases responsible for mortality. In Bangladesh, the most of the investigations on helminth infections of fishes have been done in natural ecosystem. In Bangladesh, the helminth fauna including trematodes, acanthocephala, nematodes and cestodes, mainly carryophylloid cestodes are found in both marine and fresh water fishes. It is known that in Bangladesh the temperature never falls below 4°c that provides a suitable temperature for an excellent survival and distribution of carryophylloid cestodes [2].

Among the freshwater fishes, the catfishes are more important as fish food in Asia, Africa even in USA. More than 100 species of the genus *Clarias* have been described all over the world. In Bangladesh family Claridae has only one indigenous species the *Clarias batrachus* (L.) and the African magur *Clarias gariepnus* (Burchell, 1822) (Synonyms : *C. lazera* and *C. mossambicus*) has introduced in 1982 in this country. To promote catfish culture for an optimum production one of the main significant factors to be stressed is the disease dangers and the parasites. For this reason the research aim and objective were :

– To identify the community of parasites, and the prevalence and intensity in the two species of *Clarias* (*C. batrachus* and *C. gariepinus*).
– To find the organal distribution of the recovered parasites in different region of the alimentary canal of the fishes.

Materials and methods

A total of 1000 *C. batrachus* and 500 *C. gariepinus* were examined for the present investigation. After collection, the fishes were kept in plastic bag and transport to the laboratory. At first, the total number of fishes was identified, grouped,

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differentiate the sexes and their total lengths and total weights were measured. Identification of host fishes was made by using the keys of following Laggler [3], Shafi and Quddus [4].

Each organ of the viscera was separated and kept separately in different petridishes containing physiological saline solution and examined thoroughly for helminth parasites. The oesophagus, stomach, intestine and rectum were separated and split open carefully by a longitudinal incision through their entire length and were shaken well with physiological saline solution and the contents were scrapped with a blunt-end of scalpel to separate parasite. The collected parasites were examined by both macro and microscopically into different groups : Trematoda, Cestoda and Nematoda. The parasites belonging to the above groups were isolated and counted; in several cases some larval stages were also recorded. All the worms then preserved in 70% ethyl alcohol. For cleaning, the parasites were directly put into lacto phenol at normal temperature from 70% ethyl alcohol. The parasites were mounted both temporarily and permanently. For taxonomic classification of helminth parasites Yamaguti [5-8] and available reference papers were consulted.

Result

The present investigation was done on two species of genus *Clarias*, *Clarias batrachus* and *Clarias gariepinus*. Out of 1000 *C. batrachus*, 806 were found infected (80.60%) and out of 500 *C. gariepinus*, 186 were found infected (37.20%) with different helminth parasites. In the present study, sixteen species of parasites from different helminth groups found in *C. batrachus* while, only four species found in *C. gariepinus*. The intensity of parasites at per infected *C. batrachus* was 13.74 (SD ± 12.67) and in *C. gariepinus* was only 1.60 (SD ± 0.958). Prevalence of infestation in male *C. batrachus* and *C. gariepinus* were 79.39% and 36.42%, while in female were 82.67% and 37.61% respectively. Intensity of parasites in male *C. batrachus* and *C. gariepinus* were 13.52 ± 12.79 and 1.44 ± 0.832, on the other hand, intensity of parasites in female were 14.06 ± 12.48 and 1.68 ± 1.018 respectively (table 1).

Table 1 : Diversity of helminth parasites in *Clarias batrachus* and *Clarias gariepinus*

Factors	***Clarias batrachus***	***Clarias gariepinus***
Number of fish examined	1000	500
Number of fish infected	806	186
Prevalence of infestation	80.60%	37.20%
Total number of parasites collected from host fishes	11071	298
Total number of parasites species collected from host fishes	16	2
Mean intensity per infected fishes ± standard deviation	13.74 ± 12.67	1.60 ± 0.958
Prevalence of infestation in Male	79.39%	36.42%
Prevalence of infestation in Female	82.67%	37.61%
Intensity of parasites in male	13.52 ± 12.79	1.44 ± 0.832
Intensity of parasites in female	14.06 ± 12.48	1.68 ± 1.018

In *C. batrachus*, among the sixteen parasite species, ten were Cestods : *Djombangia penetrans, Pseudocaryophyllaeus indica, Capingentoides batrachii, Lytocestus parvulus, Lytocestus indicus, Marsipometra confusa, Stocksia pujehuni, Caryophyllaeus laticeps, Bothriocephalus scorpii and Bothriocephalus salvelini;* three Trematods (*Lissorchis fairporti, Holorchis legendrei and Allocreadium isoporum,* three Nematods : *Spirocamallanus olsenia, Procamallanus bengalensis* and Larvae of *Paraquimperia tenerrima*. Only four species of parasites were collected from *C. gariepinus*, among them, two were cestodes : *Djombangia penetrans, Lytocestus parvulus,* one Trematoda : *Allocreadium isoporum* and one Nematoda was *Procamallanus bengalensis*.

In the present investigation, the cestodes were found most dominant and numerically higher group of parasites comprising 65.8% of the total number of parasites recovered from *C. batrachus*, and 73.82% in *C. gariepinus*. *Djombangia penetrans* was the most numerically dominant in both the species of catfish (Table 2). In *C. batrachus*, the prevalence and mean intensity of this parasite were 20.5% and 8.88 ± 5.24. The occurrence of *Djombangia penetrans* accounting for 16.45% of the total parasites and 25% of the cestode group in *C. batrachus* (Table 2 and 3). *Djombangia penetrans* in *C. gariepinus*, showed prevalence 18.6% and 6.59 ± 0.59 as mean intensity, and accounting for 42.28% of the total number of parasite and 57.27% of the cestode group (Table 2 and 3). *Pseudocaryophyllaeus. indica* was recovered only from *C. batrachus with* prevalence 14.8% and 8.36 ± 3.85 as mean intensity. The occurrence of this cestode was recorded 11.18% of the total number of parasites and 17% of the cestode group, *Capingentoides batrachii* in Clarias *batrachus* showed prevalence 13.5% and mean intensity 7.56 ± 3.25 and 9.21% of the total parasite and 14% of the total number of cestodes (Table 2 and 3).

In Clarias *batrachus*, *Lytocestus parvulus* showed 13.9% prevalence and 6.29 ± 2.75 mean intensity when counted 7.89% of the total parasites and 12% of the cestode group. *Lytocestus parvulus* in *C. gariepinus*, showed 16.2% prevalence and intensity 1.16 ± 0.46 (Table 2). This parasite found 31.54% of the total parasites and 42.73% of the total number of cestodes (Table 3). In Clarias *batrachus*, *the* numerically recessive four cestodes were, *Lytocestus indicus*, *Marsipometra confusa*, *S. pujehuni* and *Caryophyllaeus laticeps* showed low prevalence (12%, 9.8%, 7.3% and 5.7% respectively) and mean intensity (6.02 ± 2.47, 5.95 ± 2.37, 4.99 ± 1.66 and 5.11 ± 1.5 respectively). The above four parasites found as 6.58%, 5.27%, 3.29% and 2.63% respectively of the total parasites (Table 2 and 3) and 10%, 8%, 5% and 4% respectively of the cestode group. In *C. batrachus*, *Bothriocephalus scorpii* and *Bothriocephalus, selvelini* also with low prevalence (4% and 3%) and mean intensity (5.48 ± 1.32 and 4.17 ± 0.89). These parasites found 1.98% (*B. scorpii*) and 1.32% (*B. selvelini*) of the total parasites while, 3% (*B. scorpii*) and 2% (*B. selvelini*) of the cestode group (Table 2 and 3).

In *C. batrachus*, three species of trematode parasite were observed while in *C. gariepinus*, it was only one. The digenian trematode *Allocreadium. isoporum* collected from *C. batrachus*, accounted for 4.7% of the total parasites and 49.01% of the trematode fauna. The prevalence was found 9.5% with 5.47 ± 1.89 as mean intensity (Table 2 and 3). *Allocreadium isoporum was* the only trematode found in *C. gariepinus*, with prevalence 7.4% and 1.03 ± 0.27 as mean intensity, this parasite accounting for 12.76% of the total parasites recovered from *C. gariepinus*.

In *C. batrachus*, *Capingentoides batrachii*, *Lytocestus parvulus*, *Lytocestus indicus and Lissochis fairporti* were found in stomach, anterior and posterior intestine. Among the nematode, in *C. batrachus*, *Procamallanus bengalensis* was found in oesophagus in a very few number and most of the *Procamallanus bengalensis* in *C. batrachus* and *C. gariepinus* were collected from stomach. In *C. Batrachus*, among the nematode, quimperridae larva was numerically dominant. In *C. gariepinus*, *Lytocestus parvulus* and *Allocreadium isoporum* were collected from stomach, anterior and posterior intestine. In *C. batrachus*, *Spirocamallanus olsenia* and *Pseudocaryophyllaeus indica* were collected from stomach and anterior intestine. *Allocreadium isoporum*, *Stocksis pujehuni*, *Holorchis legendrei*, *Marsipometra confusa*, *Caryophyllaeus laticeps*, *Bothriocephalus scorpii* and *Bothriocephalus salvelini* parasites were collected from anterior and posterior intestine (Table 4).

Discussion

During the two year of investigation, *C. batrachus* showed higher prevalence and intensity of infestation by helminth parasites than the *C. gariepinus*. The prevalence of infestation of *Allocreadium isoporum*, and *Lytocestus parvulus* were more in *C. gariepinus* than *C. batrachus*, while, the prevalence of *Djombangia penetrans* and *Procamallanus bengalensis* were higher in *C. batrachus* than *C. gariepinus*. In *C. gariepinus*, there was no infestation in oesophagus and the body cavity. In *C. batrachus*, stomach was (55.55%) found as a crowded habitat for the helminth parasites and similar findings were observed in *C. gariepinus* (59.73%).

In *C. batrachus* and *C. gariepinus*, the parasite fauna had been observed to occupy the stomach, anterior and posterior intestine, few in oesophagus and only encysted larval form of *Paraquimperia tenerrima* was found to be attached to the stomach, anterior intestine, liver and fat bodies. It was also noted that, a particular parasite was found to infect one or more organ in the both host fishes.

Each parasite species prefers to live in a definite zone of the microhabitats, though some can migrate to other organs, which are normally not their usual sites of infection. Dogiel *et al.* [9] suggested five factors that directly influence the parasite

Table 2 : Prevalence and intensity of parasites in *C. batrachus* and *C. Gariepinus*

Parasite groups	Parasite species	*Clarias batrachus*		*Clarias gariepinus*	
		No. of hosts infected	**Prevalence of infestation (%)**	**No. of hosts infected**	**Prevalence of infestation (%)**
Cestoda	*Djombangia penetrans*	205	20.5% (8.88 ± 5.24)	93	18.6% (6.59 ± 0.59)
	Psudocaryophyllaeus indica	148	14.8% (8.36 ± 3.85)	—	—
	Capingentoides batrachii	135	13.5% (7.56 ± 3.25)	—	—
	Lytocestus parvulus	139	13.9% (6.29 ± 2.75)	81	16.2% (1.16 ± 0.46)
	Lytocestus indicus	121	12% (6.02 ± 2.47)	—	—
	Marsipometra confusa	98	9.8% (5.95 ± 2.37)	—	—
	Stocksia pujehuni	73	7.3% (4.99 ± 1.66)	—	—
	Caryophyllaeus laticeps	57	5.7% (5.11 ± 1.5)	—	—
	Bothriocephalus scorpii	40	4% (5.48 ± 1.32)	—	—
	Bothriocephalus salvelini	35	3% (4.17 ± 0.89)	—	—
	Total	**1051**		**174**	
	Lissorchis fairporti	75	7.5% (4.81 ± 1.52)	—	—
Trematoda	*Holorchis legendrei*	47	4.7% (3.83 ± 94)	—	—
	Allocreadium isoporum	95	9.5% (5.47 ± 1.89)	37	7.4% (1.03 ± 0.27)
	Total	**217**		**37**	
Nematoda	*Spirocamallanus olsenia*	136	13.6% (6.5 ± 2.51)	—	—
	Procamallanus bengalensis	107	10.7% (5.61 ± 1.96)	35	7% (1.14 ± 0.03)
	Paraquimperia tenerrima	153 (Larvae)	15.3%	— (8.37 ± 3.6)	—
	Total	**398**		**35**	

Table 3 : Percentage of helminth parasites in *C. batrachus* and *C. gariepinus*

Parasites	*Clarias batrachus*			*Clarias gariepinus*		
	No. of parasites collected	**Occurrence group**	**% in total**	**No. of parasites collected**	**Occurrence group**	**% in total**
Djombangia penetrans	1821	25	16.45	126	57.27	42.28
Psudocaryophyllaeus indica	1238	17	11.18	—	—	—
Capingentoides batrachii	1020	14	9.21	—	—	—
Lytocestus parvulus	874	12	7.89	94	42.73	31.54
Lytocestus indicus	728	10	6.58	—	—	—
Marsipometra confuse	583	8	5.27	—	—	—
Stocksia pujehuni	364	5	3.29	—	—	—
Caryophyllaeus laticeps	291	4	2.63	—	—	—
Bothriocephalus scorpii	219	3	1.98	—	—	—
Bothriocephalus salvelini	146	2	1.32	—	—	—
Total	**7284**	**100**	**65.8**	**220**	**100**	**73.82**
Lissorchis fairporti	361	34.02	3.26	—	—	—
Holorchis legendrei	180	16.97	1.63	—	—	—
Allocreadium isoporum	520	49.01	4.70	38	100	12.76
Total	**1061**	**100**	**9.59**	**38**	**100**	**12.76**
Spirocamallanus olsenia	845	31	7.63	—	—	—
Procamallanus bengalensis	600	22.01	5.41	40	100	13.42
Paraquimperia tenerrima	1281	46.99	11.57	—	—	—
(Larvae)						
Total	**2726**	**100**	**24.61**	**40**	**100**	**100**

fauna of fish. These include age, diet, abundance of fish, independent number of parasite fauna within the fish and the season. Occasionally fish parasites are distributed randomly throughout the population [10]. But more often they are over dispersed and aggregated in a few fish.

The cause of abundance of parasites in the stomach and intestine is thought to be due to easy availability of nutrient substance in the intestine. Markov [11] reported that the fish parasites alike to other vertebrates, subsists either on the physiological condition of the anterior region of the intestine of the catfish is more suitable to this digenean trematode than to the caryophyllid cestodes.

Out of sixteen parasites collected in the present observation, the seven species have been observed in *C. batrachus* for the first time in Bangladesh. *Caryocephallaeus laticeps* collected by Pallas, [12] from *Cyprinus* sp. in Europe. *Allocreadium isoporum* described by Looss, [13] from *Cyprinus carpio* in Europe. *Bothriocephalus scorpii identified* from fresh water teleosts. *Bothriocephalus salveline* recorded from *Salmo alpinus* by Yeh, [14]. *Lissorchis fairporti* collected from *Ictiobus cyprinella* and *I. bulbulus* in lowa by Magath, [15]. *Holorchis legenderi* from *Mullus surmuletus* a marine fish by Dollfus, [16]. *Mersipometra confusa* from *Polydon spathula* (a teleost).

Table 4 : Organal distribution of helminth parasites in *C. batrachus* and *C. gariepinus*

Host	Name of the parasites	Oestophagus	Stomach	Anterior intestine	Posterior intestine	Body cavity	Total
Clarias batrachus	*Djombangia penetrans*	0	1366	455	0	0	**1821**
	Psudocaryophyllaeus indica	0	1201	37	0	0	**1238**
	Capingentoides batrachii	0	816	190	14	0	**1020**
	Lytocestus parvulus	0	790	69	15	0	**874**
	Lytocestus indicus	0	615	93	20	0	**728**
	Marsipometra confusa	0	0	560	23	0	**583**
	Stocksia pujehuni	0	0	343	21	0	**291**
	Caryophyllaeus laticeps	0	0	275	16	0	**291**
	Bothriocephallus scorpii	0	0	201	5	0	**146**
	Bothriocephallus salvelini	0	0	141	5	0	**146**
	Lissorchis fairporti	0	18	331	12	0	**361**
	Allocreadium isoporum	0	0	511	9	0	**520**
	Holorchis legendrei	0	0	173	7	0	**180**
	Procamallanus bengalensis	9	591	0	0	0	**600**
	Spirocamallanus olsenia	0	754	91	0	0	**845**
	Paraquimperia tenerrima (Larvae)	0	0	0	0	1281	**1281**
	Total	**9**	**6151**	**3470**	**160**	**1281**	**11071**
Clarias ***gariepinus***	*Djombangia penetrans*	0	63	30	0	0	**93**
	Lytocestus parvulus	0	63	30	0	0	**93**
	Allocreadium isoporum	0	0	47	21	0	**68**
	Procamallanus bengalensis	0	56	0	0	0	**56**
	Total	**0**	**178**	**91**	**29**	**0**	**298**

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Host specificity of parasitic isopods in marine fishes

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Abstract : Isopods associate with many species of commercially important fishes around the world and cause significant economic losses to fisheries by killing, stunting, or damaging these fishes. Twenty-six species of marine fishes were infested by 12 species of parasitic isopods along the Tamil Nadu coastal environment, Southeast coast of India. Nature of infestation of the isopods on different species of fishes was described. Parasites were found in the, branchial and buccal regions and on body surface. Most of the isopods (e.g., *Joryma brachysoma* and *Nerocila phaeopleura*) were host-specific, while *Ryukyua circularis* and *Glossobius* sp. showed less specificity. The prevalence rate of parasites during different months and the range of host specificity were studied. A significant reduction in the gill surface was observed due to infestation. The infestations such as, hemorrhagic lesions, anemia, encapsulation, inflammation and penetration of dactylus usually pressure atrophy often accompanied by the presence of larger parasites. This may lead to huge economic losses in commercial species of fish.

Key words **:** *Marine fishes; Isopod parasites; Infestation; Distribution; Host specificity*

Introduction

The parasitic isopods include members of Cymothoidae, Gnathiidae and Bopyridae families. Cymothoidae and Gnathiidae prefer fishes as host, while Bopyridae are generally found on decapod crustaceans. Cymothoidae inhabit freshwater, brackish water and marine environment, and are ectoparasite of various fish species. They may be observed on the body or within the buccal cavity or gill cavity of the host (Trilles 1969; Brusca 1981). Parasites have received considerable scientific attention because they cause severe damage to fishery resources. Isopod occurs very commonly as parasites in food fishes are usually large and fierce looking and cause considerable damage to their host (Overstreet 1978). A few related works are available on the nature of infestation of isopods parasites in fishes (Williams and Williams 1994; Ravichandran et al. 1999, 2000, 2001, 2009, 2010; Ravichandran 2007; Grutter 2003; Cuyas et al. 2004; Rameshkumar and Ravichandran 2010; Rameshkumar et al. 2011).

Rokichi (1985) studied the specificity the zoogeography and the vertical distribution of isopod parasites in host systems in the northwestern African shelf. Host specificity is the tendency of a parasite to occur on one or a few host species and is a product of co-existence between both parasite and host lineages (Timms and Read 1999; Poulin 2007). Up to date, there are no detailed studies on the host specificity and infestation of isopod parasites on fishes in Indian waters. The present study was carried out to understand the host specificity of isopod parasites and the degree of infestation in fishes.

Materials and Methods

An extensive survey was undertaken over a period of one year from December 2008 to November 2009, along the Tamil Nadu coast (Southeast coast of India). Fishes were collected from major landing centres from January to November 2009, except in December 2008 and May and examined thoroughly for the presence of isopod parasites. The number of parasite, the site of attachment and their orientation were recorded for each host. The parasites were than carefully removed and transferred to 10% aqueous sodium bicarbonate solution to dissolve the mucus attached to the parasites then preserved in 70% alcohol or 2% formalin for further studies. Total length, width, weight and sex of the hosts were recorded.

Results and Discussions

Distribution

A total of 223 parasites representing 12 species and nine genera were collected from 26 species of fishes. The majority were ectoparasites. Their distribution of existing parasites host systems along the Tamil Nadu coast was mostly on pelagic fishes.

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Seasonality of infestation

The maximum degree of infestation was recorded maximum in February 2009 (40.6%) and the minimum in June 2009 (10.3%) (Fig.1). The intensity of infestation ranged from 1.0 in October and November and 1.8 in January (Fig. 2).

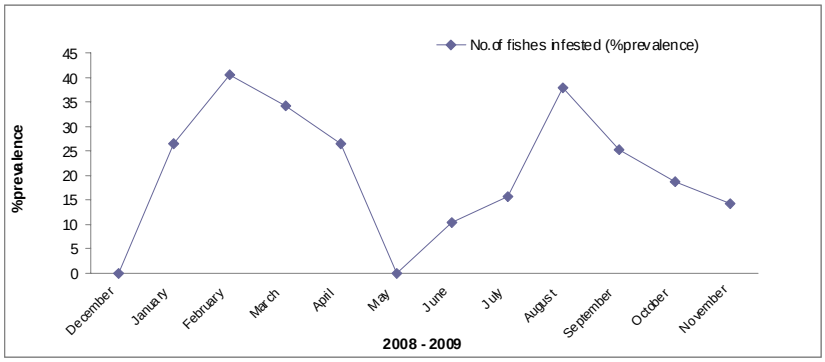

Fig. 1. Prevalence in infestation of parasites on fishes, expressed as number of infected in each sample (%), observed during this study. No samples were examined in December 08 and May09.

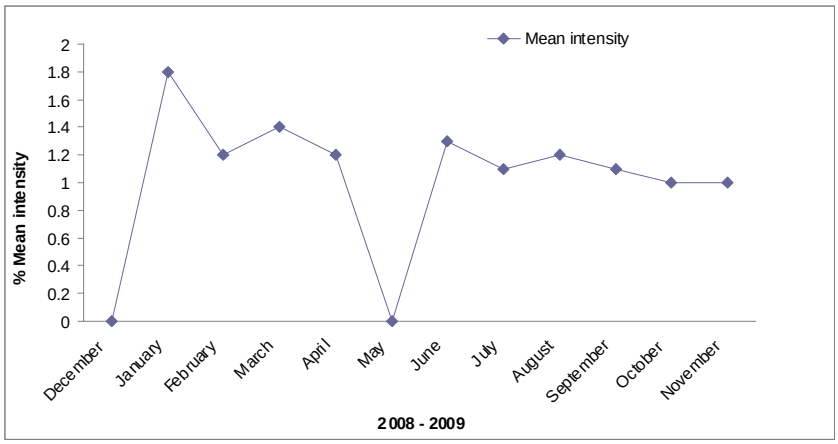

Fig. 2. Mean intensity in infestation of parasites on fishes expressed as number of parasites on infested fishes (%). No samples available in Dec 2008 and May 2009.

Localization of parasites

Each isopod species was recorded on a single host taxon but the fish species, *Cymothoa indica* and *Alitropus typus* were each hosts on two species of parasites, which were found in the mouth of the hosts, the remaining parasites were attached to the body surfaces of the host. Isopod parasites represented by 12 species were recorded viz, *Joryma brachysoma, J.tartoor, Joryma hilsae*, *Nerocila phaeopleura*, *Nerocila poruvae*, *Nerocila longispina*, *Alitropus typus*, *Anilocera* sp, *Ryukyua circularis*, *Cymothoa indica*, *Lironeca puhi* and *Glossobius* sp. (Table.1).

Table 1. Presence of the isopod parasites in different part of the fish body

Branchial cavity	Buccal cavity	Body surface
Joryma hilsae	*Cymothoa indica*	*Nerocila phaeopleura*
Joryma brachysoma	*Glossobius* sp	*Nerocila poruvae*
Joryma tartoor	*Alitropus typus*	*Nerocila longispina*
Lironeca puhi	*Joryma brachysoma*	*Joryma brachysoma*
Alitropus typus	*Nerocila longispina*	*Anilocera* sp
Nerocila poruvae		
R.circularis		

Branchial parasites

The maximum infestation was recorded in the branchial chamber of host. They were found either in the gill chambers of host. The orientation of the branchial parasites varied in most of the hosts. *Joryma hilsae, J.tartoor, Lironeca puhi, Alitropus typus, R.circularis* and *Nerocila poruvae* infest the host with its ventral region facing the inner surface of the opercula and dorsal region deeply plunged in the gill arches towards the lower half of the operculum (Fig.3).

Fig. 3. *Joryma brachysoma* on *Secutor* sp

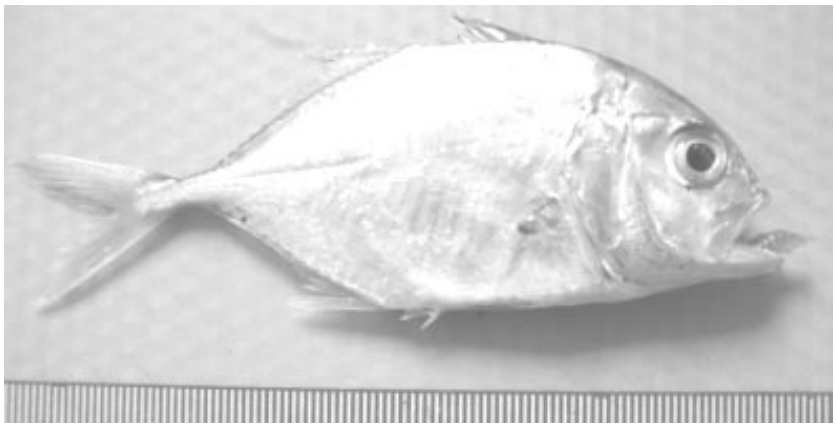

Fig. 4. *A.typus* on *Carangoides* sp

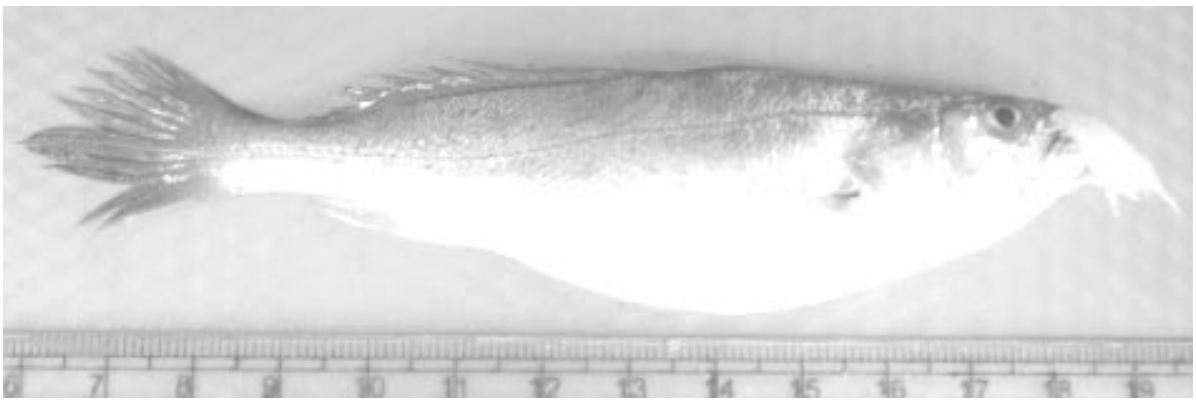

Fig. 5. *Nerocila longispina* on *Otolithes ruber*

Buccal parasites

Species of *Cymothoa* and *Glossobius* were found in the buccal cavity. *Glossobius* sp, *Alitropus typus*, *Joryma brachysoma* and *Nerocila longispina* were found attached to the bucco-pharyngeal region of their hosts (*Trichurus lepturus* ,*Carangids* sp and *Otolithes ruber* respectively) (Figs.4&5). These parasites are normally recorded as the mouth aperture. The parasites were attached in such a way that the broader posterior part of the parasite was lodged in the wider portion of the floor of the buccal cavity and the narrow anterior part was either located towards the mouth opening the mouth. The male counter part however showed an orientation with the cephalon directed either towards the anterior or to the posterior part of the mouth of the host.

Body surface parasites

Nerocila phaeopleura infesting the host of fish *Sardinella* sp, *Carangoides* sp, *Trichurus lepturas*, *Hilsha ilisha*, *Chirocentrus dorab*, *Sphyrnea jello*, and *Rastrelliger kanakurta* were found attached to the dorsalolateral region and near the pectoral fin (Fig.6). The digestive secretions/excretions of isopods apparently cause significant damage to the host's tissues.

Fig. 6. *N. Phaeopleura* on *Sardinella* sp

Narrow and Broad Host Specificity

Joryma tartoor, Joryma hilsae, Nerocila poruvae, Nerocila longispina, Alitropus typus, Anilocera sp, C. indica, R. circularis, L. puhi and *Glossobius* sp, have narrow host specificity is limited to the environment of one host species. *Nerocila phaeoplepura* and *Joryma brachysoma* have a broad host specificity is maximum to the environment of different host. *Nerocila phaeopleura*, in being host specific, would thus seem to fit in with an apparently generic characteristic and it seems reasonable to assume that the major host species. The constant position and the deep wound caused by *N. phaeopleura* suggest that the position on the host is constant and that the animal does not move about. The location of the parasite in this position can be explained by the interaction of two factors which can be called the horizontal and vertical components.

Parasitic crustaceans are the largest fish parasites, which cause considerable damage to their hosts. Isopods inhabiting the buccal cavity and branchial chamber inflict damage to gills through attachment and feeding and that the extent of damage is directly proportional to the size of the parasite and duration of settlement. Infestation causes serious problems to host animals either directly or indirectly affecting the physiological status of host.

The present study showed that *Joryma brachysoma, J.tartoor, Joryma hilsae*, *Nerocila poruvae, Nerocila longispina*, *Alitropus typus, Anilocera sp, Cymothoa indica, Lironeca puhi* and *Glossobius* sp have narrow host specificity. Brusca (1981) suggested that specific physical properties of the estuaries could favour broader spreading of the isopods, so that fish infestation was generally higher. Rokichi (1985) stated that the distribution of *Cymothoa plebia* is limited to the environment of one host species *Brachydeuterus auritus* and considered the parasite as phylogenetically order. The incidence and intensity

of crustacean parasites along the South-West coast of India noticed the higher incidence and intensity of infestation in females were than that in males (Radhakrishnan and Nair 1983). Romestand and Trills (1979) stated that the size of fishes that can be infested will be within certain maximum-minimum variables depending upon the species of host. Similar observation was made in *Epipenaeon elegans chopra* from the Persian Gulf (Dowson 1958) where infestation was also found to be parasites were more in females than in males.

The feeding activity of the Cymothoidae isopods in the gill chamber can cause damage to the host in several ways, such as tissue and blood loss, reduction of the gill filaments, and reduction of the breathing efficiency. The presence of these ectoparasites also affects the metabolism, and can cause a decrease in the growth rate (Thatcher and Neto 1994). Thatcher (1993) revealed that the Cymothoidae *Anphira branchialis* can also cause the growth of tumors in the piranha *Serrasalmus* sp.

Trilles (1964) significantly showed that a number of cymothoids, including *N. orbignyi* and *N. bivittata* are specific in their choice of hosts, whereas other genera are less specific. The results of this investigation indicate that *N. phaeopleura*, although comparatively primitive in being an external parasite and being highly host specific is also highly specialised to a mode of life upon a pelagic, fast swimming host. It lives on a highly specific region of the body. This position is determined by the needs of the parasite and the limitations exerted by the morphology and habits of the host.

The parasite occupies the entire branchial chamber of the host may produce pressure on the gill surface and thus affecting the efficiency of respiration. Although, the infestation did not cause immediate death, it had affected the normal growth of the host fish. They may lead to economic losses among some fish species.

Acknowledgement

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Antibacterial effect of some locally available plant extracts against some fish pathogenic bacteria

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Abstract : Aquaculture is a growing industry in India. Bacterial disease is a growing threat to aquaculture. The use of commercial antibiotics in aquaculture increases antibiotic resistance among pathogenic bacteria in exposed ecosystem and also creating environmental and public health hazards. For this reason many countries have banned the use of antibiotics in aquaculture. As a consequence nowadays scientists are in search of alternate antibiotics.

Therefore, this study was conducted to evaluate the antibacterial activity of locally available plant extracts against pathogenic bacteria of aquatic animals namely, *Aeromonas hydrophila, Aeromonas sobria, Vibrio paraharmolyticus.* Five plants viz: *Terminalia arjuna* (Arjuna), *C. asiatica* (Thankuni), *Ziziphus mauritiana* (Kul), *Murraya koenigii* (Kari), *Ocimum sanctum* (Tulsi) were selected for in vitro antibacterial activity assay. The Minimum Inhibitory Concentration of the methanolic extracts of the leaves of the said plants was determined by Disc Diffusion method. The plant extracts except tulsi showed antibacterial activity against all the three bacteria. Significant inhibitory activity was found in *C. asiatica, T. arjuna, M. koengii, Z. maurtitiana* respectively. Methanol was used as positive control and Tetracycline hydrochloride was used as negative control. Higher concentration of methanolic extract showed better result consistently for all the plants than lower concentration. The most effective inhibitory activity was observed in *C. asiatica* against *A. sobria*.

Results of the present study indicate the potential of these plants for further work on isolation and characterization of the active principle responsible for antibacterial activity and its exploitation as therapeutic agent.

Keywords **:** *Antibiotic, Aeromonas hydrophila, Aeromonas sobria, plant biomolecules, Vibrio paraharmolyticus.*

Introduction

Occurrence of bacterial diseases is very much threatening to the intensive fish farming system in India. Thus proper medication is needed to maintain animal health and manage fish population. Applications of commercial antibiotics are in practice but indiscriminate use of current antibiotics raises super resistant bacterial strains (Ahmed et al., 1998). Many countries also banned the use of antibiotics since their prolonged exposure to environment is associated with harmful side effects and is a concern of public health (Cuhna, 2000). Since plant based drugs cause much lower incidence of adverse reactions compared to synthetic pharmaceutical (Shariff et al., 2006), scientists felt the urgency to develop an alternative approach of herbal medication towards management of diseases.

In the present study antimicrobial activity of four different plant species are tested against four gram negative bacteria, *Aeromonas hydrophilla*, *Aeromonas sobria*, *Vibrio parahaemolyticus, Pseudomonas aeruginosa* isolated from the fishes collected from local farms. All the four plants viz: *Ziziphus mauritiana* (in Bengali Kul), *Centella asiatica* (in Bengali Thankuni), *Terminalia arjuna* (in Bengali Arjun), *Murraya keonigii* (in Bengali Curry) are widely exploited by the local community for its medicinal value.

Ziziphus mauritiana leaves are helpful in liver trouble, asthma and fever (Morton, 1987; Michel, 2002). The extract of *Z. spinachristi* was found to contain beautic acid and ceanothic acid, cyclopeptides, as well as saponins, glycoside and flavonoids, lipids and proteins (Abalaka et al., 2010).

Centella asiatica is an herb growing in wet places through out India is used in ayurvedic preparations either as whole plant or as leaves in the fresh or extract form (Sharma, 1992). This herb comprises of three active components, namely, asiaticoside, madecassoside and Asiatic acid (Zainol et al., 2008).

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Bioactive fractions of the active extracts of *T. arjuna* yielded three known oleane compounds: arjunic acid (Kumar et al., 1987), arjugenin (Row et al., 1970) and arjunetin (Honda et al., 1976).

Mahanimbine and koenigine, two carbazole alkaloids were isolated from the leaves of *M. koenigii* showed antioxidant activity and antimicrobial activity. Koenigine also showed a high degree of radical-scavenging (Banerjee et al., 2010).

Materials and Methods

Plant materials

The four plants viz: *Ziziphus mauritiana* (in Bengali Kul), *Centella asiatica* (in Bengali Thankuni), *Terminalia arjuna* (in Bengali Arjun), *Murraya keonigii* (in Bengali Curry) were collected from the garden of the University. The plants were identified based on their physical characteristics by the Department of Botany, University of Kalyani. Fresh plant leaves were thoroughly washed several times in running tap water and twice with sterile distill water, dried in blotting paper under shade for about a month.

Preparation of extract

The dried leaf samples were crushed to powder in mixer grinder. 5 gms of leaf dust of each of the four plants were soaked in 20 ml of absolute methanol at room temperature for two days. The extracts were then filtered using standard whatman filter paper. Then the filtrates were transferred to petridishes and allowed to evaporate until dried to paste and stored at 4°C. The dried extracts were re-suspended in the same solvent in two dilutions. Final concentrations were made to 1mg in 10 µL and 1mg in 100 µL.

Microorganism used

The pure strains of four species of gram negative bacteria, *Aeromonas hydrophila*, *Aeromonas sobria*, *Vibrio parahaemolyticus*, and *Pseudomonas aeruginosa* obtained from the Microbiology laboratory of the National Institute of Cholera and Enteric diseases (NICED), Kolkata. Subculture of each bacterium was maintained in nutrient agar and respective selective medium. *Aeromonas hydrophila*, *Aeromonas sobria* and *Pseudomonas aeruginosa* were then inoculated to freshwater carp fishes as they are common fish pathogens. *Vibrio parahaemolyticus* was inoculated to fresh water shrimp as it is a common pathogen of shrimp. After the fishes and shrimps showed symptoms, tissues were removed aseptically from the live host for bacteriologicalculture to confirm the respective bacteria as the cause of death. The isolated bacteria were then again cultured on nutrient agar and respective selective medium (Hi- Media) and confirmatory biochemical tests were performed. These bacteria served as test pathogens for antibacterial activity assay. A direct suspension of the test organisms was prepared in broth and the optical density (OD) of the solution was adjusted to 0.5 at 456 nm which corresponded to 1x 10^7 CFU.

Screening for antibacterial activity :

The bioassay was done using standard disc diffusion method adopted from Taylor et al., 1995. Bacteria inoculums were spread on the agar plates using sterile cotton swabs. The 5 mm diameter sterile Whatman No.1 filter paper discs were soaked

Table I : Antimicrobial activities of the methanolic extracts of the four plant material against bacterial strains based on Disc diffusion method.

Bacterial strains	Zone of Inhibition (mm)									
	Ziziphus mauritiana		Centella asiatica		Murayya koenigii		Terminalia arjuna		Methanol	Tetracyclin
	1mg/10µl	1mg/100µl	1mg/10µl	1mg/100µl	1mg/10µl	1mg/100µl	1mg/10µl	1mg/100µl		
Aeromonas hydrophilla	15±0.75	0	30±0.5	20±0.75	18±0.5	12±0.5	20±0.75	15±0.5	0	30±0.5
Aeromonas sobria	22±0.5	10±0.6	32±0.75	18±0.5	25±0.5	10±0.2	30±0.75	20±0.75	0	40±0.5
Vibrio parahaemolyticus	17±0.65	14±0.5	16±0.7	15±0.5	20±0.7	17±0.2	15±0.5	11±0.75	0	25±0.5
Pseudomonas aeruginosa	0	0	12.75±0.6	10.63±0.5	18.25±0.6	15.13±0.75	0	0	0	21.75±0.6

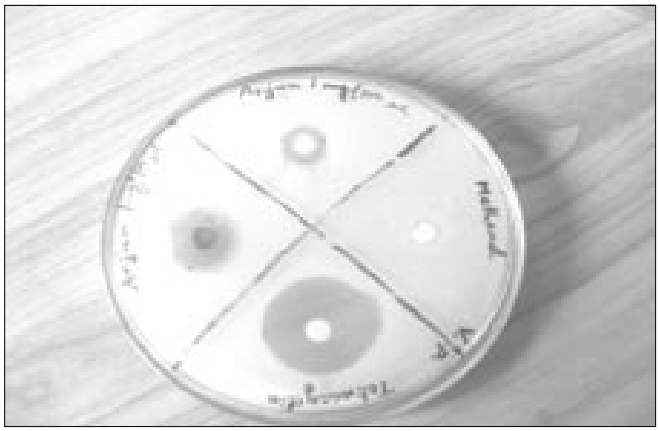

Fig. 1 : Antibacterial effect of *Terminalia arjuna* (Arjun) plant extract on *Vibrio parahaemolyticus*.

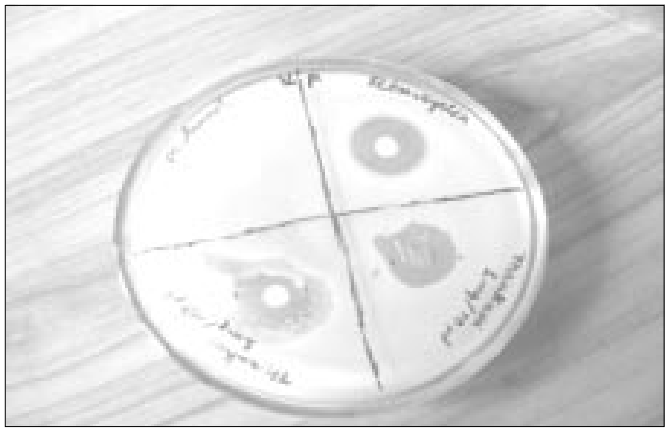

Fig. 2 : Antibacterial effect of *Centella asiatica* (Thankuni) plant extract on *Vibrio parahaemolyticus*.

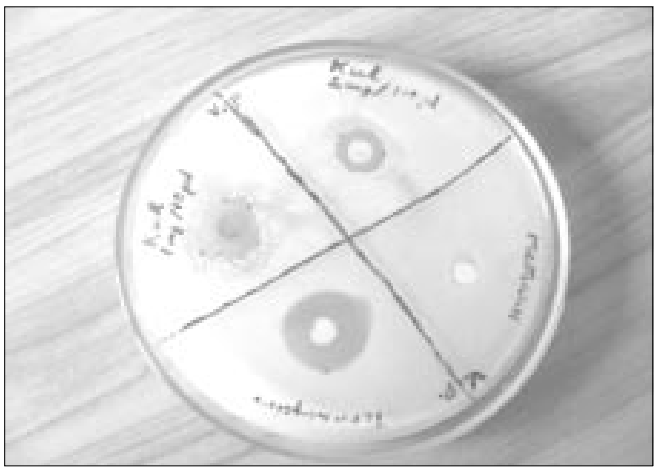

Fig. 3 : Antibacterial effect of *Ziziphus mauratiana* (Kul) plant extract on *Vibrio parahaemolyticus*.

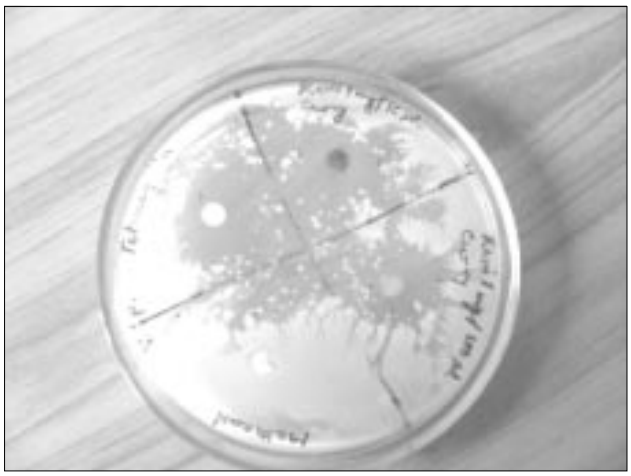

Fig. 4 : Antibacterial effect of *Murayya keonigii* (Curry) plant extract on *Vibrio parahaemolyticus*.

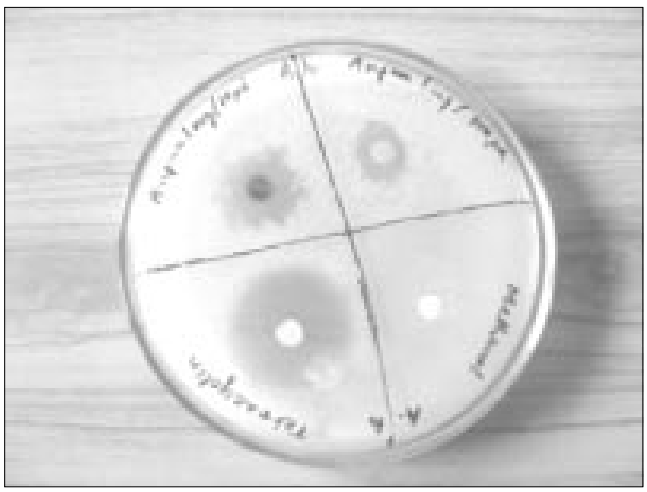

Fig. 5 : Antibacterial effect of *Terminalia arjuna* (Arjun) plant extract on *Aeromonas hydrophilla*.

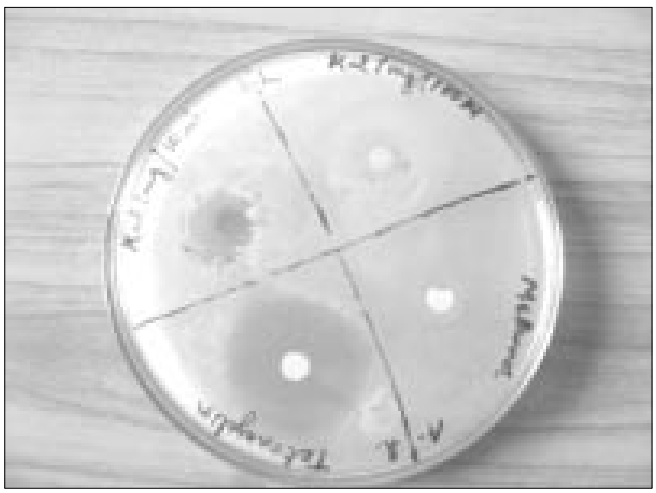

Fig. 6 : Antibacterial effect of *Ziziphus mauritiana* (Kul) plant extract on *Aeromonas hydrophilla*.

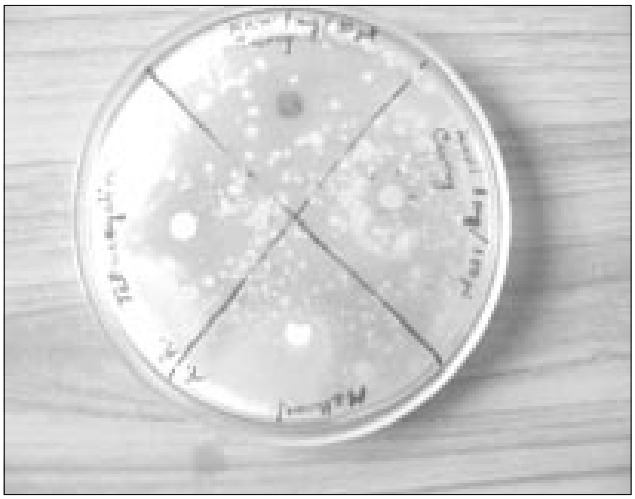

Fig. 7 : Antibacterial effect of *Murayya keonigii* (Curry) plant extract on *Aeromonas hydrophilla*.

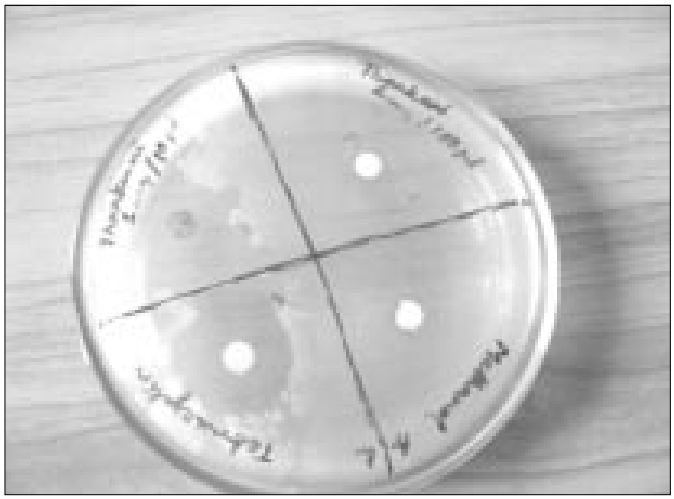

Fig. 8 : Antibacterial effect of *Centella asiatica* (Thankuni) plant extract on *Aeromonas hydrophilla*.

and saturated in extracts. Prepared discs were placed on the inoculated agar plate. The cultures were then incubated upside down for 24 hrs at 37^{o}C. The paper discs were impregnated in the same amount of methanol which was used as negative control while, tetracycline hydrochloride paper discs were used as positive control. Tetracycline hydrochloride solution was prepared by mixing 0.8 ml of tetracycline in 9.2 ml distilled water. Final concentration of tetracycline was 0.25 mg/ ml. Results were recorded as presence or absence of zone of inhibition (Lennette, 1995). The inhibitory zone around test paper disc indicated absence of bacterial growth and it was reported as positive. Absence of zone was indicated as negative. The tests were repeated thrice to ensure the results.

Results and Discussions :

The in vitro potency of the anti microbial activity of the methanolic plant extracts against these four bacteria were assessed by measuring the diameter of the clear zone around the discs placed on the petriplates. The methanolic extracts of different plants showed various inhibitory results against different bacteria. The mother solution i.e. 1mg/10µl showed better result than the solution having concentration 1mg/100µl in every cases. Previously it was found that the ethanol extract of *Centella asiatica* was active against *E.coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923, *Vibrio parahaemolyticus* ATCC 17802 *Pseudomonas aeruginosa* CC 27853 (Mamtha et al, 2004). Our observation for *Centella asiatica* also showed similar results by inhibiting *Pseudomonas aeruginosa* and *Vibrio parahaemolyticus*. Along with that it was also found effective against best antimicrobial potency was showed against *A. hydrophila* and *A. sobria.*

The present study showed that methanolic extracts of *Murraya keonigii* has significant antibacterial results against all the test bacteria though less effective than *Centella asiatica.* Previous records on antibacterial activity of this plant having tremendous medicinal value are present. The petroleum ether extract of *M. keonigii* leaf inhibited the growth of *S. aureus, B. subtilis, E. coli and P. vulgaris* (Rahman and Gray, 2005). Aqueous extract of *M. keonigii* leaf can be effective on the growth of *A. hydrophila, C. freundii, S. putrifacien, V. alginolyticus, V. harveyi and V. vulnificus* respectively (Lee Seong Wei et al, 2008).

It is known that *T. arjuna* has antibacterial activity. Earlier, it was found that biomolecules isolated from bark extracts of *T. arjuna* possessed antibacterial activity, which can specifically act against *S. epidermidis* (Singh et al, 2008). Inhibitory role of aqueous leaf extracts on *P. aeruginosa*, was assessed by Ramya et al. (2008) along with many other bacteria. In our study, methanolic leaf extract of *T. arjuna* showed significant inhibitory results against all the three bacteria excepting *P. aeruginosa* which could not be inhibited by Arjun leaf extract.

Previous results on inhibitory role of methanol *Ziziphus* leaf extract against *B. subtilis*, *E coli*, *P. fluorescens*, *S. aureus*, *X. axonopodis*, *V. malvacearum*, *A. flavus*, *D. turcica* and *F. verticillioide* (Mahesh and Satish, 2008) are available. In our study

methanolic leaf extract of Kul showed inhibitory activity against the other three bacteria, *A. hydrophila*, *A. sobria*, *V. parahaemolyticus* but with much less potential than the other extracts. It was not effective against *P. aeruginosa* though it could inhibit *P. fluorescens* (Mahesh and Satish, 2008). Thus it can be concluded that its inhibition potency is organism or species specific.

It is to be mentioned that zone of inhibition was not observed around any control group i.e. in methanol treated discs whereas, the inhibition zone diameter around the standard antibiotic (tetracyclin) impregnated discs was highly significant between 45- 30 mm.

The present study shows the potential antibacterial effect of these locally available plant extracts against the common bacterial pathogen of aquaculture. Therefore, these plant materials should be further screened for commercial use as antibiotics against the bacterial diseases causing great economic loss in the fish industry.

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Studies on parasitic diseases of fish in east Kolkata wetland and their anti-parasitic treatment which threat to environment

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Abstract : East Kolkata Wetland, only Ramsar wetland of West Bengal a vast low land located at eastern fringes of Kolkata. This unique system where treatment of the sewage is done, then allowed for pisciculture. This wetland is also important for its fishery activity and is now producing about 13000 tonnes of fish per year. Here fish production is affected by aquatic pollution through different industrial effluent discharged in it and disease is a significant problem to the majority of producers affecting all development stages. As the majority of diseases occur during winter from low temperature, although in some cases disease problems occur throughout the year where parasitic, protozoan, bacterial and fungal pathogens are indicated as the most likely causes. The copepod parasite, *Argulus sp., Lernaea sp.* affected most businesses with a variety of possible bacterial and fungal species being second and affecting a greater range of production systems. In summer high temperatures caused fish seed mortalities in production and distribution. Kerosene oil, endosulfan and the organophosphates, methyl parathion and Malathion were substances utilized in anti-parasitic treatments of fish and in pond preparation which can have effects of long persistence in the environment and or humans through mechanisms of bio-accumulation and direct toxic effects on non-target organisms. So, herbal medication should be taken.

Key words **:** *East Kolkata Wetland, Environment, Fish, Herbal medication, Parasitic Disease*

Introduction

East Kolkata Wetland is famous all over the World for its ecological importance. It was declared as Ramsar wetland on November 2002 in Ramsar convention bureau. East Kolkata wetland EKW) stretches its area from North 24 Parganas to South 24 Parganas districts of West Bengal and located at eastern side of Kolkata metropolis. It is known as kidney of Kolkata city. In this shallow water body, treatment of sewage has done and then allowed for pisciculture. Everyday about 250 million liter of waste water along with solid waste is discharged here though different canals. Huge amounts of hazardous substances, heavy metal, sulfide, grease, oil originated from different industries of the surroundings, domestic waste water and industrial effluent from Kolkata city also discharged here though Tiljala, Bagjola,vangor canal and pollute the aquatic environment. Thus fish production is hampered. Other than fish production is suffered by the fish disease. Mainly diseases are broken out during winter season. Although Fishes are suffered by parasitic, bacterial, fungal diseases, the copepod parasite Argulus sp. Lernaea sp. are the main causative agents of fish disease of the most of the water bodies. Low temperature of winter and excessive temperature during summer are also responsible for death of fish seeds. A number of chemicals like Kerosene oil, endosulfan and the organophosphates, methyl parathion and Malathion are used in anti-parasitic treatments of fish and in pond preparation. These substances have effects of long persistence in the environment and or humans through mechanisms of bio-accumulation and direct toxic effects on non-target organisms. In our laboratory we are trying to utilize herbal medication for the diseased fish which not only help to prevent parasitic diseases of fish but also help to reduce the aquatic pollution of the wetland to reduce the uses of the chemicals that are used as anti parasitic agents.

Methodology

Preliminary survey has done in East Kolkata wetland area to collect information on fish production, their disease problem and disease management taken by the fish farmers. Some indigenous ornamental fishes that are common in this wetland are collected from the ornamental fish farms in this area and also collected from the wetland. This fishes has bought to the laboratory. Then fishes are thoroughly checked, scraping of different parts of fishes has dried and stained in giemsa or silver

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nitrate or double stained in eosin and haematoxylin. Then slides have prepared and observed under microscope. On the other hand infected fishes are treated with herbal crude extract by deep bathing.

Over view on East Kolkata Wetland :

Table 1. Represent land use pattern of EKW

Different land use classes in EKW

Land use	Area
Water body	5,852.14 ha of which fish farming roughly constitutes about 3,898.70 ha. (45.93%)
Agriculture Land	Approximately 4,959.86ha.(38.92%)
Garbage Disposal Site	602.78 ha. (4.73%)
Urban Settlement Area	91.53 ha.(0.73%)
Rural Area	1234.99 ha.(9.69%)
Total Area	12500.00 ha*

*Additionally 241.30 ha. Are eing added to the system for making the system integral.

Table 2. Represent information on EKW

State	West Bengal
District	Kolkata
Co-ordinates	22^0 25' to $22^0$40' N and $88^0$20' to $88^0$35'E
Area	12741 hectares
Ownership	Government of West Bengal and Private
Declared as Ramsar wetland	19th August 2002
Ramsar criteria	1
Area	12,741 Hectares (Source: State Govt. report.)
Ramsar wetland type	O (Permanent fresh water bodies)
No. of Bheris	264

Table. 3

Pollutants	**Comments**
Sewage and waste water	600 million lit/day
Garbage	>2500 tones
Heavy metals	Cr, Cu, Pb, Etc
Sulphate	_
Oil, grease	_

Table. 4

Name of the heavy metal discharged through effluents	**Name of the industries**
Chromium (Cr)	Tannery and leather industries
Copper (Cu)	Metal handicraft and electroplating industries
Lead (Pb)	Tannery and battery manufacturing industries
Rubidium (Rb)	Painting and Dye industries

Result and Discussion

a. Industries located surround East Kolkata Wetland and their Pollutants

b. Parasitic diseases shown in the fishes of East Kolkata Wetland :

Different types of fish diseases are observed in the fishes collected from East Kolkata wetland area and they are represented below in fig1, fig 2 and fig:3

c. Symptoms of the parasitic diseases of fish and affected body parts :

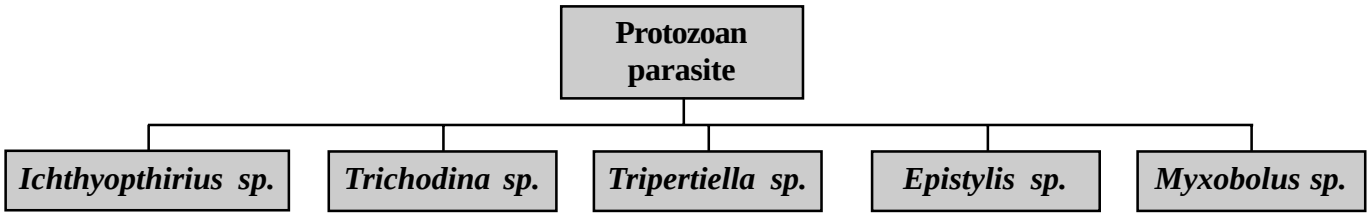

Fig: 1 : represent the causative agent of protozoan parasite of fish diseases.

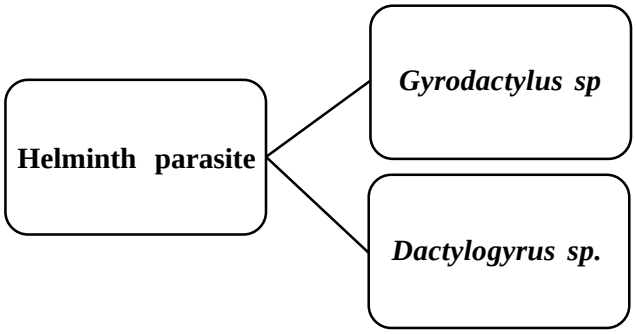

Fig. 2 : Represent the helminth parasite of fishes,

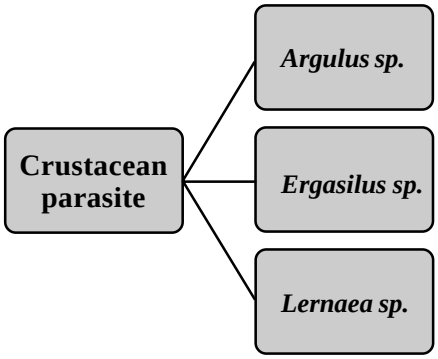

Fig. 3 : crustacean parasite represented here

Table no. 5

Name of parasite	Affected parts	Symptoms
Ichthyopthirius sp	Body,fins,gill,tail	The ich symptoms appear as white spots that look like grains of salt. Opportunistic ich parasite burrow under the skin and starts sucking blood and other fish juices and the fish trys to protect itself by growing a covering over the invaders-hence the characteristic white spot appears
Trichodina sp	Body, gill	They are probably not harmful when present in small numbers but in severe infection acts as true ectoparasite feeding on gill and skin tissue leading to necrosis and erosion of the tissues and respiratory trouble of the fishes.
Epistylis sp	Body, gill	The symptom by this stalked ciliate appears lesion pale & white in colour and resembles a fungus disease.
Myxobolus sp	Gill	Cyst appear in the form of small white spots on gills and sometimes in fins and are less pathogenic can infect other internal organs that cause high mortality.
Gyrodactylus sp	Body	With this infection the fish growth is hampered, erratic movement occurs and in heavy infestation the fishes are feeble and rest near the water margin.
Dactylogyrus sp.	Gill	With this monogenean infection, the gill fade ness and excessive mucous secretion take place causing severe respiratory trouble
Argulus sp.	Body, gill	Commonly known as fish louse, feed on body fluids and are especially harmful to small fishes. Reddish lesions occur at the site of attachment which causes secondary infection
Ergasilus sp.	Gill	Also known as gill maggot. Attacks the gill and sometimes skin of the fishes. Appear as whitish green threads hanging out of fish gill. Heavy infestation result in severe gill damage, anemia and death usually due to secondary infection.
Lernaea sp.	Body surface	The fish scrapes itself against objects, whitish-green threads hang out of the fish's skin with an inflamed area at the point of attachment.

d. Chemicals used as anti-parasitic reagents and their hazardous effects :

In East Kolkata Wetland different chemicals are used to prevent parasitic diseases of fishes, some chemicals are also applied during pond preparation. All these chemical substances have hazardous effect on target organisms as well as non target organisms. It has also hazardous effect on aquatic environment. These chemical substances present in environment thorough bio accumulation and affects nervous, immune, respiratory system and produce genetical disorder. These chemicals and their hazardous effects are represented in **Table no. 6**

Table no. 6

Name of chemicals	Type	Use	Hazardous effect
Endosulfan	Pesticide	To control parasitic disease	Insoluble in water and present in environment for a long period through bioaccumulation and affect nervous system, immune system and produce genetical disorder
Kerosene oil	Pesticide	Pond preparation	present in environment for a long period through bioaccumulation and affect nervous system, skin and mental ability
Methyl parathion	Pesticide	To control parasitic disease	Affect respiratory and nervous systems
Thion (mathion)	Pesticide	Pond preparation	Affect nervous system as well as genetical dispute shown
Butox	Pesticide	To control parasitic disease	Skin disease and respiratory problem occur
Tetramycin	Fungicide & antibacterial reagent	To prevent Fish diseases	Hazardous effect absent

e. Herbal medication practiced to prevent fish diseases

Chemicals that are used as anti- parasitic substances and used during pond preparation have used on target and non target organisms. That is why we are experimented with herbal product and get good results when applying it on diseased fish. In **Table no.7** those herbs are represented that we are used as anti parasitic substances to prevent parasitic diseases of fish.

Crude extracts of garlic and water in particular ratio helps to prevent parasitic disease of fish due to presence of allium and allicin in it. If Garlic paste and common salt in 2:5 raio with adequate amount of water is applied on diseased fish, it is found that this mixture gives better results in case of bacterial and fungal disease. It is also found that turmeric, lime and common salt in 1:2:10 ratio gives good result in bacterial and fungal diseases of fish. Neem extract along with lime in 1:2 ratio have good anti parasitic activity against fish diseases. Mixture of crude extract of neem and turmeric and water is beneficial for copepod diseases of fish. Zingerone, gingerols present in ginger act as anti allergic and ginger's crude extract with adequate amount of water gives good result in case of allergic diseases of fishes. Extract of tulsi, ginger, pan lata give good results if it applied on copepod disease affected fishes. Herbs are used from ancient age. We get best result from ginger to prevent the parasitic diseases of fish collected from east Kolkata wetland. Herbal medication has no hazardous effects on the environment and it has no side effects on target organism as well as non target organisms. Herbal medication is also important due to it is cost effective, easily available and no side effects in it.

Conclusion

In East Kolkata Wetland, fish production is hampered by fish diseases and aquatic pollution. Maximum diseases are outburst during winter. Here Fishes suffer more or less Parasitic, bacterial, protozoan and fungal diseases throughout the year.

Table no. 7

Name of herbs	Scientific name	Active ingredient	Characters
Ginger	*Zingiber officinale*	zingerone, shogaols, gingerols	Anti- allergic, anti-bacterial
Turmeric	*Curcuma longa*	Curcumin, C.I.75300, or Natural Yellow 3.	Anti-bacterial
Neem	*Azadirachta indica*	Azadirachtin, azadirachtol, azadiradione, gedunin, nimbiol, nimboeinol, nemolinone, nimbocetin	Antiseptic
Garlic	*Alliumsativum*	allicin,alliin,ajoene, diallylsulfide,dithiin	Antibiotic, Antiseptic
Pan lata	*Derris sp.*	Rotenone, dehydrorotenone, lupeol, oleanolicacid, ursolicacid	Anti- allergic, anti-bacterial
Tulsi	*Ocimum sanctum*	Bornylacetate, cadinene	Anti- allergic

Fishes of most of the ponds are attacked by copepod parasite *Argulus and Lernaea*. Winter temperatures were associated with disease, whilst in summer high temperatures caused fish seed mortalities in production and distribution. Different chemical substances utilized in anti-parasitic treatments of fish and in pond preparation which can have effects of long persistence in the environment and or humans through mechanisms of bio-accumulation and direct toxic effects on non-target organisms. So, herbal medication should be taken. A detailed study regarding this is under processed in our laboratory.

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Studies of the parasitic infestation in indigenous ornamental fish Silver Danio, *Danio devario* with its prophylactic measures

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Abstract : Ornamental fish culture and breeding is becoming an important component in fishery sector. India is one of the mega diversity countries which is bestowed with a number of aquatic biodiversity not only in the food fishery sector but also in the ornamental fishery sector and in ornamental fishery West Bengal particularly enriched with a number of indigenous ornamental fishes which are the source of income generation in the local people in many ways like seed collection, preparation of brooders, breeding and culture purposes. Among the indigenous ornamental fishes the Silver Danio- *Danio devario* is one of the potential fish which has body silvery, olivacious green on back, a dark longitudinal band in the middle of sides extending from caudal fin base to below dorsal fin origin. This fish has high demand as aquarium fish in native as well as export market. For the study of parasitic infection a number of silver danio were collected from the natural habitats of Howrah district of West Bengal in different seasons and different types of parasitic infections were observed. Among the crustacean parasite- *Ergasilus sp.* and *Argulus sp.* were identified, among the protozoan parasite *Trichodina sp*., *Tripertiella sp., Epistylis sp., Ichthyoptyhries sp.* were identified. And among the helminth parasite *Dactylogyrus sp*. and *Gyrodactylus sp*. were identified. These particular infections are major reasons for huge economic loss in the aqua business producing lower quality of ornamental fishes with reduced colouration which are of low value and actually do not confirm quality for the export market. These parasitic infections are being treated with different types of herbs extracts to control these diseases and the detailed study regarding this is under processed in our laboratory.

Keywords **:** *Control, Indigenous, Ornamental, Parasitic,*

Introduction

Ornamental fish culture is becoming an important component in Indian fisheries along with food fishes for both employment generation and export earnings (Sinha etal, 2010). India is one of the mega diversity countries having vast potential of ornamental fishes. Specially in the Ornamental sectors it's contribution is of immense important and particularly in case of West Bengal the sector is much more explored than other states of India. Specially in the native ornamental fishes i.e. the indigenous ornamental fishes are the source of income generation among the local people through the aquarium business of these fishes. Ornamental fish farming is a sub sector of aquaculture and many technologies currently applied to ornamental fish production arise from food fish and live stock farming sector (Rana, 2008). Among the indigenous aquarium fishes the silver danio- *Danio devario* is one of the potential fishes which is exported to different countries from India in terms of US$ 0.263 and Rs. 6.00/- per fish in Indian money(Sinha etal, 2010). So the fish dominates the local as well as export market for it's colouration and structural organization.

Description of the fish

Body silvery, olivacious green on back, a dark longitudinal band in the middle of sides extending from caudal fin base to below dorsal fin origin.The silver danio found in lower region hill stream, plain rivers and lentic wet lands like beels with clear slow running water. This species usually swim on the lower surface of upper column zone in shoal.

Determination of sex

Body shape slender in male but slightly convex in female. Dorsal profile is slightly convex in male. In female the ventral edge is comparatively less keeled. Mouth blunt in male but in female it is faint. In male the dorsal caudal and anal fins are reddish yellow in colour during breeding season. Fins are pale yellowish in colour during breeding season in female. In natural population the sex ratio is 2:1 (Male: Female).

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Material and methods

Survey design :

First of all an arial survey was conducted to Howrah district of West Bengal to identify the ornamental prone areas of the district. Then the areas were selected –Pakuria, Dopmjur, Kadamtala. Amta and ramrajatala. After that a number of survey were conducted to those areas in a year from November 2009 to October 2010 dividing the period into three seasons i.e. Pre monsoon, Monsoon and post monsoon. Simultaneously a number of silver danio was randomly collected from the natural habitat of that fish from the selected ornamental prone areas.

Laboratory preparation :

After collecting those fishes, the fishes were brought to laboratory and checked thoroughly for the infestation of parasites. For this purpose the following steps were done-

- Scrapings from the body, gill, fins and tails were taken
- Thin smear was *prepared* in the slide from those scrapings.
- The smear was then allowed to semidry.
- After that the smears were either single stained with Giemsa or Silver nitrate or double stained with Haematoxylene and Eosin.
- After staining the slides were observed under microscope for the idemtification of parasites.

Result and Discussion

Parasites are generally opportunistic pathogenic organism, causing diseases under congenital condition. Usually parasites are present in almost all animals in low numbers, but generally do not cause any disease condition but if present in large numbers they can turn fatal for the host (Sanil et.al 2008).The stained Slides Showed Different Types of protozoan, and Helminthes and Crustacean parasites of the fish – *Danio devario*.

Identification of Protozoon parasites

Among the protozoon parasiotes the fish was infected with a number of *Ichthyopthirius* sp, *Trichodina* sp, *Tripartiella* sp and *Epistylis* sp were identified.

Description and Symptoms of the Protozoan parasites

A. Ichthyopthirius multifilis :

Ich is the most common disease in aquaria and is caused by the ciliate parasite, *Ichthyopthirius multifilis*. Symptoms include white glistening spots or salt like specks on the body and fins, excessive slime on body, difficulty in breathing, clamped fins and loss of appetite. The parasite lacks host specificity can infect any fish species, making it the most destructive fish parasite in ornamental fishes.

B. Trichodina sp :

Dogiel (1961) reported that trichodiniasis is caused by Trichodina spp; the infection being stimulated by the high density of fish in ponds. A disc shaped ciliate protozoan found on the skin and gills of many fresh water fishes. Circular rows of denticles and a ciliary girdle give this parasite a unique symmetry. Probably not harmful when present in small numbers but on stressed and young fishes, especially when organic load in the water is high, they proliferate rapidly and behave like true ectoparasites, starts feeding on gill and skin tissues leading to necrosis and erosions of tissues. Lack of host specificity coupled with their pathogenicity.

C. Tripartiella sp :

Another disc shaped urceolarid protozoan parasite that resembles with *Trichodina* sp. but differs in size other internal characters. The parasite found on gill, skin, fin & tail region of fresh water ornamental fishes.They become dangerous when present in large numbers and causes tissue necrosis & erosions of the surrounding tissue.

Table 1 : Distribution and Biodiversity of the Protozoan parasites

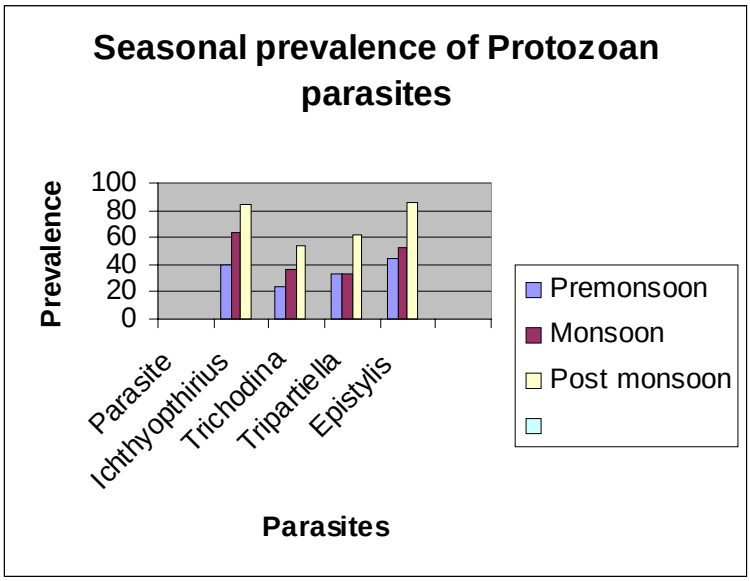
Host	Collection site/District	Parasite	Phylum	Infected Organ
Danio devario	Howrah	*Ichthyopthirius sp.*	Ciliophora	Body, Gill, Fin, Tail
		Trichodina sp.	Ciliophora	Body, Gill
		Tripartiella sp.	Ciliophora	Body, Gill
		Epistylis sp.	Ciliophora	Body, Gill

D. Epistylis sp :

A stalked ciliate which is commonly found in fresh water containing a high organic loads. Lesions appear pale and white in color and resemble a fungal disease. Microscopically, it appears as a ciliated crown with a long stalk which is prone to frequent contractions. This disease is usually not fatal in itself but may open the fish up to secondary infection.

Prevalence of Protozoon parasites

According to our study we have taken 50 numbers of fishes randomly in each seasons thoroughly checked those fishes for the study of infection. We observed that 40% of fishes were infected with Ichthyopthirius sp in Pre monsoon while 64% and 84% fishes were infected during the Monsoon and post monsoon seasons. On the other hand in case of Trichodina sp the prevalence rate is 24%, 36% and 54% in Premonsoon, Monsoon and Post monsoon respectively. In case of Tripartiella sp the prevalence rate is 34%, 34% and 62% in Pre monsoon, Monsoon and Post monsoon respectively. And in case of Epistylis sp the prevalence rate of infection is 44%, 52% and 86% in Premonsoon, Monsoon and Post monsoon respectively. The details of the protozoan prevalence is shown in the chart below.



Identification of Helminthes Parasites

Among the Helminthes parasites two types of parasites were identified i.e. *Dactylogyrus* sp and *Gyrodactylus* sp.

Description and Symptoms of the Helminthes parasites

Dactylogyrus sp :

Monogenean parasite including *Dactyloyrus* sp is considered as an ectoparasite which can cause huge damage to the host when present in large numbers. The parasite possess a multiple hooked attachment organ called an opisthaptor which disrupts the integrity of the host's skin and mucus membranes. It actually infects the gill of the fishes in which condition the fish feels suffocated and becomes stressed and generally fading of body colouration is seen.

Gyrodactylus sp :

Another monogean worm present on the body surface of fresh water fishes is *Gyrodactylus* sp. It also causes huge damage when present in large numbers. In general there is growth retardation in afflicted fishes for the disease is encountered.

Table 2 : Distribution and Biodiversity of Helminthes Parasite

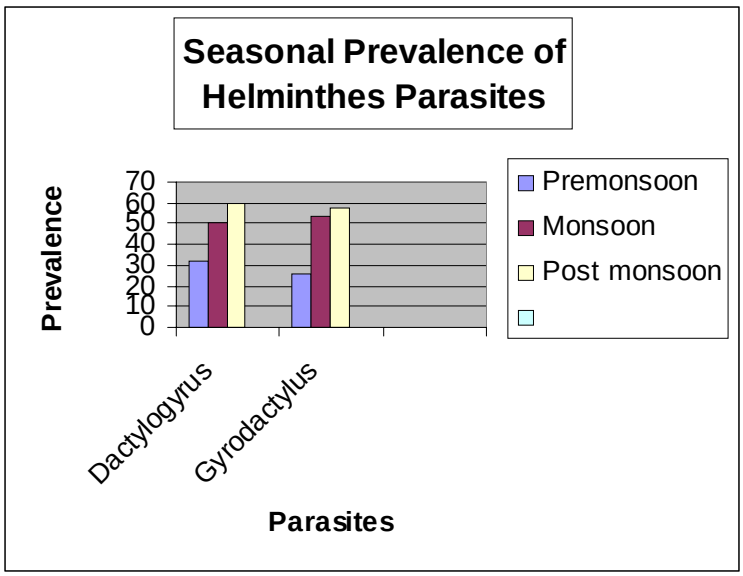
Host	Collection site/ District	Parasite	Phylum	Infected Organ
Danio devario	Howrah	*Dactylogyrus* sp	Platyhelminthes	Gill
		Gyrodactylus sp	Platyhelminthes	Body

Prevalence of Helminthes parasites

50 numbers of fishes were randomly selected in each season from the natural habitat of Howrah district and the prevalence of helminthes parasites were checked. According to our observation 32% fishes are infected with Dactylogyrus sp in the pre monsoon season. While 50% and 60% fishes are infected with this parasite during the monsoon and post monsoon season.On the contrary 26%,54% and 58% fishes are infected with the Gyrodactylus sp during the pre monsoon, monsoon and post monsoon season. The chart below shows the details.

Identification of Crustacean parasites

Among the Crustacean parasites the prepared slides showed the infestation of two types of parasites i.e. *Ergasilus* sp and *Argulus* sp.



Description and Symptoms of the Crustacean Parasites

Ergasilus sp :

The parasite is also known as “Gill maggot” which infest the gill of the fishes. The adult female *Ergasilus* sp is parasitic. The parasite looks as white bodies less than 2mm long. Surfacing, lethargy and restlessness occur during heavy infestation and the intensity of infection is directly proportional to the size of the fish(Das, 1997).

Argulus sp :

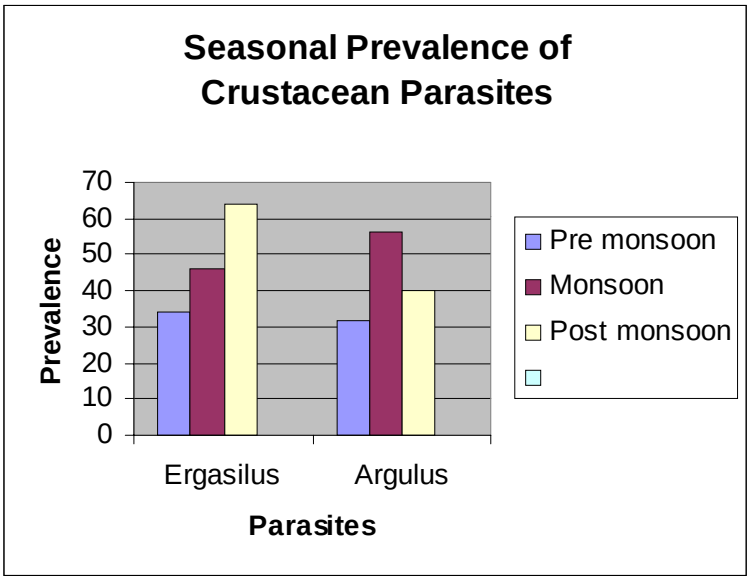
The parasite is generally known as “Fish louse” with flattened mite-like crustacean that attaches itself to the gill and some times the body of the fish. They irritate the host fish which have clamped fins, becomes restless and may show inflamed areas where the lice have been situated. The fish scratches itself against objects. The parasite can be visible with naked eye in huge infestation.

Table 3 : Distribution and Biodiversity of Crustacean parasites

Host	Collection spot/ District	Parasites	Phylum	Infected organ
Danio devario	Howrah	*Ergasilus*	Crustacea	Gill, skin
		Argulus	Crustacea	Skin, fin

Prevalence of Crustacean Parasites

Here also 50 number of fishes were collected from its natural habitat and the prevalence of crustacean parasites were estimated. So according to our investigation the prevalence of *Ergasilus* sp is 34% in the pre monsoon and it is 46% and 64% during the monsoon and post monsoon seasons respectively. On the other hand the prevalence rate of *Argulus* sp is 32%, 56% and 40% during the pre monsoon, monsoon and post monsoon season respectively. The details has been showed in the chart below.



Prophylactic control

Generally Protozoan ectoparasites like *Ichthyopthirius* sp, *Trichodina* sp and *Tripartiella* sp are treated with Sodium chloride, Malachite green and with other commercial products by administrating different doses. But at the same time those chemicals lefts some residual effects on the fish species. So in this respect we tries to eradicate the diseases with the application of some medicinal plants. In this regard Chitmanat etal 2004 recommended the use of Garlic-*Allium sativum* and Indian almond-*Terminalia catappa* to eliminate *Trichodina* sp from Tilapia fish. More over Abo-Esa 2008 recommended the use of Ginger-*Zingiber officiale* to eradicate the *Trichodina* sp. from Cat fish –*Clarias gariepinus.* Ekanem.etal 2004recommended the use of *Mucuna pruriens* and *Carica papaya* to eliminate the *Ichthyopthirius* sp from fresh water fishes.

In case of helminthes parasite according to Abo-Esa 2008 managed to eradicate some of the Dactylogyrus sp from Cat fish –*Clarias gariepinus.* and rest of the other parasites are still to be examined and recommended by any scientist. In this respect we tryinr to eradicate some of the parasites in our laboratory, Some good result have achieved also and rest is being done in our laboratory.

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Random amplified polymorphic DNA and drug sensitivity analysis of recent clinical isolates from Indian kala-azar patient

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Abstract : Kinetoplastid protozoan parasites belonging to the Genus *Leishmania* cause a great loss to mankind throughout the world in terms of morbidity and mortality. The estimated annual new cases are 1.5 to 2 million with up to 350 million people at risk of infection and disease. In our previous study, we typed the nine clinical isolates from confirmed KA and Post Kala-azar Dermal Leishmaniasis (PKDL) patients by RAPD-PCR method using eight selected primers. We identified one isolate (study code T5) with different RAPD PCR profile from other clinical isolates and also from the WHO reference strain for *Leishmania donovani*, DD8. In the present study, we have extended our RAPD PCR work and checked the drug sensitivity of the isolates for Sodium Antimony Gluconate (SAG), the first line drug for KA and also for Miltefosine (ML), the oral cancer drug, now in use for treating KA of India. While all clinical isolates of KA showed sensitivity to SAG and ML, T5 showed unresponsiveness to SAG. The RAPD PCR banding pattern of T5 with two random primers (OPA1 and OPA4) also confirmed our earlier observations that T5 is not *L.donovani* and clinical isolates may behave differently towards the drugs, SAG and ML.

Key words **:** *Indian Kala-azar, Leishmania donovani, L. tropica, Miltefosine, Random Amplified Polymorphic DNA, Sodium Antimony Gluconate.*

Introduction

Visceral Leishmaniasis (VL) or Kala-Azar (KA) is a serious global health problem. It is caused by infection of the reticuloendothelial system of the vertebrate host including humans by *Leishmania* species transmitted by the female sand fly vector. The endemic countries are India, Nepal, and Bangladesh. Other endemic areas for VL are East Africa, the Mediterranean, and Brazil, annual burden being 5,00,000 cases (Desjeux, 2004).The disease manifests with intermittent fever, hepatosplenomegaly, weight loss, and pancytopenia and is fatal if the patients left untreated (Herwaldt, 1999).Till date there is no vaccine for the disease. Chemotherapy is the only weapon to combat the disease (Croft et al 2006).

Urea stibamine, the pentavalent antimony compound, was first emerged as an effective chemotherapeutic agent against Indian kala-azar (Brahmachari, 1922). Other antimony drugs namely, sodium stibogluconate (Pentostam) and meglumine antimoniate (Glucantime), are still the first choice for treatment of leishmaniases despite extended treatment regimens, parenteral administration, and toxic side effects (Herwaldt, 1999; Murray et al 2005). Standard treatment regimen is daily injections 20 mg pentavalent antimonial compounds per kg body weight for 28 days. Pentamidine along with Amphotericin B represents the second line of treatment in regions of India where there is a high frequency of resistance to antimony but both the drugs have tremendous side effects (Davidson and Martino, 1996; Amato et al 1998). Recently several lipid-based formulations of Amphotericin B with reduced levels of toxicity have been used but these are not affordable by all (Jha et al 1995; Sundar et al 1997; Guerin et al 2002).

Miltefosine (ML) is hexadecylphosphocholine, an alkylphosphocholine analogue that was originally developed for the treatment of cutaneous metastasis from mammary carcinomas (Unger et al 1992). ML has been hailed as first potential oral treatment of human leishmaniasis (Sundar et al 2000). So, chemotherapy for Indian Kala-azar is besieged with problems of proper drug formulations.

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Before going for any drug regimen, the identification of the parasites is a must. Characterization and identification based on morphotaxonomy do not hold good for the parasites belonging to *Leishmania spp* as they are indistinguishable morphologically and sometimes also clinically. This difficulty has been overcome by various modern techniques involving biochemical, immunological or molecular criteria. With the advent of PCR based characterization procedures, the researches in the field of molecular taxonomy, phylogenetics and evolutionary biology have received tremendous impetus. The Random Amplified Polymorphic DNA (RAPD) analysis is one of the most reliable, easy to perform method that has extensively been utilized for typing *Leishmania* sp all over the World (Tibayrenc et al 1993). It can differentiate between different Old World and New World species of *Leishmania* (Oliveira et al 2007; Hamad et al 2010). In the present study, we have extended our RAPD PCR work for recent (2009-2010) clinical isolates of KA. One isolate (T5) was not matching with WHO reference strain, DD8. This finding prompted us to check the responsiveness of the clinical isolates to Sodium Antimony Gluconate and Miltefosine, previous one is still the first line drug for Kala-azar while the latter one is now introduced to treat Indian Kala-azar patients including those who are not responsive to SAG (Sundar and Jha, 2002).

Materials and Methods

Chemicals and Reagents

The parasite *in vitro* culture chemicals like medium M199, medium RPMI-1640, sodium bicarbonate, streptomycin, and penicillin and other reagents used were obtained from Sigma chemical (St. Louis, MO, USA). Fetal Bovine Serum (FBS) was purchased from Invitrogen, USA. Random primers were procured from Eurofins Scientific GmbH, Germany. Sodium Antimony Gluconate was purchased from Albert David (India) Pvt. Ltd., Kolkata.

Reference strains and Clinical isolates

World Health Organization (WHO) reference strains for *Leishmania donovani* (DD8) and *L.tropica* (K27) were kindly provided by Dr. L. F. Schnur, Hebrew University-Hadassah Medical School, Jerusalem, Israel. We have collected six clinical isolates of KA from patients admitted in the Calcutta National Medical College, Kolkata. All the parasites were maintained in culture medium M199 supplemented with 10% Fetal Bovine Serum (FBS).

Preparation of nucleic acid from the reference strains and clinical isolates of KA

Total nucleic acids from five clinical isolates and two WHO reference strains were prepared according to Bhattacharya et al (1993). In brief, *Leishmania* promastigotes were suspended in 0.02M Phosphate Buffered Saline (PBS) pH 7.4. The pellets from different isolates and standard strains were obtained by centrifugation at 6000 rpm for 10 min at 4°C. Pellets were washed in PBS and resuspended in 400 µl of NET buffer. DNA was prepared by digestion with 100µg/ml Proteinase K in the presence of 1% SDS keeping overnight at 37°c. This was followed by Phenol Chloroform extraction. Ethanol precipitated DNA was dissolved in TE buffer and stored at -20°C for further experiments.

RAPD PCR amplification of the DNA of reference strains and clinical isolates of KA

DNA amplification using random primers were preformed according Manna et al (2005) with the following two random primers, OPA 1(5' -TGC CGA GCT G-3'), and OPA 4(5'-GTG ACG TAG G-3'). Sequences of the primers were selected randomly with the requirement that their GC contents are 60% to 70% as Leishmania genome is GC rich. The primers also do not have any self complementary ends.

The procedure, in short, was as follows, reaction mixtures contained 10x PCR buffer, 200µM of each dNTP, 1 µM of each primer, 25 ng total nucleic acid and 1.2 units of Taq DNA polymerase and 50 mM $Mgcl_2$. The volume of the reaction mixtures were made up to 20 µl with PCR grade distilled water. Amplification was carried out for 30 cycles in a thermal cycler with the following programme: 5 minutes at 94ºC, 1 minute at 94ºC, 2 minutes at (50ºC -60ºC), 2 minutes at 72ºC. After the completion, all tubes were kept for 5 minutes at 72ºC for extension. The products were electrophoresed in 1.2% agarose gel in TAE buffer and photographed for analysis.

Drug Sensitivity Assay

The stock concentration of SAG was 100mg/ml from which we made three dilutions (1µg/ml, 10µg/ml, 100µg/ml respectively). For ML, the stock concentration was 1mg/ml from which we made four dilutions (0.01µg/ml, 0.1µg/ml, 1µg/ml, 10µg/ml respectively). Sterile Phosphate buffered saline (PBS) used as diluent. Promastigotes (10^5/ml) were taken in 2ml culture medium in triplicate for each concentration for both the drugs. Parasites with flagellar movement were considered as viable. Cell counts were taken with the help of haemocytometer.

Results

In the present study, RAPD PCR analysis of five recent clinical isolates with primers OPA1 (Figure 1) and OPA 4 (Figure 2) supported our previous observations (Khanra et al, unpublished data) that the clinical isolate, T5 (lane 7 of both the Figures 1 & 2 respectively) has shown similar banding profiles with K27, WHO reference strain for *L.tropica* (lane 8 of both the Figures 1 & 2 respectively) while other clinical isolates (T2, T3, T4, T7 in lanes 3, 4, 5, 6 respectively for Figure1 and Figure 2) showed same pattern of amplification as that of DD8, the WHO reference strain for *L. donovani* (lane 2 for both the Figures 1 & 2 respectively). We calculated Jaccard's Similarity Co efficient (Sneath and Sokal, 1973) with the data obtained from these two primers and saw that T5 showed 100% similarity with *L.tropica* WHO reference strain, K27 and approximately 15% similarity with *L.donovani* WHO reference strain, DD8 and also with other clinical isolates of KA studied in the present work.

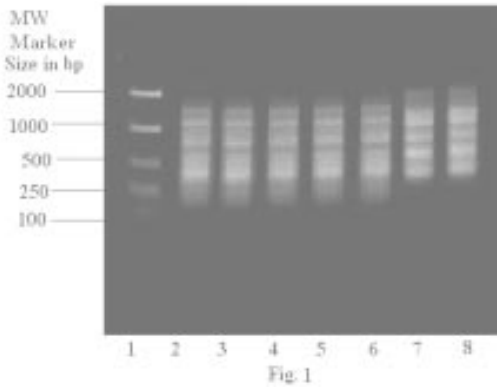

Fig. 1

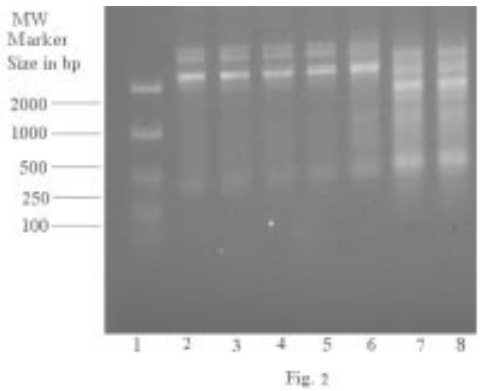

Fig. 2

Fig.1 and Fig. 2 *RAPD-PCR amplification of 25ng of total nucleic acid of different clinical isolates of Kala-Azar along with DD8 and K27 using primer OPA1 (**Fig.1)** and OPA 4 (**Fig. 2)** respectively*

Lane 1 = MW marker (100 – 2000 size in bp); Lane 2 = DD8; Lane 3 = T2; Lane 4 = T3; Lane 5 = T4; Lane 6 = T7; Lane 7 = T5; Lane 8 = K27

DD8 and K27 are WHO reference strains for Leishmania donovani and Leishmania tropica respectively, T2, T3, T4, T5 and T7 were clinical isolates of Indian Kala-azar (KA) patients

We screened the clinical isolates for their drug responsiveness to SAG (Figure 3) and ML (Figure 4). We noticed T5 responded to SAG (Figure 3B) differently than the other clinical isolates (Figure 3A for T7). The isolate (T7) seemed to stop dividing and died within fifth day while T5 not only thriving but also dividing. The growth rate was much reduced compared to the control culture but they sustained even after fifth day at highest conc. of SAG used (100µg/ml). They survived even after eight days if fresh medium was being added (data not shown). Regarding response to ML, all isolates showed sensitivity to the drug including T5 but here again, T5 showed some uniqueness in the response pattern.

While other isolates (T7 for example in Figure 4A) died gradually at 120-hour for drug conc. 10µg/ml, T5 parasites died abruptly (Figure 4B) within 24 hours of initiation of treatment with the same conc. of ML. For drug conc. 1µg/ml, T5 died within three days while T7 survived till fifth day and then disappeared from the medium. For drug conc. 0.1µg/ml, T5 died within four days and T7 again survived till fifth days. The drug conc. 0.01 µg/ml had different effects on T5 and T7. T5 was more resistant to this conc. than T7. This conc. was growth limiting for T7 but not for T5 as evident from the growth curves depicted here.

Discussion

In India, Kala-azar is caused by *L.donovani* and is endemic in the eastern part of the country (Salotra and Singh, 2006) while *L.tropica* , the causal agent of cutaneous leishmaniasis is found only in the western part (Kumar et al 2007). In our pilot study, it was observed that though T5 was collected from confirmed Kala-azar patient admitted to Calcutta National Medical College, Kolkata, it does not belong to *L.donovani* species. On contrary, T5 showed extreme homology with *L.tropica*. There is none but one report which stated that *L.tropica* causes Indian Kala-azar (Sacks et al 1995) and the authors also suggested that this might be related to the drug unresponsiveness in the field. When we screened all clinical isolates for their

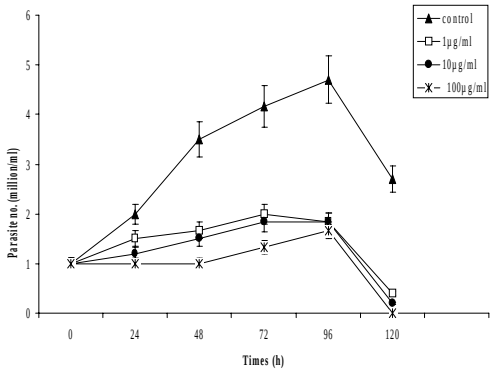

Fig.3A

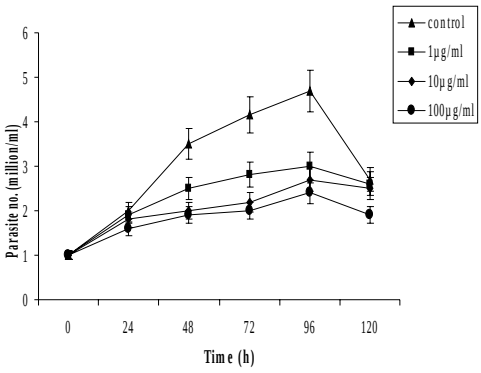

Fig.3B

Fig. 3 Growth Curves of the clinical isolates of Kala-Azar for Sodium Antimony Gluconate (SAG) sensitivity: *Control tubes contained 1X10^5 Leishmania clinical isolate, T7 (A) and T5 (B) per ml medium M199 supplemented with 10% Fetal Bovine Serum and no drug. Experimental tubes (three sets) contained 1X10^5 Leishmania clinical isolate, T7 (A) and T5 (B) in the same conditions along with three different SAG concentrations (1µg/ml, 10µg/ml and 100 µg/ml respectively). Flagellar movement and increase in parasite number was considered as markers of viable parasites and was counted with the help of haemocytometer. Control and experimental tubes were taken in triplicate and one representative data set out of three repeat experiments is presented here.*

The level of significance is p < 0.01.

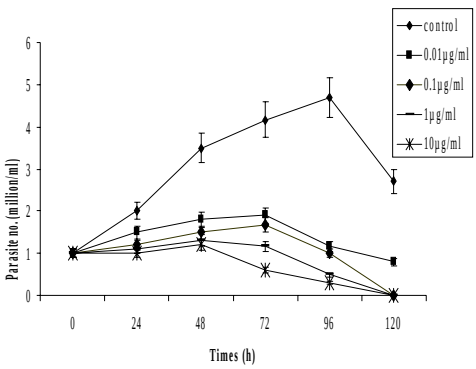

Fig.4A

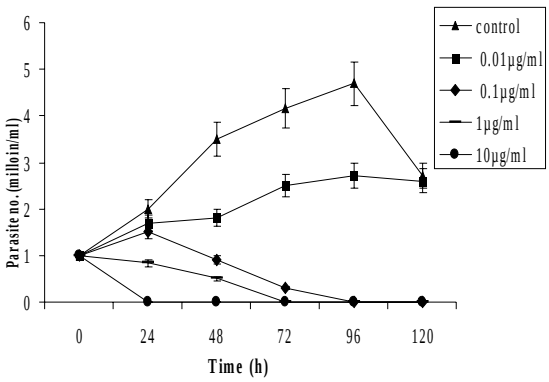

Fig.4B

Fig. 4 Growth Curves of the clinical isolates of Kala-Azar for Miltefosine (ML) sensitivity: *Control tubes contained 1X10^5 Leishmania clinical isolate, T7 (A) and T5 (B) per ml culture medium M199 supplemented with 10% Fetal Bovine Serum and no drug. Experimental tubes (four sets) contained 1X10^5 Leishmania clinical isolate, T7 (A) and T5 (B) in same conditions along with four different ML concentrations (0.01 µg/ml, 0.1µg/ml, 1 µg/ml & 10 µg/ml respectively). Flagellar movement and increase in parasite number was considered as markers of viable parasites and was counted with the help of haemocytometer. Control and experimental tubes were taken in triplicate and one representative data set out of three repeat experiments is presented here.*

The level of significance is p < 0.001.

drug responsiveness to SAG and ML, we noticed that T5 was not responding to SAG the ways other clinical isolates did. The sensitivity experiments was carried out on promasitgote (vector) stage of the parasites in culture which is reported to be less sensitive compared to the amastigote (vertebrate host) stage , still it gave the hint of differential response of T5 towards SAG with respect to other isolates (data with T7 shown here). Our works on sensitivity of amastigotes for SAG in macrophage model are underway. For responsiveness to ML, again T5 showed different behavior than other isolates. Though the promastigote stage is sensitive stage to ML (Verma and Dey, 2004), we have to conduct the experiments taking amastigote stage in host macrophage system as this mimics the actual situation of drug treatment to the KA patients. Our present study indicated the urgent need for more systematic screening of each clinical isolate of KA patients before any treatment schedule starts as the drug for *L. donovani* may not work well for *L.tropica* and thus may exacerbate the disease.

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First record of a gregarine parasite from an earthworm of Bangladesh

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Abstract : The present communication deals with the description of a new species of the genus *Monocystis* (Apicomplexa:Eugregarinorida). During the study of aseptate gregarines from the earthworm hosts of Bangladesh, one species of the genus *Monocystis* Von Stein, 1848 has been recorded which appears to be the first record from Bangladesh. Keeping it in mind extensive survay on aseptate gregarines of Oligochaetes have been done. In this paper the description, taxonomy and systematics of *Monocystis ayeshae* n. sp., its morphology and morphometric comparisons with closely related species are presented.

Key words **:** *Gregarine, earthworms, Bangladesh.*

Abbreviations

SD = Standard Deviation
SE = Standard Error
CV(%) = Coefficient of Curvature
TL = Trophozoite Length
TW = Trophozoite Width
DN = Diameter of nucleus
LG = Length of Gametocyst
WG = Width of Gametocyst
OL = Oocyst Length
WO = Oocyst Width

Introduction

Gregarines are a diverse and successful group of protozoan parasites belonging to the phylum Apicomplexa, order Eugregarinorida Lager, 1900. The Eugregarinida are all parasitic and are restricted to invertebrates (Clopton 2002). Investigation on the incidence of aseptate gregarines from the annelids have immense important because of severe pathogenecity of the parasites. Aseptate gregarine fauna has been reported from various parts of the world including India. Bhatia (1929) listed fifteen species of *Monocystis* and seven species of related genera from the seminal vesicles of a single species of common earthworm, *Lumbricus terrestris.* Twenty seven species have been recorded from *L. rubellus* as stated by Meier (1956). Levine (1977a) listed seventy species under the genus *Monocystis.* Later on, five more species were added: two by Segun (1978), one each by Kalavati (1979), Subbarao *et al* (1979) and Pradhan and Dasgupta (1982). Prior to that, Kar (1946) described *M. megascolexae*. India has gained momentum since 1980 (Bandyopadhyay *et al*, 2001, 2002, 2005a, 2005d, 2006c, 2007, 2008) Pradhan and Dasgupta 1983a, 1983b, Roy Choudhury and Haldar, 1984. In course of the ongoing survey of the aseptate gregarines in India, 15 species of *Monocystis* have been recorded. Out of them 13 species have been reported from Oligochaete hosts. However, especially in Bangladesh, the search is far from this type of study. The biodiversity survey of gregarines from the Satkhira district of Bangladesh revealed a new species of the genus *Monocystis* Stein, 1848 from the seminal vesicles of the earthworm, *Metaphire posthuma*. This paper presents the description of a new species of the genus *Monocystis*.The morphometric comparison with other related species and the discussion of its taxonomy and systematics have been incorporated. Out of twelve host specimens studied about nine hosts were infested with this new species. The infestation rate of *Monocystis* was very significant.

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Materials and Methods

Oligochaetes, host specimens were collected from the Satkhira district of Bangladesh. The host earthworms were collected during winter season (November 09 – February 10) and the collected earthworms were kept in soil in a tub and taken to the laboratory alive. Some of the earthworms were dissected while alive and their seminal vesicles were carefully removed. These were placed on clean glass with a drop of 06% Nacl solution. A thin film of seminal fluid was drawn out on a slide covered with a cover slip for examination of living protozoan under a light microscope. After initial study of living protozoans the content of the seminal vesicles semidried and fixed in Schaudin's fluid for 20 minutes. The smears were stored in 70% ethanol for removal of mercuric chloride. The slides were then passed through a descending series of alcohol (5 minutes each) and placed in distilled water. These were transferred to a 3% iron alum solution (Over night) and stained with Heidenhain's hematoxylin solution for 20 minutes. Differentiation was done with 1% iron alum solution under the low power objective lens of the light microscope. The slides were then washed thoroughly, dehydrated in an ascending series of alcohol, cleared in xylin and mounted in DPX. Camera Lucida drawings of different stages of gregarines were made and photomicrographs were taken with an olympus phase contrast microscope (15 × 40 magnification, Model $CH^{-}2$, 396250) fitted with digital camera. All measurements were taken with the aid of calibrated ocular micrometers (µm). In each case minimum and maximum values are given, followed in parentheses by arithmetic mean, standard deviation and sample size, n = 12. The methods of describing shapes of planes and solids have been done following clopton (2004).

Results

Monocystis ayeshae n.sp. (Fig: 1-4, Table-1, 2).

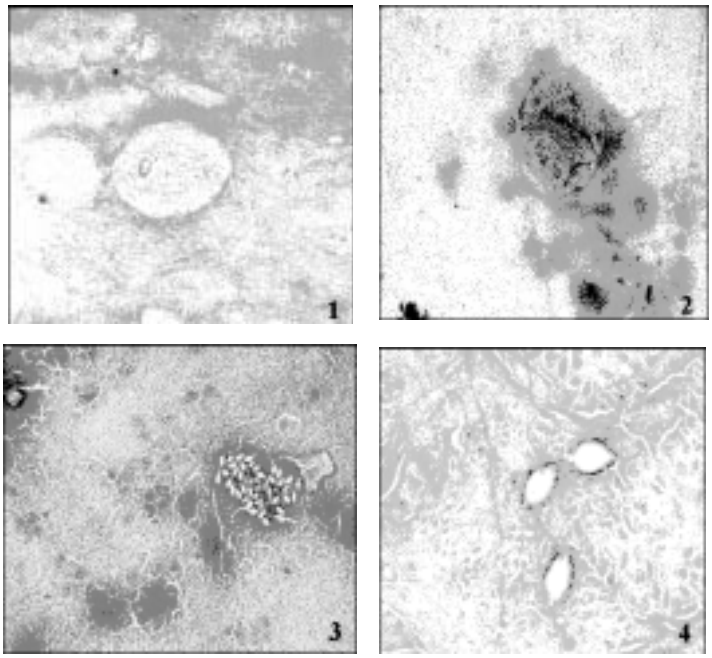

Fig 1-4. Photomicrographs of different stages of the life cycle of *Monocystis ayeshae* n.sp. obtained from the seminal vesicles of the earthworm *Metaphire posthuma*.

Fig 1. A mature trophozoite, **Fig 2.** Gametocyst, **Fig 3-4.** Oocysts.

Trophozoites are oval in shape, middle portion is slightly wide. Mucorn not visible. It measures 92.82-97.24 µm (95.21±1.98) × 50.83-57.46 µm (53.40±3.24), n=12 and has alveolated protoplasm without any inclusions. Ectosarc thin, endosarc is more granulated. Nucleus is large and also oval in shape. It is placed at the anterior part of the trophozoites. There is no chromatin bodies in the nucleus. Nucleus measures 13.26-17.68 µm (16.02±1.91) in diameter. Gametocyst almost rounded and consists two unequal sized of gametocytes. Gametocyst measures 132.6-141.44 µm (136.83±4.04) × 97.24-101.66 µm (99.26±1.98). The large gametocyte is bean shaped or kidney shaped whereas the small gametocyte is broadly elliptoid. Oocysts are with pointed end and navicular in shape. It measures 8.84-15.47 µm (11.78±2.17) × 4.42-8.84 µm (6.81±1.75).

Taxonomic summary

Phylum : Apicomplexa Levine, 1988;

Order : Eugregarinorida Leger, 1900;

Family : Monocystidae, Biitschili, 1882;

Subfamily : Monocystinae, Bhatia, 1930

Genus : *Monocystis* Stein, 1848;

Typehost : *Metaphire posthuma.*

Type material : *Monocystis ayeshae* sp.nov

Type locality : Satkhira, Bangladesh.

Site of infestation : Seminal Vesicles.

Prevalence : 9/12 = (75%)

Holotype : BD/05/10 deposited in the museum of the Parasitology Laboratory, Department of Zoology, University of Kalyani, Kalyani -741235, West Bengal, India.

Paratype : BD/06/10 deposited in the museum of the Parasitology Laboratory, Department of Zoology, University of Kalyani. Kalyani–741235, West Bengal, India.

Specific epithet : The name of the species is given after the name of Prof (Dr.) Ayesha Khatun, retired Professor of Zoology, Principal, Eden Girls' College, Dhaka, Bangladesh and former Director General of Secondary and Higher Education, Dhaka, Bangladesh for her contribution in the field of Parasitology.

Discussion

Spherical to cylindrical gamont with irregular mucorn and little different anterior end if at all, seminal vesicles or coelom dwelling, solitary and are parasitic to earthworms. Oocysts of this species are bioconical and systemetical. Inclusion of the present form under the family Monocystidae (Biitschli, 1982) Sub-family monocystinae (Bhatia, 1930) and genus *Monocystis* Stein, 1848 is on the basic of aforesoid properties. The present species differs from other described species namely *Monocystis llyodi*, Ghosh 1923, *Monocystis pheritimi*, Bhatia and Chatterjee, 1925, *Monocystis lalbagensis*, Bandyopadhyay *et al*, 2001, *Monocystis metaphirae* Bandyopadhyay *et al* Mitra, 2006, in shape and size in following significant ways:- In the present study the trophozoite is oval in shape, middle part wider just like an egg, where as in *M. llyodi* the trophozoite rounded to oval some what fusiform. It is variable spherical, ovoidal, ellipsoidal or dump-bell shaped in *M. pheretimi,* cone shaped in *M. lalbagensis,* bean shaped with broad anterior end and posterior end in *M. metaphirae.*

The shape of the nucleus of the present study is also oval, large without any karyosome. While it is spherical, ovoidal, ellipsoidal or elongated in other mentioned species. Position of the nucleus is also differs in different species of described *Monocystis*.

Endoplasm with scattered granules in *M. llyodi*, in *M. metaphirae is* vacuolated with few black granules which are completely absent in the former one, but in *M. lalbagensis* and *M. pheritimi* poses vacuolated endoplasm.

The gametocyst of the present form is oval with two unequal sized gametocytes, which resembles the shape with the other described species.

Oocyst of the present study is navicular with pointed ends while it is bioconical in *M. lalbagenensis*, navicular in *M. metaphirae.*

So it is described that the width of trophozoites, endoplasmic organization, shapes of the gametocyst etc. are different in present form. Moreover their hosts are different such as *M. metaphirae* is from *Metaphire houlleti* (Perrier). *M. llyodi, M. pheritimi and M. lalbagensis* are from *Pheritima posthuma* (L. vaill). The present species is also from *M. posthuma of Bangladesh.*

In India, a good number of aseptate gregarines parasites have so far been reported from oligochaetes but it found for the first time from *M. posthuma* of the south region of Bangladesh. On the basis of above taxonomic and comparative study based on the morphology of the trophozoite, gametocyst and oocyst, the species is described here as a new species under the genus *Monocystis* and hence *Monocystis ayshae* n. sp. is designated.

Table 1 : Summary of morphometric data of 12 specimens of trophozoites, gametocysts and oocysts are given below.

Parameters	**Range (R)**	**Mean (x^-)**	**SD**	**SE**	**CV(%) n=12**
Trrophozoite length (TL)	92.82-97.24	95.21	1.98	0.57	2.07
Trophozoite width (TW)	50.83-57.46	53.4	3.24	0.93	6.06
Diameter of Nucleus (DN)	13.26-17.68	16.02	1.91	0.55	11.92
Length of gametocyst (LG)	132.6-141.44	136.83	4.04	1.16	2.95
Width of gametocyst (WG)	97.24-101.66	99.26	1.98	2.07	1.99
Oocyst length (OL)	8.84-15.47	11.78	2.17	0.62	18.42
Oocyst width (Ow)	4.42-8.84	6.81	1.75	0.5	25.69

Table 2 : Morphometric comparison of the new species with previously described sp. of the genus n. sp.

Species / Characters	***Monocystis Hyodi*, Ghosh, 1923**	***Monocystis pheritimi*, Bhatia and Chatterjee. 1925**	***Monocystis lalbagensis* Bandyopadhyay *et al*, 2001**	***Monocystis metaphirae* Bandyopadhyay *et al* Mitra, 2006.**	**Present study (n=12)**
Host	*Pheritima posthuma* (L.Vaill)	*Pheritima posthuma.*	*Metaphire posthuma*	*Metaphire houlleti* (Perrier).	*Metaphire posthuma.*
Locality	Culcutta	Lahore (Pakistan) and Mumbai.	Lalbag, Murshidabad.	Madhyammgram, North 24 Parganas.	Satkhira, Bangladesh.
Site of infestation	Seminal vesicle	Not stated.	Seminal vesicle	Seminal vesicle.	Seminal vesicles
Trophozoite shape	Rounded or oval, somewhat fusiform.	Variable, spherical ovoidal, ellipsoidal or dumb-bell shaped.	Cone shaped, pellicle is distinct but very delicate.	Bean shaped with broad anterior end and the posterior end is comparatively narrow.	Trophozoites are oval in shape, middle portion is slightly wide. Mucorn not visible.
Trophozoite size	100 µm in length.	Up to 200×50µm	62.4-108.1µm× 33.2-53.2 µm.	9.40-151.0 (119.0) µm× 53.0-81.0 (66.0) µm.	It measures 92.82-97.24 µm (95.21±1.98) × 50.83-57.46 µm (53.40±3.24)
Ectosarc	Smooth	Smooth.	Not stated.	Thin and smooth.	Ectosarc is thin.
Endosarc	Finley granular with scattered granules	Vacular.	Endoplasm is excavated by vacuoles	With evenly distributed vacuoles	Endosarc is more granulated.
Nucleus shape	(Shape not stated) with small ecentric karysome.	Spherical or ovoidal with eccentrically placed karyosome.	Spherical.	Almost rounded.	Nucleus is large and also oval in shape. It is placed at the anterior part of the trophozoite. There is no chromatin bodies in the nucleus.
Size of nucleus	Not stated.	Not stated.	8.3-12.5 µm	4.0-16.0 µm.	It measures 13.26-17.68 µm (16.02±1.91) in diameter.
Gametocyst shape	Spherical.	Spherical.	Ovoidal.	Ovoid.	Rounded. Large gamet bean shape and small one is ellipsoid.
Gametocyst size	84 µm in diameter.	80 µm in diameter.	71.0-83.2 µm× 45.0-76.1 µm	85.0-102.0 µm.	It measures 132.6-141.44 µm (136.83±4.04) × 97.24-101.66 µm (99.26±1.98).
Oocyst shape	Not stated.	Not stated.	Biconical.	Navicular.	Navicular with pointed ends.
Oocyst size	Not stated.	Not stated.	6.4-6.6 µm×3.1-3.33 µm.	6.5-11.0 (9.0) µm× 4.0-7.5 (5.5) µm.	It measures 8.84-15.47 µm (11.78±2.17) × 4.42-8.84 µm (6.81±1.75).

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Molecular characterization and phylogenetic analysis of mosquito-pathogenic *Bacillus cereus* (HM026606)

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Abstract : Out of 1012 *Aedes albopictus* larvae collected from different breeding habitats of Hooghly district, west Bengal, 205 were found to be unhealthy and 2.5% of unhealthy larvae were naturally infected by *Bacillus cereus. Bacteria* formed white, circular, and flat colonies. Bacteria were gram positive, ellipsoidal/oval spore forming aerobic rods. Although the isolate was positive for catalase, urease, gelatinase, lipase and H_2S production, but negative for indole production, nitrate reduction, Vogues-Proskauer test, oxidase test and acid/ gas production from carbon sources. The organisms were sensitive to recommended doses of kanamycin, gatifloxacin,gentamycin, levofloxacin, doxycyclin, nalidixic acid, streptomycin, rifampicin, vancomycin, ofloxacin, but found resistant to ampicillin. The phylogenetic tree showed that the strain Ts-118 branched with *Bacillus cereus* strain XW2b and *Bacillus cereus* strain 14893 with 97% and 100% bootstrap support, respectively. In the laboratory, *B. cereus* suspension (8.5×10^7 bacteria/ml) resulted 83.46% death of *Ae. albopictus* larvae after 6 hours.

Keywords **:** *Bacillus cereus, mosquito-pathogen, 16S rRNA gene sequence, phylogenetic tree*

Introduction

There are more than 2500 species of Culicinae of which the main genera are *Aedes* with more than 900 species (Kettle, 1984). *Aedes* is the best known vector of yellow fever and dengue fever. Some *Aedes* species may act as vectors of some filariasis and viral disease (WHO, 1997). Dengue fever (DF)/dengue haemorrhagic fever (DHF) outbreaks have been reported in various parts of India during the past four decades (Yadava and Narasimha, 1992). An estimated 100 million cases of dengue fever, 500,000 cases of dengue haemorrhagic fever and 25,000 deaths have been reported annually (Gubler *et al*., 1998). Although *Aedes aegypti* is the principal vector for dengue transmission (Yadava and Narasimha, 1992; Das *et al*, 2000**)**, *Aedes albopictus* has been known to be present in some of the peri-urban and rural areas **(**Das *et al*, 2000**)** and also considered as a potential vector of dengue and chikungunya along with *Ae aegypti* (Singh and Pauri, 1967). Adult *Aedes aegypti* and *Aedes albopictus* may be differentiated by the patterns of white scales on the dorsal side of the thorax. For *Aedes aegypti*, the scale pattern consists of two straight lines surrounded by curved lyre-shaped lines on the side (Harwood and James, 1979; WHO, 1995).On the other hand, *Aedes albopictus* has only a single broad line of white scales situated in the middle of the thorax (WHO, 1995). In *Aedes aegypti* larvae, the comb teeth have well developed lateral denticles but the pecten teeth have less defined denticles. Whereas in *Aedes albopictus* larvae, the comb teeth have no lateral denticles but the pecten teeth have three well defined pointed denticles (Cheong, 1986; WHO, 1995). *Aedes* density is found significantly higher in rainy season than the other two seasons due to the availability of preferable breeding sites like tree holes, bamboo pots and other rejected containers (Rudra and Mukhopadhyay, 2010).The bacterial strains of *Bacillus* sp can survive under adverse conditions due to their resistance of their endospores to air drying and to other stresses. The primary habitat of majority of *Bacillus* species is the soil (Winogardsky, 1949). These organisms are now regarded as r-strategists (Campbell, 1989) and become metabolically active in the suitable substrates. *Bacillus cereus* is widely distributed in nature, usually found in soil, milk, cereals and other dried food stuffs etc. The bacteria in insects have been studied as pathogens of useful insects as well as the pathogens of insects pests and vectors or as insect- borne pathogens in humans and other animals. The interest for using bacteria as mosquito control agents was favoured by the increasing number of mosquito species resistant to chemical insecticides. For this purpose particular attention was given to the spore-forming bacteria of the genus *Bacillus*. A great number of bacteria isolated from insects can be regarded as facultative pathogens. The entomopathogenic bacteria

Bacillus thuringiensis ver. israelensis (Bti) has high mosquito larvaecidal activity. At sporulation these bacteria can produce proteinaceous prasporal crystalline proteins lethal to mosquito larvae upon ingestion (Gill *et al.,* 1992). Although *Bacillus sphericus* spore/crystal toxins have been proved to be powerful agent for controlling mosquito vectors (Poopathi *et al.,* 1999; Chatterjee *et al*, 2008), some studies have shown the resistance and cross-resistance against *Bacillus sphericus* and Bti in mosquito vectors (Poopathi *et al.,* 1999, 2000, 2010; Wirth *et al.,* 2000, Chatterjee *et al.,* 2008). It is a hard job to find out a new potential mosquito pathogenic strain which would be effective against mosquito larvae both in the laboratory and field conditions. Present study is an attempt to observe the role of *Bacillus cereus* as a facultative pathogen of *Ae. albopictus* larvae.

Materials and methods

Bacteria isolation and characterization :

The larvae of *Ae.albopictus* were collected from water-filled containers, cemented tanks and Tree-holes of Tarakeswar of Hooghly district, West Bengal and brought to the Parasitology and Microbiology Laboratory, Department of Zoology, The University of Burdwan, Burdwan. Moribund, paralyzed irritable/sluggish, fragile, putrefied, blackish and fluid oozing larvae were recorded as the unhealthy insects (Poinar and Thomas, 1984). The moribund larvae were surface-sterilized by 70% ethanol (5 min) and washed three times with sterile distilled water. The gut was dissected out under the laminar air flow, the content was aspirated with a sterile syringe, diluted up to 10^{-2} level with sterile distilled water and pour-plated on 5 plates at 100 µl/100 ml with nutrient agar (g/l: peptone 5, beef extract 3, agar 3, pH 7) media (Poinar and Thomas, 1984; Lacey, 1997). The predominant bacterial colonies obtained from then media were isolated, purified and characterized following standard methods (Pelczar, 1957; Sneath, 1986; Lacey, 1997). Antibiotic sensitivity test was done with standard antibiotics discs following Brown (2007). The bacterial isolates were identified both phenotypically (Sneath, 1986) and on the basis of 16S rRNA gene sequence analysis. Genomic DNA was isolated from the pure culture, about 15 kbp rDNA fragment was amplified by PCR, product was sequenced and the phylogenetic tree was prepared through the NJ method (Saitou and Nei 1987).

Toxicity test : For the determination of the toxicity of *B. cereus* against *Ae.albopictus* larvae, the bioassay tests were carried out at 35 ± 2°C using 100 larvae (late third instar) kept in 1000 ml water in glass bowls. The larvae were exposed to the dose of 5 ml of *B. cereus* suspension (8.5 x 10^7bacteria/ml) per liter of water. Each test was replicated three times along with a control and the mortality (%) was determined with the following Abbott's formula :

$$\text{Mortality (\%)} = \frac{\text{(\% mortality in the experiment)} - \text{(\% mortality in control)}}{100 - \text{(\% mortality in control)}} \times 100$$

Results and discussion

The bacterium TS118 formed circular, white, flat colonies (Table 1). Bacteria were Gram positive, spore forming aerobic rods and did not produce acid and gas from carbon sources (Table 1). The organism was positive for catalase, lipase, urease, protease and H_2S production but negative for indole production, nitrate reduction, oxidase test and Vogues-Proskauer test (Table1). The nucleotide composition is shown in Fig1. AT and GC content were 46.78% and 53.22% respectively. Fingerprint of nucleotides of *Bacillus cereus* Ts118 is shown in Fig 2.

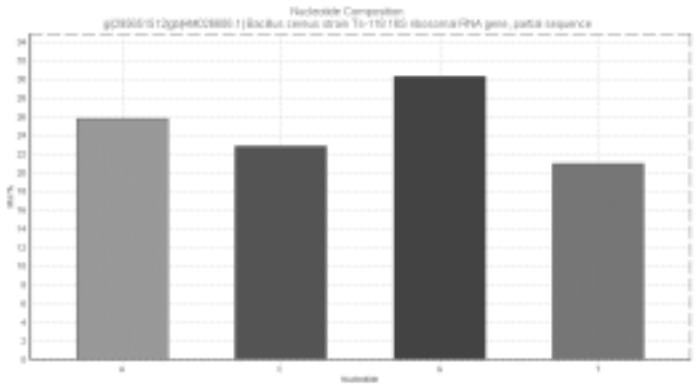

Fig. 1. Nucleotide composition of *Bacillus cereus* (Ts-118)

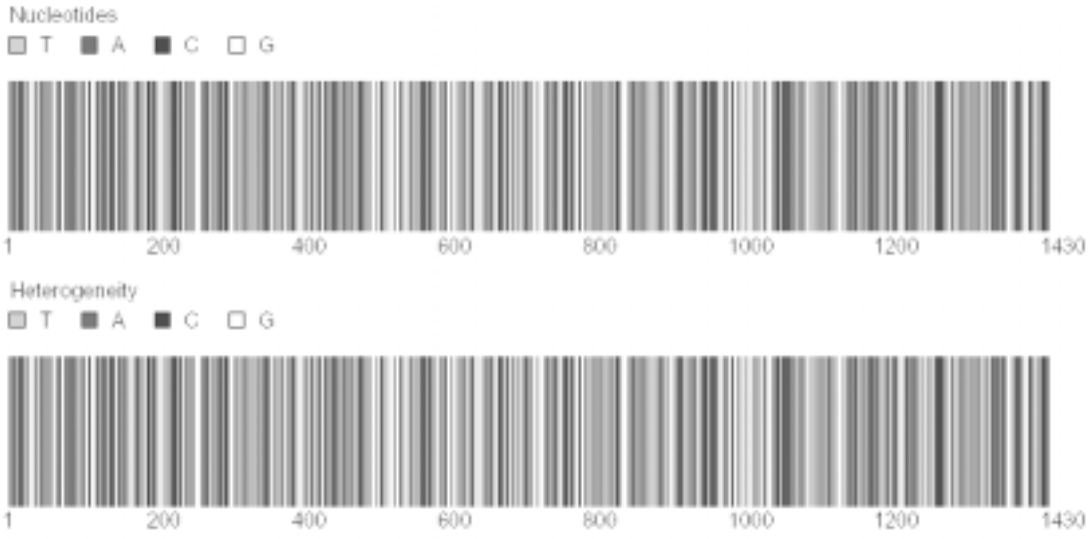

Fig. 2. Fingerprint of nucleotides of *Bacillus cereus* Ts-118

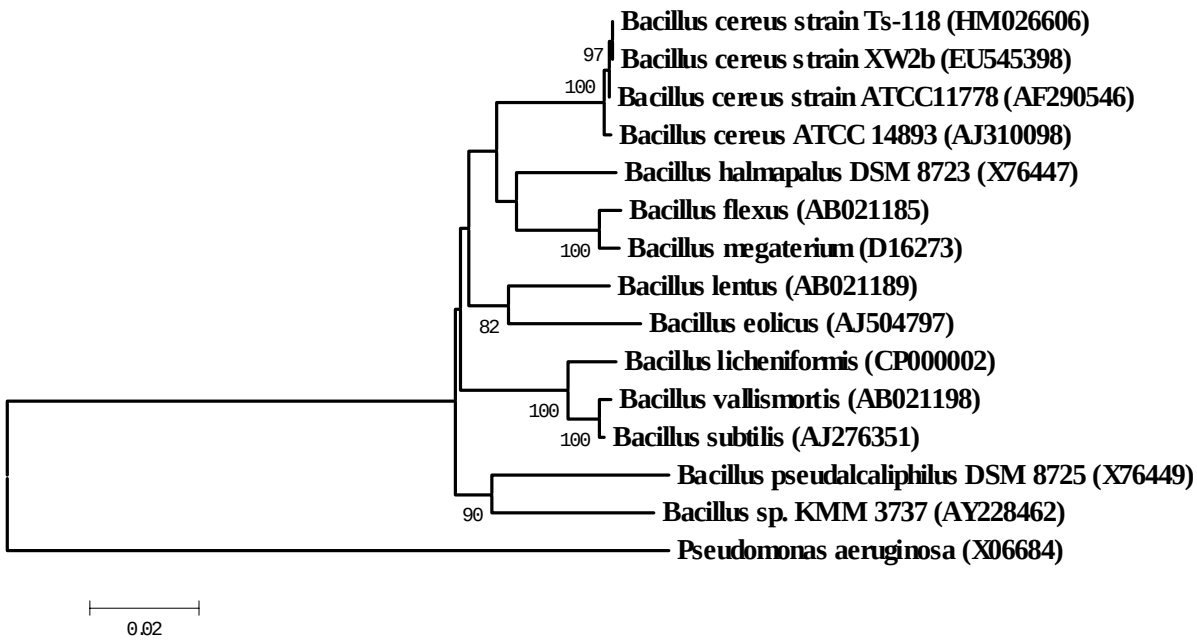

Fig. 3. Neighbor-joining tree constructed based on partial 16S rRNA genes sequences of *Bacillus cereus* strain Ts-118 along with few other16S rRNA genes retrieved from NCBI and RDP database. Numbers at nodes indicate percent bootstrap values above 80 supported by 1000 replicates. Bar indicates Jukes-Cantor evolutionary distance.

Phylogenetic affiliation of the bacterium under study (*Bacillus cereus* Ts-118) was done by 16S rRNA gene sequence analysis. BLASTN from NCBI and RDP classifier from RDP databases was done to investigate the most similar sequences. To assign the taxonomical affiliation of this bacterium, the phylogenetic trees (Fig 3) were constructed based on partial sequences of 16S rRNA genes of (*Bacillus cereus strain* Ts-118) along with other 16S rRNA gene sequences of type strain of genus *Bacillus* retrieved from NCBI and RDP databases. Phylogenetic relationships were derived from a neighbor-joining analysis of 16S rRNA gene sequence of the isolate Ts-118 described species of the genus *Bacillus.* The phylogenetic tree showed that the strain Ts-118 branched with *Bacillus cereus* strain XW2b and *Bacillus cereus* strain 14893 with 97% and 100% bootstrap support, respectively. The branch closest to this cluster contains four strains of *Bacillus cereus* (EU545398, HM026606, AJ310098, and AF290546) with 100% bootstrap support. Altogether, 1012 *Ae.albopictus* larvae were collected from different breeding habitats of Tarakeswar of Hooghly district, West Bengal, India out of which, 205 were found to be unhealthy and 2.5% of unhealthy larvae were naturally infected by *B. cereus.* The bacterial infection to different larval instars

Table 1. Phenotypic characterization of the *Bacillus cereus* (Ts118)

Character	Observation	Character	Observation
Colony character	Circular, white,	Urease production	+
	Flat, Entire	test	
Bacterium	Rods,Gram(+)ve	Oxidase	-
(l x w, µm)	(2.1-4.2 x 1)	H_2S Production test	+
		Citrate Test	+
Spore (Dia., µm)	Round, size	Gelatinase	+
	(0.95-1.15)	Casein hydrolysis	+
NaCl tolerance (%)	7	Amylase	+
Acid and gas	Negative	Lipase	+
production		Nitrate reduction	-
Catalase	+	test	
Indole production	-	Antibiotic sensitive	
Methyl red test	-	(µg/ml)	
Vogues-Proskauer Testt	-	Kanamycin (30)	
Test		Levofloxacin(5)	
Antibiotic resistant		Rifampicin(5)	
(µg/ml)		Vancomycin(30)	
Ampicillin(10)		Gatifloxacin (10)	
		Streptomycin(10)	
		Gentamycin (10)	
		Nalidixicacid(30)	
		Ofloxacin(5)	
		Doxycycline(30)	

Table 2. Population of *Ae.albopictus* larvae infected by *Bacillus cereus* at breeding habitats

Breeding habitat	Larval instar	Collected larvae	Number of unhealthy larva	*Bacillus cereus* infection (%)	Unidentified bacteria (%)
Water-filled containers, cemented tanks, tree-holes	3rd	540	131	2.46	0.54
	4th	472	74	2.53	0.67
Total/average*		1012	205	*2.49	*0.605

of *Ae.albopictus* is shown in Table 2. Infection caused by *B.* cereus (Ts-118) suggests that they would be the pathogens of *Ae. Albopictus.* In the laboratory condition, *B. cereus* (Ts-118) suspension resulted to 44.85 % and 83.46% death of *Ae.albopictus* larva after the exposure of 3 and 6 h, respectively. No larval mortality was observed in control experiment during the 6 h. *B. cereus* is able to colonize in the guts of the mosquito larvae (Plearnpis *et al*., 2001) and considered as natural pathogen of mosquito larva (Krattiger, 1997; Cooping and Menn, 2001; Wirth *et al.*, 2004; Teng *et al*., 2005, Chatterjee *et al*.,

2010). Insecticidal activity of spores of *B. cereus* against *Aedes aegypti* has been documented (Dana *et al*., 1981).Present study reveals that *B. cereus* (TS-118) is a natural facultative pathogen of *Ae.albopictus* larvae, and may be exploited in vector-control programme.

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Monoclonal antibody based latex agglutination test for the diagnosis of trypanosomiosis in cattle

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Abstract : Trypanosomosis which is an arthropod borne disease have become menace to the Indian farmers because of its significant impact on the productive status of the animals. Moreover the zoonotic effect of this disease has also been proved now. Research on newer techniques for the diagnosis of this important disease has been carried out for the past hundred years and still this search is going on for finding a more sensitive and specific test. The parasitological examination which is used for the diagnosis at the field level misses about 80% of positive cases. Keeping in view the shortcomings of the conventional diagnostic methods we carried out the present investigation for the detection of active infection of Trypanosoma evansi by monoclonal antibody based latex agglutination test (MAb-LAT). About 88 blood samples collected from cattle of karnal district of Haryana were screened initially by Wet Blood Film (WBF) immediately after collection and their corresponding serum samples were subjected to latex agglutination test. WBF could detect the presence of motile trypanosomes only in three samples (3.41%) where as MAb-LAT detected 53 samples (60.23%) positive for the circulating antigens of Trypanosoma evansi. Study found that MAb-LAT is much sensitive than the conventional parasitological examination. Moreover MAb-LAT is simple to perform**,** rapid, and cost-effective and can be used in field-level.

Keywords **:** *Diagnosis, MAb-LAT, Trypanosoma evansi, WBF*

Introduction

Trypanosoma evansi, a blood protozoan parasite, causes a serious disease known as 'surra' in domestic and wild animals. It is a mechanically transmitted arthropod borne disease and *Tabanus* spp. has been implicated as the main vector. It is the most widely geographically distributed pathogenic trypanosome in Africa, South and Central America and Asia (Luckins, 1998; Pathak and Khanna, 1995). In India, *T. evansi* infection is widely prevalent in different parts and is of significant economic importance in livestock production (Juyal *et al.*, 2007). Surra may occur in acute, sub-acute, chronic and inapparent forms. Acute and sub-acute forms of the disease are usually fatal. In buffaloes, cattle and camels, the disease is usually chronic, though acute cases have also been reported. Recently a case of surra infection in a man has been reported in India (Joshi *et al.*, 2005; Powar *et al.*, 2006). This report assumes significance, for it indicates possible zoonotic threat in future (Laha and Sasmal, 2007).

Though trypanosomes have been studied over the past 130 years, their definite diagnosis still suffers from low sensitivity and specificity. Trypanosomosis is responsible for fluctuating nature of parasitaemia, which is often difficult to be detected by the commonly used parasitological methods. Though, animal inoculation methods are more sensitive for diagnosis of surra, yet they are laborious, time consuming and unsuitable for large scale use in the field. Further, SPCA (Society for Prevention of Cruelty to Animals) does not permit the use of experimental animals, if alternatives are available.

The limitations in terms of low sensitivity of parasitological diagnostic techniques had been a driving force for research into alternate techniques such as serological and DNA based methods, which have got a great potential for unequivocal identification of the causative agent with higher sensitivity. Serological diagnosis using antibody detection is hampered by its inability to distinguish between current and past infections because of persistent titres and occurrence of false positive results. The identification of circulating variable antigen types (VAT) would be a great value in developing more sensitive

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diagnostic tests. Accurate diagnosis of 'surra' is extremely important to identify animals for treatment, to track the prevalence of the disease and to avoid misuse of the trypanocidal drugs. So development of cost-effective and field-oriented diagnostic test is required for large scale screening of animals for effective control of disease. The present study reports the use of monoclonal antibody based latex agglutination test for detection of circulating antigen of *T. evansi* in the serum of infected cattle.

Materials and methods

Collection of blood and serum samples: A total of 88 blood samples from naturally infected, suspected and healthy cattle from different places of Haryana state were collected during September, 2008 – January, 2009 in separate vials with and without anticoagulant. The blood samples with anticoagulant (heparin, 10 units/ml of blood) were used for detecting *T. evansi* in wet blood film (WBF) within three hours of collection. The blood samples without anticoagulant were collected to separate serum for use in latex agglutination test. The blood and serum samples were stored in sterilized vials at –70ºC until further use.

WBF : A drop of blood from each sample was used for preparation of WBF in triplicate on a clean glass slide and examined for *T. evansi* at a magnification of 100x and 400x according to the method of Killick-Kendrik (1968).

Monoclonal antibody based latex agglutination test (MAb-LAT): For detection of *T. evansi* antigen in cattle sera samples by MAb-LAT, latex reagent which is a suspension of latex microbeads, 0.8 µm in diameter, coated with anti-*T. evansi* murine monoclonal antibody was used. MAb-LAT was performed according to the method of Rayulu *et al.* (2007).

Twenty microlitres of the latex reagent were taken in the cavity of the slide (Himedia, India) and an equal volume of cattle serum was added to it. The reagent and the serum sample were mixed by gentle swirling motion of the slide for five to ten minutes. In case the clumps or granular aggregates formed within five minutes, the sample was scored as strong positive and within ten minutes, the sample was scored as weak positive. The five and ten min. reaction criteria had been established earlier in a larger study. Controls including known positive and negative rat serum samples were also used in parallel.

Results and Discussion

Parasitological examination by WBF: Examination of blood samples collected from cattle by WBF revealed the presence of *T. evansi* parasite in three samples (3.41%). One obvious reason for low number of positive samples by WBF is the inherent low sensitivity of the test. Similar observations have been made by numerous workers during the past two decades in India (Swarnkar *et al*., 1993; Pathak *et al*., 1993; Singh *et al*., 1995; Rayulu *et al.*, 2007) and in other countries (Masake and Nantulya, 1991; Olaho-Mukani *et al*., 1993; Davison *et al*., 2000; Ngaira *et al*., 2003). Another reason for this low number of cases could be probably due to the treatment of animals for trypanosomosis on symptomatological basis-quite common practice in the field in India, including the state of Haryana. It was difficult to extract history of treatment of the substantial number of cases, if not all from which the samples had been taken.

MAb-LAT of the sera samples: Out of 88 cattle sera samples examined, 53 samples (60.23%) showed positive for the circulating antigens of Trypanosoma evansi. by MAb-LAT. Similar observations have been reported by Nantulya (1994) using monoclonal antibody-based latex agglutination test (Suratex®), detected the antigens in 53 (88.3%) of 60 blood samples collected from experimentally infected rabbits in comparison to 22 (36.7%) and 2 (2.3%) by buffy coat and WBF, respectively. Olaho-Mukani *et al*. (1996) screened 549 camels by Suratex® and found *T. evansi* antigens in 254 (46.3%) camels. Rayulu *et al*. (2007) using latex agglutination test (LAT) declared an overall of 42.59% positive out of 1538 field samples. Overall, both MAb-LAT reagents (Nantulya's and Rayulu's) could detect far more samples positive than those detected by WBF, indicating thereby higher sensitivity of the LAT than that of WBF.

An inherent limitation of Ag-detecting LAT or any other Ag-detecting serological test is high probability of declaring the recently-treated animals as positive, since the antigens released from the killed parasites remains in blood circulation upto nearly four weeks after treatment, as observed previously in other studies (Olaho-Mukani *et al*., 1996; Thammasart *et al*., 2001; Wernery *et al*., 2001; Singh and Chaudhri, 2002). Therefore, this necessitates getting reliable history of the animal that receives anti-trypanosome treatment during past few weeks before sample collection to make LAT more dependable. In the present study we could get the history of treatment of cattle from few cases but not from all.

MAb-LAT detected far more samples positive for *T.evansi* than WBF. Most of field samples were, in fact, collected from the area where the vector density is high, so the number of animals infected in the region should be certainly higher than that those detected by parasitological examination. Moreover, the test is simple to perform neither requiring multiple and

complex procedural steps, nor the use of sophisticated equipment for reading the results. MAb-LAT was found to be a rapid, convenient, cost-effective and field adaptable test. The merits of MAb-LAT make the test suitable as a field-level test for screening of *T. evansi* infected cattle, thereby helping in effective control of the disease.

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Polymerase chain reaction based detection of *Trypanosoma evansi* in whole blood of domestic animals

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Abstract : *Trypanosoma evansi*, a blood protozoan parasite causing 'surra' in domestic animals is widely prevalent in different parts and is of significant economic importance in livestock production. Parasitaemia is often intermittent and is not always possible to demonstrate the parasites in blood and hence diagnosis of trypanosomosis is often difficult. The present investigation has been carried out with the aim of detecting *T. evansi* in cattle, buffaloes and equines in the state of Haryana by parasitological (WBF), and DNA-detecting (TE-PCR) tests. Out of 205 field blood samples, only 2% were positive for *T. evansi* by WBF while PCR using synthetic oligonulceotide primers (21 mer sense and 22 mer antisense) targeted to a repetitive nuclear DNA sequence of *T. evansi* detected 60.49% samples positive. Study revealed TE-PCR to be highly sensitive to the conventional parasitological method and could be used for early of diagnosis of trypanosomosis in domestic animals.

Keywords **:** *Diagnosis, TE-PCR, Trypanosoma evansi, WBF*

Introduction

Animal husbandry sector plays a pivotal role in the Indian economy and socio-economic development of the country. Though India owns the largest livestock population in the world, recovery of produce from this sector is lower than its potential. Loss of production on account of animal diseases alone amounts to more than billions per year. The long term debilitating effects of parasitic infestations in livestock assume greater importance in terms of production losses (Rao, 2006). This losses can be reduced by early diagnosis of the diseases to certain extend.

Trypanosoma evansi, a unicellular haemoflagellate causes surra in domestic animals which has the economic significance due to its high morbidity, mortality and chronic debilitating effects. Though this disease has been studied for past many decades, the definite diagnosis is still in menace. The parasitological examinations frequently fail to detect patent infections because parasitaemia is scanty in peripheral blood in the chronic forms (Killick-Kendrick, 1968). Serological diagnosis using antibody detection is hampered by its inability to distinguish between current and past infections because of persistent titres and occurrence of false positive results. So we relied on PCR for diagnosis of trypanosomosis which was found to be highly sensitive and specific in the present study.

Materials and method

Collection of blood : Two hundred and five blood samples from naturally infected, suspected and healthy cattle (n=88), buffaloes (n=46), and equines (n=71) from different places of Haryana state were collected in vials with anticoagulant (heparin, 10 units/ml of blood). The blood samples were subjected to Wet Blood Film (WBF) for detecting *T. evansi*. The blood sample positive for *T. evansi* parasites by WBF was inoculated in 0.2 ml volume into one rat by intraperitoneal (i.p) route for bulk harvests of parasites for extraction of DNA.

Genomic DNA extraction : Genomic DNA was extracted from field blood samples using phenol: chloroform: isoamyl alcohol (25:24:1) according to the method modified from Sambrook and Russell (2001). A set of primers specific to *T. evansi* repetitive DNA sequence probe pMUTec 6.258 as described by Wuyts *et al.* (1994) were used for amplification by TE-PCR.

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Forward primer (21 mer)

5'-TGCAGACGACCTGACGCTACT-3'

Reverse primer (22 mer)

5'-CTCCTAGAAGCTTCGGTGTCCT-3'

Polymerase chain reaction : TE-PCR was carried out in 50µl reaction mixtures containing 10x PCR buffer with KCl, 1.5mM $MgCl_2$, 200µM each dNTP, primers each at 200 pM and 2U of thermostable Taq DNA polymerase. The cycles included an initial step at 95ºC for 4 minutes followed by 29 cycles of denaturing at 95ºC for 1 minute, primer annealing at 60ºC for 1 minute and primer extension at 72ºC for 1 minute. This is followed by last cycle of denaturing at 95ºC for 1 minute, annealing at 60ºC for 1 minute and. extension at 72ºC for 10 minutes and hold at 4ºc for indefinite time. Amplification products were resolved in 2% agarose stained with ethidium bromide (5mg/ml) and visualized by UV transillumination and photographs were taken.

Results and discussion

Out of 205 blood samples examined by WBF, only four were positive for *Trypanosoma evansi* i.e., nearly 2% samples. The low number of positive samples by WBF can be due to the inherent low sensitivity of the test. Similar observations have been made by numerous workers during the past two decades in India (Pathak *et al*., 1993; Singh *et al*., 2004). Wet film examination for the parasite in the infected animals is often the only test used in the field even today, but it is probably the least sensitive test missing 50-80%. Another reason why WBF figures could be low might be probably due to the treatment of animals for trypanosomosis on symptomatological basis which is a quite common practice in the field in India, including the state of Haryana. It was difficult to extract history of treatment of the substantial number of cases, if not all from which the samples had been taken.

TE-PCR reaction was initially optimized using genomic DNA extracted from *T. evansi* infected rat blood which could obtain specific band of 227 bp and further the test was applied on field blood samples (Fig.I). Out of two hundred and five samples, 124 (60.49%) were positive by TE-PCR which included 58 cattle (65.91%), 35 buffaloes (76.09%), and 31 equines (43.66%) (Table I). However, most of these samples showed weak band of 227 bp by TE-PCR which indicated that most samples either contained low number of parasites or there were some inhibitors coming in the DNA during its extraction from blood samples. This could be the probable reason for the two WBF-positive samples showing weak positive by TE-PCR. In the present study, TE-PCR positive signal was obtained with template DNA content of 12 trypanosomes extracted from whole blood sample. The limit of detection was higher than that obtained in other studies. Wuyts *et al*. (1994) could achieve limit of detecting 0.5 pg of parasite DNA or one parasite in 10ml of blood sample. Omanwar *et al*. (1999a), however, using the same primers could detect five organisms in 10 µl crude blood samples. PCR could detect much larger number of positive samples than WBF. Several

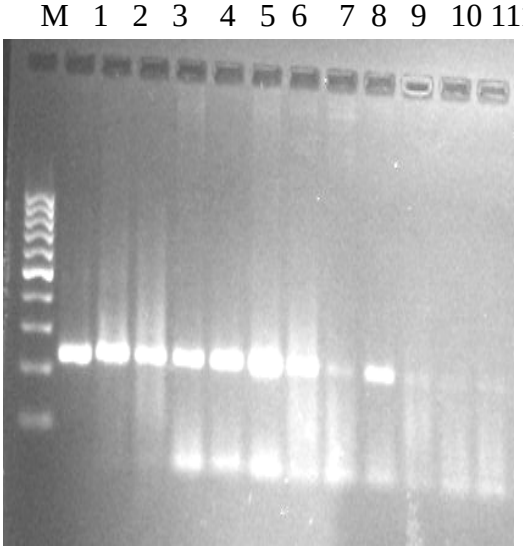

Fig. I. *T. evansi* - specific PCR using DNA extracted from field blood samples
[Lane M: 100 bp DNA ladder; Lane 1: positive control, Lane 2 - Lane 11 field samples]

Table I : Detection of *Trypanosoma evansi* in domestic animals by TE-PCR

Animal species	Number of blood samples examined	TE-PCR					
		Number Positive			Percent Positive		
		Strong	Weak	Total	Strong	Weak	Total
Cattle	88	5	53	58	5.68	60.23	65.91
Buffalo	46	5	30	35	10.87	65.22	76.09
Equine	71	0	31	31	-	43.66	43.66
Total	**205**	**10**	**114**	**124**	**4.88**	**55.61**	**60.49**

TE-PCR : *T. evansi* specific Polymerase Chain Reaction.

investigators have reached similar conclusions using PCR, Mugittu *et al*. (2001) detected trypanosome DNA in 27 (43.55%) out of the 62 parasitologically-negative samples. In another study, Omanwar *et al*. (1999b) using the Wuyts' primers in PCR detected 3 (15%) out of 20 parasitologically-negative blood samples from camels in Rajasthan.

It was concluded that TE-PCR was sensitive and could be used for the large scale screening of domestic animals for trypanosomosis. Moreover TE-PCR has an advantage of detecting the active infection because the test depends on the presence of parasitic DNA.

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Anticestodal effect of medicinal plants from West Bengal

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Abstract : An ethanolic crude extract from the leaves of *Cassia angustifolia* Vahl., *C. alata* Linn., and *C. occidentalis* Linn., (Caesalpinaceae), were tested in vitro on the domestic fowl gastro-intestinal cestode parasite, *Raillietina tetragona* to determine their anthelmintic efficacy. The parasites were exposed to varying concentrations, (viz. 5, 10, 20, 40 and 80 mg/ml) of the three crude leaf extracts and compared with praziquantel (0.0005, 0.001, 0.025 and 0.05 mg/ml) a reference cestocidal drug so as to assess the motility and mortality of the worms. Dose dependent efficacy of plant extracts were clearly observed in all experiments. All the concentrations were made in normal Phosphate buffer saline (pH 7.4) with 1% DMSO and one set of experiment containing only PBS and 1% DMSO was used as a control medium. Survivability of the parasites in the controlled medium was 81.93 ± 4.71 hours, whereas in the crude extract of *C. angustifolia* and *C. alata* at a concentration of 80 and 40 mg/ml respectively took very less time (1.67 ± 0.08 h and 1.68 ± 0.11 h respectively) for paralysis which is comparable with that of praziquantel (1.27 ± 0.13 h) at 0.001 mg/ml. concentration. *C. occidentalis* showed longer time for paralysis as compared to the other two plant extracts at 80 mg/ml concentrations (3.57 ± 0.06 h). Histological studies also showed topographical changes in the tegument of the treated parasites with all plant extracts and praziquantel as compared to control. These finding may indicate that the crude plant extracts used may have vermifugal activity on the parasites and can be an important inexpensive clinical agent in eliminating the cestode parasite from the host. However, further studies are required to determine its value as a potential anticestodal agent.

Key words **:** *Raillietina tetragona, anthelmintic, medicinal plant, cestocidal.*

Introduction

Herbal medicines play a very important role in healthcare of large population, particularly in developing countries, where they often bridge the gap between the availability, and demand for modern medicines (Akerele, 1988) and are both sustainable and environmentally acceptable. *Cassia* plant, widely distributed shrubs found in the tropical and subtropical regions including India, commonly known as Senna is cultivated throughout India and in south West-Bengal. There are three species viz. *C. angustifolia* Vahl, *C. alata* Linn, and *C. occidentalis* Linn of the family Caesalpinaceae which are widely used in traditional folk medicines as purgative, for regularization of bowel movement, and in skin diseases etc (Paria, 2005), fungicides (Ibrahim and Osman, 1995). Hydroxyanthracene derivatives were demonstrated as the active constituents of *Cassia alata* (Elujoba *et al*. 1989). While their properties as laxative and anti bacterial has been widely explored, (Peter *et al.* 2006; Makinde *et al.* 2007; Agarwal *et al.* 2010) the locals, also use aqueous concoction of the leaves of Senna as anthelmintic, however, its vermicidal and/or vermifugal properties have not been studied extensively and therefore detail investigation is awaited. Thus the present study aimed at exploring the antihelminthic activity of *Cassia* species and to further validate if the plant-derived components caused any alterations in the tegumental architecture of the parasite comparing them with a reference drug praziquantel on a parasitic model *Raillietina tetragona* (fowl tapeworm).

Materials and Methods

Preparation of crude plant extract

Fresh young leaves of the 3 plants (*C. angustifolia* vahl, *C. alata* linn, *C. occidentalis* linn) were collected from the nearby places in and around the university campus. Leaves were weighed and washed with deionized water, oven-dried at 50^{o}C and crushed into powdered form, soaked in Ethanol (90%) for 5-7 days and filtered through muslin cloth. Filtrate was evaporated in hot air oven at 55-60^{o}C and the crude extracts procured from each plant used in the experiment were weighed and stored at 4^{o}C till further used.

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Chemicals and drugs

All the chemicals used were standard analytical grades, obtained from Merck, USA. Ethanol was supplied by Bengal Chemicals, Kolkata, India, and the reference drug praziquantel with trade name Distocide (composed of 600 mg praziquantel) is a product of Chandrabhagat Pharma Pvt. Ltd., Mumbai, India.

Collection of worms and in vitro treatments of parasites

The parasites were collected from the intestine of freshly slaughtered domestic fowl and the live worms were treated *in vitro* with various concentrations, (10, 20, 40, 80 mg/ml, in 0.9% phosphate buffer saline, PBS), of ethanolic plant extract and 0.0005, 0.001, 0.0025, 0.005 mg/ml praziquantel (PZQ, the broad spectrum reference drug) along with maintenance of controls in PBS with 1% dimethylsulfoxide (DMSO) at 37 ± 1^0C.

Motility, mortality, histological studies

Paralysis and death were the parameters taken for observation for the treated parasites. Paralysis was observed to ensue within a time span that was comparable with the paralysis timing of worms treated with PZQ. Thus this concentration was selected for further histological studies where the mature proglottids of the control and the treated parasites were fixed in Bouin's fluid and processed through double staining technique using eosin and haematoxylin for histological studies (Bancroft and Stevans, 1977).

Statistical analysis

Data are presented as the mean ± standard error of mean for each group (n=6). For determining the statistical significance, standard error of mean and analysis of variance (ANOVA) at 5% level significance was employed; $p<0.001$ were considered significant.

Results

The time recorded for paralysis and death are presented in Table. It was observed that each plant extract showed a dose dependence efficacy. Paralysis at 40mg/ml concentration of plant extracts took lesser time (1.68±0.11 hrs) with *C. alata* and more time (4.13±0.13 hrs) with *C. occidentalis* while it took 2.95±0.12 hrs for *C. angustifolia*. Similarly the post paralytic time (death) showed the same pattern where *C. alata* took lesser time (8.55±0.13 hrs) and *C. occidentalis* a longer time (22.39±0.49 hrs). With other low concentrations (5, 10 and 20 mg/ml) the paralytic and death time showed the same pattern where *C. alata* showed more effect followed by *C. angustifolia.* The paralytic time of parasites treated with *Cassia alata* 40 mg/ml can be

Table : Effect of the three plant crude leaf extracts and praziquantel on *R. tetragona*.

Concentration	***Cassia angustifolia***		***Cassia alata***		***Cassia occidentalis***	
(mg/ml)	Paralysis	Death time	Paralysis	Death time	Paralysis	Death time
Plant extracts	time (hrs)	(hrs)	time (hrs)	(hrs)	time (hrs)	(hrs)
5	19.38±1.40	31.29±0.39	4.88±0.34	16.99±0.26	49.89±0.63	59.92±0.52
10	14.16±0.83	31.14±0.28	3.1±0.06	15.88±0.19	32.75±0.1	48.06±0.07
20	5.39±0.19	21.15±0.26	2.89±0.1	13.09±0.15	9.31±0.07	26.96±0.09
40	2.95±0.12	18.52±0.04	1.68±0.11	8.55±0.13	4.13±0.13	22.39±0.49
80	1.67±0.08	6.85±0.08	0.82±0.02	5.99±0.52	3.57±0.06	20.83±0.16
Praziquantel	Paralysis time (hrs)				Death time (hrs)	
0.0005	3.22±0.03				28.33±0.03	
0.0010	1.27±0.13				21.79±0.77	
0.0025	0.76±0.05				19.14±0.48	
0.0050	0.17±0.01				17.12±0.14	

Control parasite survivability time = (81.93 ± 4.71) hours.

Each values are represented as mean ± standard deviation (n= 6).

compared with that treated with praziquantel at 0.001mg/ml. The post paralytic time in plant extracts are comparatively shorter as compared to the drug. However, the control parasites showed survivability up to 81.93 ± 4.71 hours.

The control worms revealed typical cestode structure with respect to the tegument, its outer wall (OW) has a well organized basal lamina and subtegumental muscle layers (ML). (Fig 1a). In worms treated with crude plant extracts (Fig 1c-d) extensive alterations in the tegument were noticeable; infolding of the OW into the ML leading to disformity in the whole tegumental structure, and dislocation, segregation and degradation of the circular and longitudinal muscle layers. Severe infolding is found to occur in *C. angustifolia* (Fig 1c) amongst the plant extracts. All these observations were comparable with that treated with PZQ (Fig 1b) having similar morphological changes at the integument.

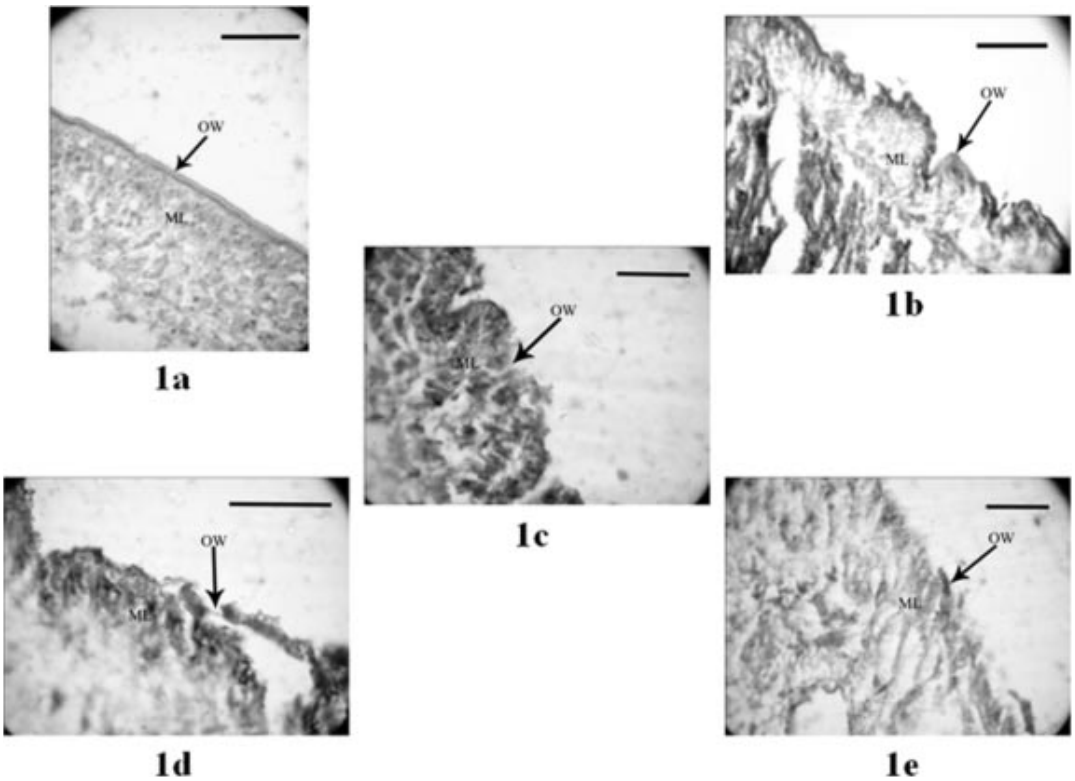

Fig.1. Light microscopic studies of proglottids of *R. tetragona*. Fig1a- Control showing well-defined tegument with no constriction in the brush border. Fig 1b-e; treated parasites with Praziquantel, *C. angustifolia, C. alata* and *C. occidentalis* respectively showed infolding regions and cracks in the tegument. *All bars* 50 µm.

Discussion

The primary evaluation on the early loss of spontaneous movement and complete immobilization of the treated worms compared to the control were observed by many workers on several ethnomedicinal plants that established their anthelmintic potency (Temjenmongla and Yadav 2005; Tangpu et al. 2006).

The present study showed a dose dependent efficacy of the crude plant extract, as an increase in the concentration of the test material caused more pronounced destructive effect on the tegumental surface of the parasites, as observed by Dasgupta *et al.* (2010). Also many chemically derived marketed drugs having broad spectrum showed dose dependent efficacy as well as tegumental alteration, enzymatic change (Markoski *et al.* 2006). Following *in vitro* incubation with various concentrations *Raillietina tetragona* showed severe tegumental damage. The plant extract caused swelling of the basal infolds and intense cracks of the tegument as observed herein, these swellings could have an osmotic basis, due to impairment of energy-dependent ion pumps (Anderson and Fairweather, 1995; Meaney *et al.* 2004). Further vacuolization of the tegument has been known to be induced by a triggering of calcium ion flux, for example like PZQ (Jiraungkoorskul *et al.* 2006) and metabolic disorder on the helminth parasite are known to enter the parasite tegument through simple diffusion and then cause

disruption of the tegumental and muscle layers (MacKinstry *et al*. 2003). The plant treated parasites also showed rupture of muscle layers and this may increase chloride ion conductance of worm muscle membrane and produces hyper polarization and reduced excitability that leads to muscle relaxation and flaccid paralysis (Martin 1985).

In the PZQ-treated parasites also, most of the damage induced was tegumental and the rapid paralysis observed thus induced Ca^{2+} influx (Redman *et al.* 1996) that account for both the body contraction seen in histological observation.

Permanent damages in the contour of the scolex and disorganization of tegumental appearance and dislocation of hooks in the scolex exhibited extensive distortion in the form of shrinkage observed in the present experiment may be attributed on the tegumental enzymes as they are the primary target to such action (Pal and Tandon, 1998).

Conclusion

Structural alterations and deformity in the tegumental interface of the parasite subsequent to the exposure to the test plant extract and the concoction of two plants indicate towards the vermicidal potential of the three *Cassia* plant. Further, biochemical and molecular analyses are required to form concrete inferences to the actual mode of action and to determine the specific active compound(s) responsible for such anthelmintic activity.

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Changes in haematological parameters in trypanosome infected *Clarias batrachus* (L.) in West Bengal

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Abstract : This study has been undertaken to investigate the haematological abnormalities of the fish due to trypanosome infection, which retard the growth of economically important geol fish *Clarias batrachus* (L.). A total of 128 fishes from several locations of ponds and water bodies of South 24 Parganas and North 24 Parganas, West Bendal, India were examined from January 2008 to April, 2010. The prevalence of trypanosome infection was 58.6%. The values of Haemoglobin, PCV, MCHC and TEC were declined in infected fishes and the values of MCV, MCH and TLC were increased in infected fishes in comparison to normal fish. Based on the average values, it was observed that the percentage of haemoglobin, percentage of PCV, percentage of MCHC and TEC were reduced by 37.81%, 15.08%, 20.06% and55.19% respectively. On the other hand, the values of MCV, MCH and TLC were increased by 67.47%, 59.51and 29.92% respectively. The shape of the RBC was decreased and most of the nuclei were fragmented into two or more equal or unequal parts. Monocyte, large lymphocyte and nutrophil were observed. They are vacuolated in infected fishes while the cell lining of eosinophil and basophil were irregular.

Keywords **:** *Trypanosomiasis, Clarias batrachus, haematology, West Bengal.*

Introduction

Live fishes are cultured in pollutant rich water. So they are prone to parasitic infection. Trypanosomiasis is dreadful to live fish. It is transmitted rapidly from fish to fish by intermediate host – Leech. So blood parameters can be served as indicators of fish health.

The study of the physiological and hematological characteristics of cultured fish species is an important tool for the development of aquaculture system, particularly in_regard to the use in detection of healthy fish from diseased or stressed animal (Rainza-paiva et al., 2000; O'Neal and Weirich, 2001). The lakes, rivers and seas have become illegally the end point of the discharge of pollutants (Elnwishy et al., 2007), the majority of fish diseases might be occurred as a result of parasitic infection or environmental pollution (Hussain et al., 2003). It is known that certain blood parameters serve as reliable indicators of fish health (Bond, 1979) Therefore, the changes associated with hematological parameters due to various parasite infections establish a database, which could be used in disease diagnosis and in guiding the implementation of treatment or preventive measures. The changes in the blood characteristics of *Clarias gariepinus* caused by stress due to exposure to environmental pollutions, diseases or attack by pathogens have been studies by a number of authors (Onusiriuka and Ufodike, 2000; Ezeri, 2001; Gabriel et al., 2001).

Trypanosoma are widely reported to dwell in the blood of fish in both freshwater and marine fishes (Becker, 1977; Joshi, 1982a, 1985, 1989; Khan, 1985; Gupta and Pilarczyk, 1994; Gupta et al., 2006; Burreson, 2007; Ruszczyk et al., 2008), but studies pertaining to concomitant hemoflagellates infection in fish are relatively scant (Mandal, 1979; Joshi, 1982b; Gupta and Gupta, 1990). The hemoflagellates are known to cause changes in the various hematological alteration are also relatively spasmodic (Tandon and Joshi, 1973; Sharma and Joshi, 1991; Gupta and Gupta, 1985; Akmirza and Tepecik, 2006). Hematological investigation in *Haeteropneustes fossilis* by Gupta et al., (2002) indicated that hemoglobin concentration (16.18%) and PCV (10.98%) decreases and WBC counts (19.03%) increases due to trypanosome infection.

The present study aims in establishing the changes in haematological parameters due to trypanosome infected *Clarias batrachus* under natural condition.

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Materials and Methods

The study has been done in examination of 128 fishes collected from ponds of several locations of South 24 Parganas (27^0 13' N - 89^0 53' E) and North 24 ($23^0$15' N - 89^0 5' E) Parganas, West Bengal. Thin hart blood smear was prepared and was allowed to air dry.

It was then fixed in absolute methanol. Slides were stained with May-Grunwald Giemsa Stain (pH 6.8), mounted in DPX and were examined under 1000 magnification of the phase contrast microscope. Blood parameters like- Hb, PCV, MCV, MCH, MCHC, TEC, TLC, Serum cholesterol and blood glucose were measured by standard protocol.

Results

A total of 128 fishes from several locations of ponds and water bodies of South 24 Parganas and North 24 Parganas, West Bendal, India were examined from January 2008 to April, 2010. It was found that only 75 out of 128, *C. batrachus* were infected. The prevalence of trypanosome infection was 58.6%.

The concentration of haemoglobin (Hb) in infected host fish ranged from 8.3 to 12.5 gm % in *C. batrachus.* The average value showed that this concentration was decreased in infected fish in comparison to normal by 37.81%.

Table I : Showing parameters to assess the haematological conditions of infected fish of *C. batrachus* with respect to normal fish. Average values were calculated and represented with standard error of mean (SE) from twenty samples (N = 20). Unlike superscripts denote significant mean difference at 5% level ($P < 0.05$).

Parameters	Normal fish		Infected fish	
	Range	Average	Range	Average
Hb (gm%)	8.3 – 12.5	10.5 ± 0.56	5.6 – 8.1	6.53 ± 0.35
PCV (gm%)	31.2 – 35.2	32.5 ± 1.53	22.4 – 30.1	27.6 ± 1.33
MCV (μm^3)	139.91 – 181.14	158.3 ± 8.07	225.5 – 772.41	486.7 ± 33.09
MCH (ñg)	48.26 – 56.05	51.17 ± 2.92	60.90 – 193.10	126.4 ± 6.95
MCHC (%)	0.309 – 0.345	0.319 ± 0.02	0.349 – 0.269	0.255 ± 0.01
TEC ($10^6/mm^3$)	1.72 – 2.23	1.83 ± 0.09	0.29 – 1.33	0.32 ± 0.02
TLC ($10^3/mm^3$)	7.92 – 12.86	10.33 ± 0.59	8.93 – 20.79	14.74 ± 0.77

The range of packed cell volume (PCV) of normal and infected fish was shown in table -I. The average value showed that this concentration was decreased in infected fish in comparison to normal by 15.08% (Table-1). The concentration of Mean Corpuscular Haemoglobin Concentration **(**MCHC) in infected fishes ranged from 0.349 – 0.269% in *C. batrachus* (table-1). The average value 20.06% was decreased by 20.06% in infected fish in comparison to normal. Total Erythrocyte Count (TEC) was different among different species. Its range in infected and normal fish of C. *batrachus* was shown in table-1. The average value was decreased in infected fishes from normal by 55.19% which was statistically significant (Paired-t Test, $P < 0.05$).Mean corpuscular volume (MCV) was different among different species. The concentration of MCV in infected host fish ranged from 225.5 – 772.41 μm^3 in *C. batrachus* (Table-1). The average value showed that this concentration was increased in infected fish in comparison to normal by 67.47% (Table-1). It was statistically significant. The range and mean values of Mean Corpuscular Haemoglobin **(**MCH) of both infected and normal fish of host species was recorded in table-1. It was revealed that the mean value of MCH was increased in normal and infected fishes by 59.51% which was statistically significant (Paired-t Test, $P < 0.05$). The Total Leukocyte Count (TLC) ranged from 8.93 – 20.79 $10^3/mm^3$ in infected fish of *C. batrachus was* recorded in table -1. The average value was increased in infected fish from normal by 29.92% which was statistically significant (Paired-t Test, $P < 0.05$).

Blood smear of trypanosome infected and none infected *C. batrachus* of host fish were stained with May-Grunwald Giemsa stain and based on their staining quality the morphology of Read Blood Cell (RBC) and White Blood Cell (WBC) (monocyte, lymphocyte, neutruphil, eosinophil and basophil) were investigated.

In normal fish, the shape of the RBC was oval in shape but a few irregular circular cells with rounded nucleus were seen at the centre of the cytoplasm (Fig-1A). The size was also same. The volume of cytoplasm was more or less four times higher than the nucleus. The colour of the cytoplasm and nucleus was reddish and purple respectively

But in infected fishes, the number of RBC was decreased gradually and the volume of cytoplasm of these RBC was also reduced gradually. The most of the nuclei were elongated and some of them were fragmented into two or more equal or unequal parts (Fig-1B). In some cases, the cytoplasm was vacuolated, tear drop like or completely lost leaving the nucleus either intact or swelling (Fig-1C&D).

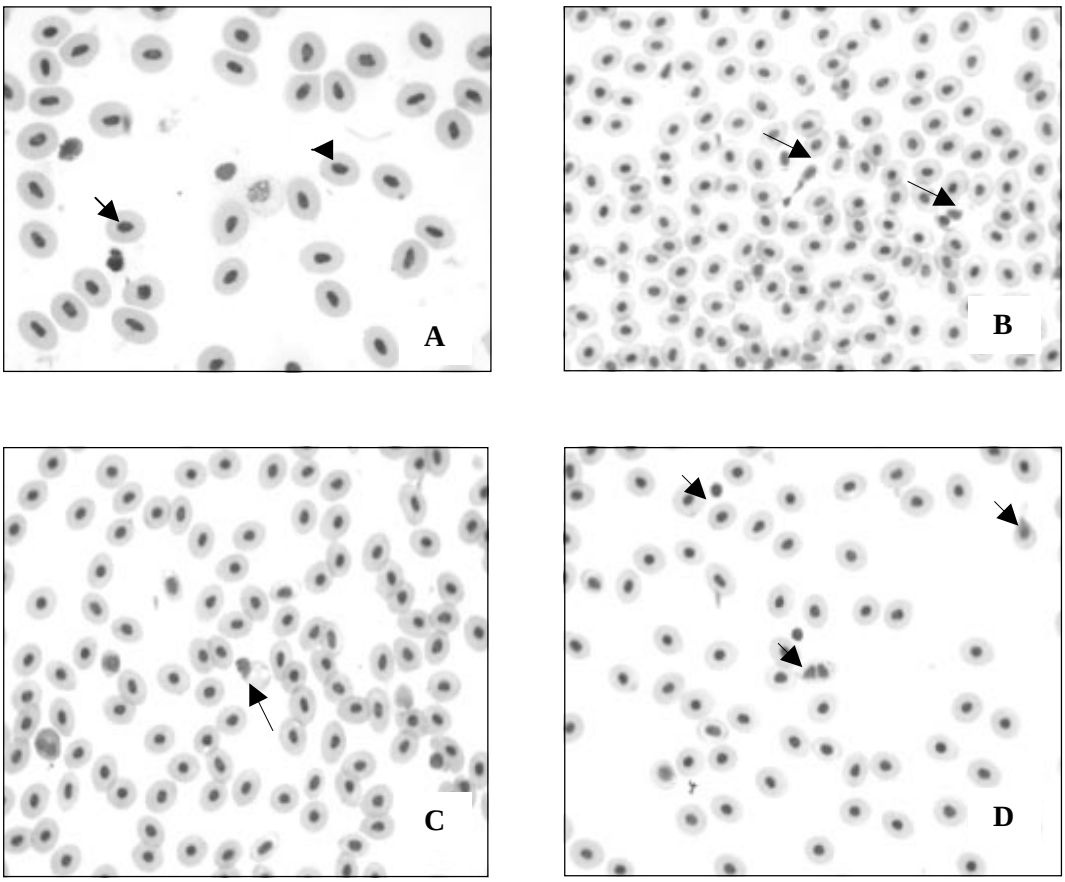

Fig 1 (A –D) : Morphology of RBC of *Clarias batrachus*. A: normal RBC in non infected host fish. B - D: abnormal RBC in Trypanosome infected host fish (B- fragmented RBC, C – vacuolated cytoplasm with extruded nucleus and D – tear drop like RBC and nucleus with out cytoplasm)

In non infected fish blood, monocytes were large, spherical in shape and nucleus was indented and surrounded by large amount of cytoplasm. Where as in trypanosome infected fish blood, monocytes were reduced in shape and both cytoplasm and indented nucleus was vacuolated. In normal blood, lymphocytes were round in shape and nucleus surrounded by a rim of large amount of cytoplasm. But in infected blood, the amount of cytoplasm was reduced and lymphocyte was migrated to periphery. Nucleus and cytoplasm were vacuolated occasionally. The normal neutrophils were circular with nucleus. But in infected blood, neutrophils were irregular in shape. Both cytoplasm and nucleus were vacuolated and sluggish. The normal eosinophil was rounded and regular in cell outline. Nuclei were bilobed, dumb bell shaped and stained purple colour.

But in infected blood, the cytoplasm of eosinophil was vacuolated. Vacuoles were enlarged gradually and nucleus was pushed to its periphery. The normal basophil was roughly oval in shape. In infected blood, morphological deformities were observed in basophil.

Discussion

The average concentration of haemoglobin was decreased by 37.81% in infected fish than normal of *C. batrachus* host fish and this occurred due to trypanosome infection. It was reported that parasitization by blood pathogen like *Trypanosoma*

sp. was metabolically depended on the blood of their host and alter the host physiology. So decrease in Hb concentration in fish was due to trypanosome infection.

The PCV was decreased significantly in all infected fishes due to the parasitization of *Trypanosoma.* During parasitization the parasite released catecholamine that mobilized RBCs from spleen (Wells and Weber, 1990). This catecholamine also induced RBC swelling as a result of fluid shift into the intracellular compartment (Chiocchia and Motais, 1989).

The average values of TEC was decreased significantly in infected fishes from normal by 55.19% in host fish(Table-1) and this value was also decreased with the increase in prevalence of infection. Besides, erythropaenia to damage of haematopoietic tissues in the kidney was due to parasitic infection.

The average values of TLC were increased by 29.92% in the infected host fish. This might be due to interference of some toxins or by products released by the haemoflagellates or a process of gradual immunization, being achieved by the reticubendothelial system of the host *C. batrachus*. This toxin or by products stimulated the haemopoietic tissues and immune system of the host by producing antibodies and chemical substances working as defense against infection (Wedmeyer and Wood, 1974; Lebelo et al., 2001 and Hassen, 2002). This could also attribute to the increase in the number of lymphocytes in the parasitized fishes (Murad and Mustafa, 1998 and Hassen, 2002). Akmirza and Tepecik (2007) reported that season also plays an important role in decreasing TLC. In a study on roach, *Rutilus rutilus* infected with *Cryptobia tincae*, they observed that the eosinophil count was increased in autumn and decreased in spring.

All the morphological abnormalities of erythrocytes found in trypanosome infected blood of host C. *batrachus,* might be due to abnormal erythropoiesis. Reduction in the volume, vacuolation of cytoplasm and fragmentation of RBC are the indication of anemia. Gradual increase in number of WBC was the peculiar effect of anemic condition and was depended on trypanosome infection. This work corroborated with work done by Joshi and Dabral (1981). They also observed that the number of neutrophils and thrombocytes would fall conspicuously due to trypanosome infection.

Acknowledgement

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On the occurrence of a protozoan parasite from edible oysters of Sunderbans region of West Bengal

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Abstract : The Sundarbans region of the West Bengal is highly diverse and ecologically important place which thrive many organisms. Oyster is one of the keystone species of this region and typically serves as habitat and shelter for many other organisms. *Saccostrea cucullata, Crassostrea gryphoides* are the common edible oysters species found in the intertidal zone of this region and can tolerate huge variation of salinity. These are used as food by local people and marketed to earn money. The population of this species has been declined due to parasitic infection and pollution. There are many protozoan parasites which infect these oysters causing diseases. During survey, a protozoan parasite of the genus *Cristigera* have been observed from mantle and gill of the edible oyster *Crassostrea gryphoides*, collected from Kaikhali of Sundarbans region, south 24 parganas. The parasite is a hymanostome ciliate measuring 22-40µm in length and 7-12µm in breadth. The macro nucleus is 3.8-9.6µm in length and 2.8-6.6µm in width. The micronucleus is 1.9-2.8µm in diameter. While œmparing the organism with other related species it appears to be new to science. Hence *Cristigera* has been designated in the communication.

Key words **:** *Sundarbans, keystone, protozoan parasites, ciliate.*

Introduction

In Protozoa, comparatively highly complicated organisms are represented by numerous ciliates. Ciliates are a diverse and successful group of protozoan parasites belonging to the phylum Ciliophora , order Hymenostomatida . Genus *Cristigera* (Roux, 1901) are common in invertebrates of marine and fresh water organisms. Species of *Cristigera* sp. are isolated from the mantle, gill, and labial palp of the oysters. Most of the species of the genus *Cristigera* are free living ciliates excepting *Cristigera susmai* (Jamadar and Choudhury, 1988). In India the *Cristigera* parasites have so far been reported from *Crassostrea cuculata* collected from the meeting place of the Hooghly River with the Bay of Bengal, Sagar Island, West Bengal, India (Jamadar and Choudhury, 1988). Species of ciliates capable of facultative parasitism are also known (Corliss, 1961). This paper deals with the incidence of a species under the genus *Cristigera* from an oyster *Crassostrea gryphoides* of Sundarbans region, West Bengal.

Materials and methods

Host specimens were randomly sampled from the Kaikhali near Jaynagar-majilpur, South 24 parganas of Sundarbans region, West Bengal and brought alive or in moribund condition to the laboratory. Oysters (n=30 each) were collected seasonally from the specified sites from November, 2009 to September 2010. Oysters were taken directly from the reefs.

In the laboratory the shell of the oyster were opened with fine knife and smeared materials were scrapped out on glass slides in 0.6% saline and observed under microscope for examination of parasites. For permanent preparations the smeared slides are semi air-dried, fixed in schaudinn's fluid and stained in Heidenhains iron alum haematoxylin.

Measurements of the parasites were taken with the aid of a calibrated ocular micrometer. All measurements are presented in hm as mean ± SD followed in parentheses by the range. Drawings were made on stained material with the aid of a mirror type camera lucida. Photographs were taken with Olympus phase contrast microscope fitted with Olympus digital camera.

Result

Morphology of *Cristigera* sp. :

The body is ovoid, much compressed, anterior end pointed and posterior end slightly broad with a distinctly convex dorsal surface and a comparatively flattened ventral surface. The cytoplasm of the body is scattered with dense granule. These dense

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granules are easily distinguishable in the live materials. The parasites measure 22-40µm in length and 7-12µm in breadth. The macro nucleus is 3.8-9.6µm in length and 2.8-6.6µm in width. The micronucleus is 1.9-2.8µm in diameter. Buccal cavity present on the ventral surface of the body, lying just above the macronucleus. The cytostome is located near the buccal cavity and possess an undulating membrane. The circular contractile vacuole present in the posterior end of the body of the parasites.

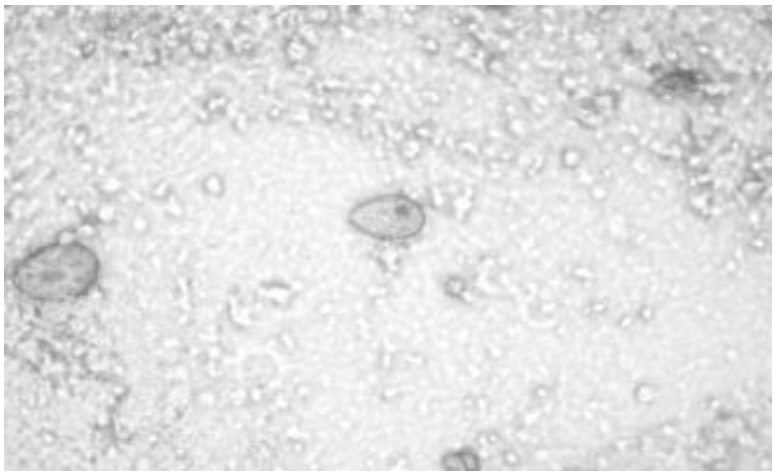

Fig. : Photomicrograph of *Cristigera* sp.

Taxonomic Hierarchy :

Kingdom	Protozoa
Phylum	Ciliophora
Class	Ciliatea
Subclass	Rhabdophorina
Order	Hymenostomatida
Suborder	Pleuronematina
Family	Cyclidiidae
Genus	*Cristigera* (Roux, 1901)

Taxonomic summary :

a) Type specimen : Syntypes, 6K/CG/02/2010; deposited in the Parasitology Laboratory, Department of Zoology, University of Kalyani, West Bengal, India.

b) Type host : *Crassostrea gryphoides*

c) Type locality : Kaikhali near Jaynagar-majilpur, 24 Parganas (South) West Bengal, India

d) Collection date : 2010, February.

e) Site of infection : Mantle, gill, labial palp

f) Prevalence : 03 out of 30 hosts sampled and examined randomly. i.e. 10% of the sampled hosts were found to be infected by this parasites.

Discussion

The most conspicuous group of Protozoa is Ciliate, containing two nuclei; macronucleus and micronucleus. Sexual reproduction is through conjugation (Kudo, 1966). We owe Kahl a great deal for his series of comprehensive taxonomic studies of free-living ciliates (Kahl, 1931, 1935). The ciliate are grouped under the four orders, of which the present species of *Cristigera* is under Hymenostomatida .The class Ciliata has completely been reclassified, following Kahl's admirable work on free-living ciliates (Kahl, 1930- 1935); however, unlike the latter, all parasitic ciliates have also been considered in the present work.

Genus *Cristigera* Roux. much compressed; with a postoral depression; peristome closer to mid-ventral line; fresh or salt water. Several species of this genus are found so far. The present form differs in morphology or morphometry from other twelve already described species of Genus *Cristigera* in shape and size in following significant ways.

The present form is smaller in size in comparison to *Cristigera paucisetosa*; *C. pleuronemoides*, *C. media*, *C. penardi* and C. suleata. The size of the body of the present form is more or less similar with the *C. phoenix* and C. minor but the present form is ovoid whereas *C. phoenix* and C. minor are elliptical in shape. The size and the shape of the body of the present form is also more or less similar with *C. setosa* and *C. cirrifera* but the cilia are restricted to anterior and posterior part of the body in *C. setosa* and *C. cirrifera* while in the present form the cilia are present throughout the body. The cilia are also present throughout the body except the anterior tip in *C. minuta* and *C. vestita*. The species under discussion resembles closely to *C. susmai* in morphometry. The only difference is that the anterior end of the present form is not as much compressed as in *C. susmai*.

The shape and size of the species also differs. It is ovoid in shape with pointed anterior end in the species under discussion while the aforesaid species is cylindrical or elliptical ovoid in shape.

On the basis of above taxonomic comparative study and based on the morphology, the species described in this paper is new to science and has been designated as *Cristigera crassostrae* n.sp.

Detailed morphometric comparison has been given in Table 1.

Table 1 : Morphometric comparison of the new species with previously described species of the genus *Cristigera* sp. (Survey on the data on the Genus *Cristigera* sp. reproduced from Kahl, 1931, 1933).

Sl. No.	Species	Author	Habitat	Body Size	Body Shape	Perisome	Somatic Cilia	Caudal Cilium
1.	*Cristigera paucisetosa*	Gourret & R. 1888	Marine	50-60 µ	Small spindle form, ventral side concave.	Peristomial membrane ½ of the body length.	Cilia restricted to anterior and posterior part.	Long
2.	*C. pleuronemoides*	Roux,1901	Marine	60-70µ	Ovoid, frontal plate not prominent, broken ciliation in middle region, cytoplasomic inclusion in the posterior part of the body.	Peristome not extended up to the middle.	Cilia restricted to anterior and posterior part.	Long
3.	*C. phoenix*	Penard,1922	Fresh water	35-50µ	Long, elliptical	_	Cilia in the middle portion, very small	Long
4.	*C. minor*	Penard,1922	Marine	25-30µ	Small, elliptical.	Not reaching the middle.	Cilia in the middle portion, very small.	Long
5.	*C. setosa*	Khal,1928	Marine	26-33µ	Ovoid body, broad frontal plate.	2/3 of the body length.	Cilia restricted to anterior and posterior part.	Long
6.	*C. media*	Khal,1928	Marine	45-50µ	Broad and ovoid.	½ of the body length with broad membrane.	Cilia through out the body except the anterior tip.	Long
7.	*C. minuta*	Khal,1928	Marine	26-33µ	Small. Ovoid, frontal plate relatively smaller, plasma with shining granules.	½ of the body length with broad membrane.	Cilia through out the body except the anterior tip.	Long

Sl. No.	Species	Author	Habitat	Body Size	Body Shape	Perisome	Somatic Cilia	Caudal Cilium
8.	*C. vestita*	Khal,1928	Marine	25µ	Cylindrical. Post oral depression small.	½ of the body length with broad membrane.	Cilia through out the body except the anterior tip.	Long
9.	*C. cirrifera*	Khal,1928	Marine	24-28µ	Ovoid body with cirri, membrane as long as the body length.	½ of the body length with broad membrane	Cilia restricted to anterior and posterior part.	Long
10.	*C. penardi*	Khal,1931	Marine	58-70µ	Post oral depression small.	1/3 of the body length.	Cilia restricted to anterior and posterior part.	Long
11.	*C. suleata*	Khal,1933	Marine	60µ	Similar to *C. phoenix* but more broad conspicuous frontal plate.	½ of the body length.	Cilia through out the body except the anterior tip.	Long
12.	*C. susmai*	Jamadar & Choudhury, 1988	Brackish water entocom mensal.	28.5-42.5µm	Ovid, much compressed, anterior end pointed and posterior end slightly broad with a distinctly convex dorsal surface and a somewhat flattened ventral surface.	1/3 of the body length.	Cilia through out the body, 5.1µm at the anterior part and 3.7 µm at posterior part of the body.	Long
13.	**Present form**		Brackish water.	22-40µm	Ovoid body with pointed anterior end.	1/3 of the body length.	Cilia through out the body, very small.	Long

Acknowledgments

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Variation in the diversity of protozoan parasites of fishes in Manipur : climate change - a possible cause

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Abstract : Climate change is predicted to have important effects on parasitism in freshwater ecosystems, with consequences for human health and socio-economics. The distribution of parasites will be directly affected by global warming, but also indirectly, through effects on host range and abundance. In general, transmission rates of parasites are expected to increase with increasing temperature. Evidence from a research done in Manipur suggests that distribution of some protozoan parasites of fishes increases, a few decreases while some are newly recorded. These protozoans include Myxozoans, Ciliates and Trypanosomes. Among the Myxozoans, species of *Myxobolus* increases while those of *Henneguya* and *Myxidium* are found to be decreased. But the species of *Thelohanellus* remains without any change. Among the Ciliates, Trichodinid ciliophorans (*Tripartiella*) increased in large numbers. The study also reports the new record of the species of *Ichthyophthirius* and *Trypanosome* recovered from fresh water fishes of Manipur which otherwise were not observed in earlier works. The finding of these species that prefer warm climate suggests the possible change in diversity may be due to the increase in temperature.

In Manipur Wetlands had decreased to 19 from 500 and temperature had increased from 20^0C to 38^0C during summer from 1950's to 2009 (Profile on state Environment Report of Manipur, 2006-07). The study believes that variation in the species diversity may be directly related with climate change and indirectly with the drying up of wetland, alteration in fish metabolism and physiology, increase in the concentration of host in small area, alteration of host range etc. (Parry *et al.,* 2007; Scavia *et al.,* 2002; Schindler, 2001.)

Key words **:** *Protozoa; Climate change; Myxozoan parasites; Ciliophoran; Trypamosoma.*

Introduction

In Manipur, temperature has shot up in the region with official sources quoting a rise of almost 5^oC on an average in the last two decades. Due to scarcity of rainfall, the entire state of Manipur was declared to be draught-hit by the Central Government on July 14, 2009. In the early 20^{th} Century, Manipur had about 500 wetlands. But today even the few existing ones are on the verge of drying up because of climate change and human interference. Survey conducted by the Environment and Ecology Wing of State Forest Department pointed out that only19 wetlands are left in Manipur (Profile on State of Environment Report of Manipur 2006-07). This will highly affect the aquatic organism of the state which belongs to one of the Mega Biodiversity Hotspot region. All aquatic ecosystems are influenced by climate change (Parry *et al.* 2007, Scavia *et al.* 2002, Schindler, 2001). A relative small temperature changes alter fish metabolism and physiology with consequences for growth, fecundity, feeding behavior, distribution, migration and abundance (Ficke *et al.* 2007, Reis *et al*. 2006a, b, Roessig *et al*. 2004). Accordingly the parasites which are associated with fish may also be changed. Harvell *et al.* (1999, 2002) and Marcogliese (2001) pointed out that climate change will have a profound impact on the spread of parasites and disease in aquatic ecosystems. Not only will climate change affect parasite species directly, but also through changes in the distribution and abundance of their host (Dobson & Cauper, 1992 and Marcogliese, 2001).

A survey was conducted for 8 years with a view to assess the distribution pattern of protozoan parasites in fishes and disease outbreak caused by this parasites in relation to climate change in the state of Manipur. This communication reports some preliminary findings regarding the impact of climate change on some commonly found protozoan parasites of fishes in Manipur.

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Materials and Methods

Fishes of small size (10 – 15cm in length) were collected from different wetland areas and observed externally for the presence of any parasites at the collection site if immediate transportation to the lab were not possible. Otherwise they were brought to the lab and properly examined for the parasites. Almost all the organs of the host were examined carefully and smear of each organ were made on clean grease free slides. For identification of Myzoxoan and Trypanosome parasites, smeares were air dried, fixed in acetone free methanol, stained with Geimsa and washed with buffer solution. It was then mounted with DPX and observed under microscope. For Ciliate protozoans, smear were air dried, stained with 2 – 3% silver nitrate solution for 8 – 10 minutes and washed two to three times in distilled water and then exposed to UV light. During exposing the slide was dipped in distilled water in a Petridish (Klein, 1958). The exposure was done for 45 minutes to 1 hours after which the slide was observed under microscope for identification. Photomicrograph was taken with the help of CCD camera fitted to Trinocular Microscope, Model No. GE – 52 TRH.

Results

In Manipur research on protozoan parasites of fishes started from 2002. The study reveals the presence of different protozoan parasites in different fishes of Manipur. The protozoans include the species belonging to the genus *Myxobolus, Thelohanellus, Henneguya, Myxidium, Trichodina, Tripartiella, Ichthyophthirious, Trypanosoma* etc.

GENUS - *MYXOBOLUS* BÜTSCHLI (FIG: 1)

***Myxobolus* Bütschli, 1882 :** Klass Ordn., des Tierreiches, Protozoa. 1: 590 – 603 (Type species – *Myxobolus muelleri* Buetschli, 1982; Type host – *Leuciscus cephalus*)

Myxosoma Thélohan, 1892; *Bull. Soc. Philom.* 4: 165 – 178.

Lentospora Plehn, 1905; *Arch. Protistenk.* 5: 145 – 166.

Facieplatycauda Wyatt, 1977; *J. Protozool.* 26(**1**): 47 – 51.

Rudicapsula Kalavati and Narasimhamurti, 1984; *Z. Parasitenkd.* 70(**1**): 21 – 27.

Myxobolus are the myxozoan species having two polar capsules at one end and spores without any caudal prolongation. This type of protozoa is found infecting most of the fish species found in Manipur throughout the year. During the year 2002 to 2006 only 10 – 15 species were recorded from the state. The number gradually increased year after year. During 2009 – 10 as many as 36 species including known ones were recorded. Most of the species are not host specific and tissue specific except *Myxobolus neurophilous* which is the only species that specific to *Channa gachua* (Chanid). The variation in the population is presumed to be related with increase in temperature during the last few years in the state as the species of *Myxobolus* favour warmer climate which is in conformity with the observation of Hiner and Moffitt (2001).

GENUS - *THELOHANELLUS* KUDO (FIG: 2)

***Thelohanellus* Kudo, 1933;** *Trans. Am. Microsc. Soc.* 52: 195 – 216. (Type species – *Thelohanellus pryriformis* (Thélohan, 1892) Kudo, 1933; Type host – *Tinca tinca*)

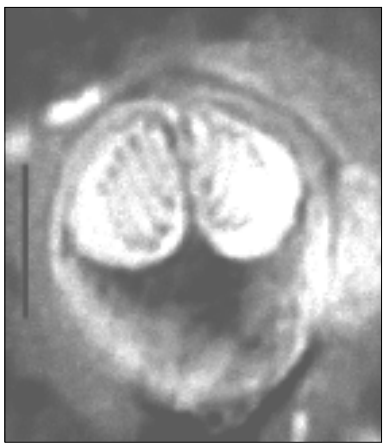

Fig. 1 : A *Myxobolus* species

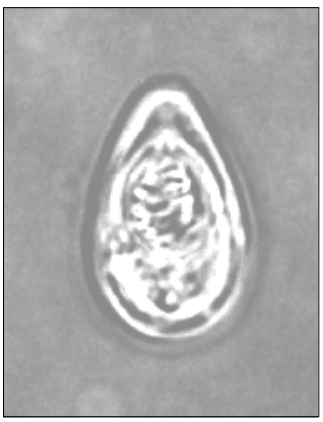

Fig. 2 : A *Thelohanellus* species

Thelohanellus are the myxozoan species having only one polar capsule at one end and spores without caudal prolongation. This type of protozoa is found mostly infecting the major and minor carps of Manipur. They are only found during the months of March to May and September to November every year when the temperature is moderate. During the year 2002 to 2006, 12 – 15 different species of *Thelohanellus* were recorded including known and new. Not much change in species were observed in the subsequent years. During the year 2009 – 2010 only 16 species were. The finding indicates that this myxozoa is also temperature dependent. They are not observed during the months of June to August when the temperature is high and December to February when the temperature is very low in the state.

GENUS - *HENNEGUYA* THÉLOHAN (FIG: 3)

***Henneguya* Thélohan, 1892;** *C. R. Acad. Sci.* 115: 1091 – 1094. (Type species – *Henneguya psorospermica* Thélohan, 1895; Type host – *Perca fluviatilis*)

Henneguya are the myxozoan species having two polar capsules at one end and spores with a long caudal prolongation which are mostly bifurcated. In Manipur this type of protozoa is found infecting the Chanid and Anabantid fishes during the cold season only i.e during the months of November to March every year. During 2002 – 2006, 9 – 10 species of *Henneguya* were recorded including known and new species. The number gradually decreased in the subsequent years and only 2 – 4 species were observed during the year 2009 – 2010. The finding infer that this type of protozoa might not be able to tolerate warm climate. Rise in winter temperature in the State in the recent years may have helped in the reduction in the number of *Henneguya* species.

GENUS – *MYXIDIUM* BÜTSCHLI (FIG: 4)

***Myxidium* Bütschli, 1881;** *Zool. Jahrb. f.* 1880, 1: 162 – 164 (Type species – *Myxidium leiberkuehni* Bütschli, 1881; Type host – *Exos lucius*)

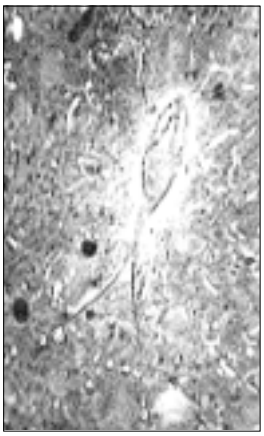

Fig. 3 : A *Henneguya* species

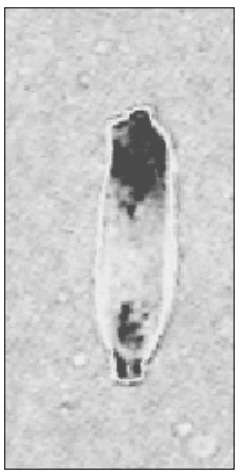

Fig. 4 : A *Myxidium* species

Myxidium are the myxozoan parasites having two polar capsules, one each on both end and spores with no caudal process. In Manipur this type protozoa is found infecting only *Tricogasteri faciatus* and *Clarias magur* during winter season. During the year 2002 – 2006 only two species were recorded which are all known species. The number gradually decreased in the subsequent years. But during the year 2009 – 2010 no *Myxidium* species were recorded from any fish found in Manipur. From this it can be assumed that *Myxidium* has almost vanished from the state.

GENUS - *TRICHODINA* EHRENBERG (FIG: 5)

***Trichodina* Ehrenberg, 1838;** Leopold Voss. 547 (Type- species - *Trichodina pedculus* (O.F.Muller, 1786) Ehrenburg, 1838; *Numulella conchiliospermmatica* Carus.1832; *Trichodina fultoni* Davis, 1947 in part; *Urceolaria discina* Lamarck, 1816; *Urceoloria stellina* Dujardina, 1814; Type host - *Hydra fascia, Hydra viridis*)

Acyclochaeta Zick, 1928; *Z. Wiss. Zool.* 137: 356 – 403.

Anhymenia Fabre- Domergue, 1888; *J. Anat. Physiol.* 23: 214 – 260.

Cyclocyrrha Fabre-Domergue, 1888 ; *J. Anat. Physiol.* 23: 214 – 260

Paravauchomia Raabe, 1963; *Acta Protozool.* 1: 121 – 138.

Trichodina are the ciliophoran species having denticulate ring composed of denticles with straight or curved blades, distinct rays of various shapes and length, adoral spiral describing an arc of 330^0 – 450^0. This type of protozoa is found infecting the gills and body surface of major carps and *Mistus* found in Manipur. They favour warm climate and are obtained during the months of May to September every year. During winter when the temperature falls down their presence are not observed. During the year 2002 – 2006 only two species were recorded with low population. The number increased in the subsequent years. During the year 2009 – 2010 as many as 5 species were recorded and the population drastically increased. In a slide smeared from one gill clip of an infected carp nearly hundreds of individuals were observed. Such populations were not seen in the previous years. This indicates that warm climate stimulate their activities.

GENUS - *TRIPARTIELLA* LOM (FIG: 6)

***Tripartiella* Lom, 1959;** *Acta Parasitol. Polon.* 7: 573 – 590. (Type- species - *Tripartiella copiosa* Lom, 1959: Type-host - *Rhodeus sericues*)

Trichodina - proparte Davis, 1947; Dogiel, 1940; *Tr. Len. Obsh. Yestiestvoispitatieley.* 68: 8 – 31.

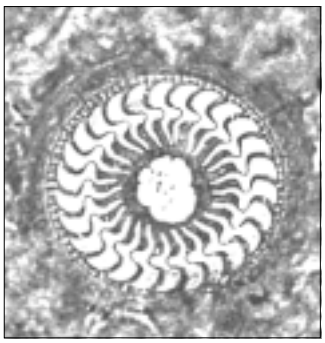

Fig. 5 : A *Trichodina* species

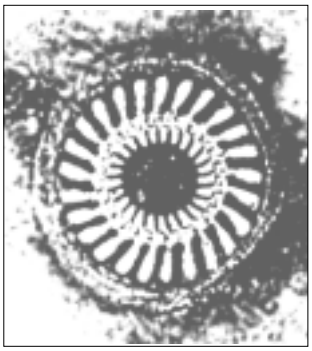

Fig. 6 : A *Tripartiella* species

Tripartiella are the ciliophoran parasites having denticles consisting of blades, rays and central parts and adoral spiral making turn of 180^0 – 270^0. This type of protozoa is found infecting only the gills of Major carps inhabiting Manipur during the months of May to September. During the years 2002 – 2006 only one species were recorded with low population but during the years 2009 – 2010 three species were recorded with unexpected rise in population. A slide smeared from one gill clip contained hundreds of individual while the host seemed to be non symptomatic when observed.

GENUS – *ICHTHYOPHTHIRUS* FOUQUET (FIG: 7)

***Ichthyophthirius* Fouquet, 1876** (Type- species – *Ichthyophthirus multifiliis* Fouquet, 1876)

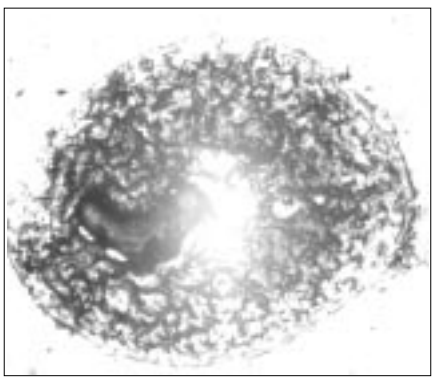

Fig. 7 : An *Ichthyophthirius* species

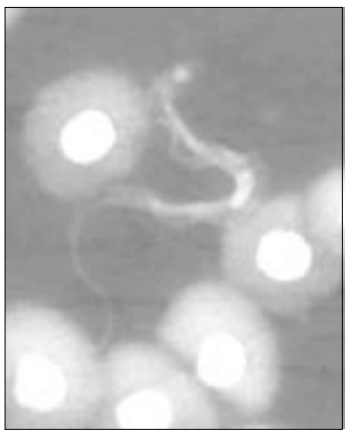

Fig. 8 : A *Trypanosoma* species

Ichthyophthirius are the ciliate parasites having ovoid body, uniform ciliation, a big rod shaped or horse shoe shaped nucleus inside the body. This type of parasite is found infecting the gills of carps in Manipur. It was recently recorded from the gills of *Labeo rohita* in April, 2010. Before this no *Ichthyophthirius* species were recorded from the state. This will proved that parasite species entered new areas searching for suitable habitat in response to climate change.

GENUS – *TRYPANOSOMA* GRUBY(FIG: 8)

***Trypanosma* GRUBY, 1843**

Trypanosoma are the blood flagellate protozoa having highly flattened body, pointed at flagellate end, and bluntly rounded or pointed at the other end. There is a blepharoplast from which the flagellum arises and nuns toward opposite end, making the outer boundary of the undulating membrane; in most cases flagellum extends freely beyond body. This type of protozoa was recently recorded from the blood smear of *Clarias magur* collected from Utlou, Manipur. Till date no report on the presence of such protozoa from Manipur was recorded.

Discussion

Most of the Scientists agree that climate change has great impact on the parasites and infectious diseases of aquatic animals. Numerous host – parasite systems are affected by climate. Climate change directly affect parasitic file cycles or indirectly affect host populations and communities (Mouritsen and Poulin, 2002). Asha Gupta and Usharani (2009) reported that the average temperature during summer is 32^0C in Manipur in 2008. Again according to Manipur Weather report the average temperature during the year 2009 is 35^0C – 37^0C and that of 2010 is 33^0C – 36^0C upto September and sometime reach 41^0C. With such changes in temperature in the state Manipur some of the protozoan parasites have undergone population change. Species of the genus *Myxobolus* had increased in number from 2002 – 2010 in which maximum species were recorded during 2009 – 2010. Species of ciliate trichodinid that favour warmer climate also increased in number. *Ichthyophthirius* and *Trypanosoma* were also recovered from the state. These two parasites were not reported from any part of the North East in the previous years and the present communication is the first report of the two species. This predicts that many new parasites that were not observed before existed through transmission processes from other regions due to global warming (Poulin, 2006).

On the other hand, myxozoan species of the genus *Henneguya* and *Myxidium* had negative response to warm climate. They only favour winter season. The species of *Myxidium* could not be recovered during 2009 – 2010 in Manipur. This may be due to many reason, 1st – they cannot withstand high temperature (cannot complete life cycle), 2nd – reduction in host population (Channid fishes). In Manipur, the production of Channid fishes (commonly harvested during winter) were greatly reduced during the last 2-3 years. 3rd – reduction in habitat i.e. wetland areas, 4th – cannot expand host range. These findings confirm that "Parasites may respond to increasing temperatures more strongly than their host" (Poulin, 2006).

Thus, there will be correctness in the prediction that Global warming brings alteration in host range, parasite range, host immunocompetence, parasite virulence, parasite transmission rates, disease occurrence and parasite infection etc. Even the parasites that are uncommon to humans may also be transmited to human being. White Pelican (Avian Piscivores) acting as as vectors for *Myxobolus cerebralis* (causal organism for whirling disease in salmonid fishes) can be taken as an example for expansion of host range of the myxozoa (Koel *et al.*2020). After all human being are the last target for all the parasites.

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Diversity of Tick (Acari) in West Bengal

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Abstract : Though the study of ticks in India was initiated by Linnaeus in 1758, the first record of tick from West Bengal was done by Rudow (1870). He described an ixodid tick *Amblyomma bengalense* from *Python* sp. Later many workers studied the taxonomy of ticks of the state and till date 32 species under 9 genera of the family Ixodidae are known from West Bengal. Ticks have been recorded from all the districts in which Darjiling occupies the highest position in number of species. The tick species recorded from the state are known from outside India and show maximum similarity in species composition with palaearctic region (25%). *Amblyomma supinoi* is known only from the Indian state West Bengal.

Key words : *Tick, Acari, W.B.*

Introduction

The ticks belong to the order Metastigmata or Ixodida under the class Acari. They occur throughout the world, but are more frequently encountered in tropical and subtropical realms. The ticks are distinguished from other acarines by their larger size (2,000 µm to over 30,000 µm) and few other peculiarities. A hypostome armed with retrose teeth is of unique feature which serves to anchor the tick to its host. A complex sensory setal field, Haller's organ, is located on the dorsal side of tarsus-I, providing sites for contact or alfactory chemoreception. There are other distinguishing characters like stigmata situated posterior to coxa IV or dorsal to coxa III-IV, palp with only three or four segments, 2-segmented chelicerae, etc. Of the three families under Ixodida, the Ixodidae or hard ticks and Argasidae or soft ticks are found plenty in India but the third family Nuttalliellidae is known only from Africa.

The ixodid ticks are scutate with terminal capitulum, well marked sexual dimorphism, spiracle posterior to coxa-IV and pads and with porose areas and festoon. The argasid ticks are non-scutate with leathery integument, slight sexual dimorphism, small spiracle situated anterior to caxa-IV and pads and without porse areas and festoon.

The ticks live as ectoparasite of vertebrates and feed obligatorily on the blood of mammals, birds and reptiles. There are tick species recognized as significant pests of man and animals. In temperate and tropical countries, they surpass all other arthropods as transmitters in the number and variety of diseases of domestic animals and man. The ticks serve as reservoir and vectors for many infective viruses, rickettsia, bacteria, sporozoans and spirochaetes. They also cause paralysis and anaemia to hosts. Ticks are the main vectors of Kayasanur Forest Disease (KFD) in man and monkeys in Karnataka State. Other arboviruses like Kaisodi, Ganjam and Bhanja have also been isolated from ticks in India. Ticks are oviparous. Their life history passes through egg, larva, nymph and adult stages.

The study of ticks in India was started by Linnaeus in 1758. But the first record of tick from West Bengal was done by Rudow (1870). He described an ixodid tick *Amblyomma bengalense* from *Python* sp. The presence of the species was reported doubtful by Neumann (1911) and since then there is no record of the species from the State. Sharif (1928) reported that Neumann (1899) first recorded *Aponomma gervaisi* (Lucas) from West Bengal and later Warburton (1910) recorded the same species from Zoological Garden, Calcutta. Later, medical entomologists and acarologists got interested about tick fauna of the state and described and recorded a good number of genera and species of ticks. The detailed accounts of studies so far done have been summarized by Sanyal and De (1991, 1992, 2001).

Status of tick diversity

The analysis of diversity of tick species in West Bengal vis-à-vis in India (12 genera and 107 species) clearly indicates that the state alone represents 30% of the total Indian tick fauna. The total number of taxa of ticks so far known from

West Bengal is represented by 32 species under 9 genera and a single family Ixodidae. It is presumed that no record of argasid tick from the state may be due to poor abundance of these ticks and lack of attention to this family by the acarologists.

Of the 32 species of tick known from the state, only three species *viz*., *Hyalomma brevipunctata* Sharif, 1928 from Medinipur, *Haemaphysalis darjeeling* Hoogstraal and Dhanda, 1970 from Darjiling and *H. ramachandrai* Dhanda, Hoogstraal and Bhat, 1970 from Darjiling and Jalpaiguri have been described as new to science from the State. The analysis also showed that *Boophilus microplus*, *Haemaphysalis bispinosa* and *Hyalomma anatolicum anatolicum* are most dominant species being present in all the districts of the State.

Table I and II (Sanyal and De, 2001) showing host and distribution of tick species in different districts of the State, indicate that the species are well adapted on different hosts in different states from plains to higher altitude of the Himalaya.

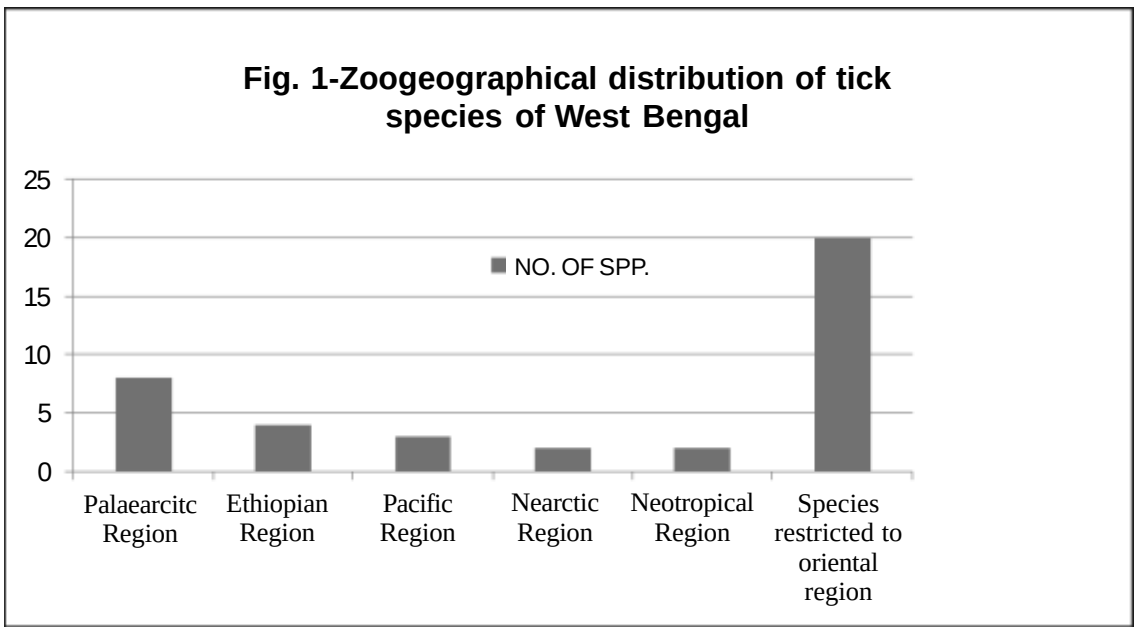
Table I. Species of ticks known from different districts of West Bengal

Sl. No.	Districts in West Bengal	Species of ticks
1.	Kolkata (Calcutta)	*Amblyomma helvolum* Koch, *A. javanense* (Supino), *Aponomma gervaisi* (Lucas), *Boophilus microplus* (Canenstrini), *Dermacentor auratus* Supino, *Haemaphysalis aponommoides* Warburton, *H. bispinosa* Neumann, *H. indica* Warburton, *Hyalomma anatolium anatolicum* Koch, *Rhipicephalus sanguineus* (Latrielle), *R. turanicus* Pomerantzev
2.	North 24-Parganas	*Amblyomma testudinarium* Koch, *Boophilus microplus*, *Dermacentor auratus*, *Haemaphysalis bispinosa*, *Hyalomma anatolicum anatolicum*, *H. hussaini* Sharif, *Rhipicephalus haemaphysaloides* Supino
3.	South 24-Parganas	*Boophilus microplus*, *Haemaphysalis bispinosa*, *Hyalomma anatolicum anatolicum*
4.	Barddhaman	*Aponomma lucasi*, *Boophilus microplus*, *Haemaphysalis bispinosa*, *Hyalomma anatolicum anatolicum*
5.	Medinipur	*Boophilus microplus*, *Haemaphysalis bispinosa*, *Hyalomma anatolicum anatolicum*, *H. marginatum isaaci* Sharif, *H. Brevipunctata* Sharif, *Nosomma monstrosum* (Nuttall & Warburton), *Rhipicephalus sanguineus*
6.	Nadia	*Boophilus microplus*, *Haemaphysalis bispinosa*, *Hyalomma anatolicum anatolicum*, *Rhipicephalus turanicus*
7.	Hugli	*Boophilus microplus*, *Haemaphysalis bispinosa*, *H. indica*, *Hyalomma anatolicum anatolicum*, *Rhipicephalus turanicus*
8.	Bankura	*Boophilus microplus*, *Haemaphysalis bispinosa*, *Hyalomma anatolicum anaatolicum*, *H. marginatum isaaci*
9.	Puruliya	*Boophilus microplus*, *Haemaphysalis bispinosa*, *Hyalomma anatolicum anatolicum*, *H. marginatum isaaci*, *Rhipicephalus sanguineus*, *R. turanicus*
10.	Jalpaiguri	*Amblyomma javanense*, *A. supinoi* Neumann, *A. testudinarium*, *Boophilus microplus*, *Dermacentor auratus*, *Haemaphysalis aborensis* Warburton, *H. bispinosa*, *H. cornigera shimoga* Trapido and Hoogstraal, *H. hystricis* Supino, *H. obese* Larrousse, *H. ramachandrai* Dhanda, Hoogstraal & Bhat, *H. spinigera* Neumann, *Hyalomma anatolicum anatolicum*, *Rhipicephalus haemaphysaloides*

Sl. No.	Districts in West Bengal	Species of ticks
11.	Darjiling	*Amblyomma testudinarium*, *Boophilus microplus*, *Dermacentor auratus*, *Haemaphysalis aborensis*, *H. aponommoides*, *H. birmaniae* Supino, *H. bispinosa*, *H. darjeeling* Hoogstraal & Dhanda, *H. himalaya* Hogstraal, *H. hystricis*, *H. montgomeryi* Nuttal, *H. ramachandrai*, *H. spinigera*, *Hyalomma anatolicum anatolicum*, *Ixodes acutitarsus* (Karsch), *I. granulatus* Supino, *I. ovatus* Neumann, *Rhipicephalus haemaphysaloides*
12.	Koch Bihar	*Boophilus microplus*, *Haemaphysalis bispinosa*, *Hyalomma anatolicum anatolicum*
13.	West Dinajpur	*Boophilus microplus*, *Haemaphysalis bispinosa*, *Hyalomma anatolicum anatolicum*
14.	Murshidabad	*Boophilus microplus*, *Haemaphysalis bispinosa*, *Hyalomma anatolicum anatolicum*
15.	Birbhum	*Boophilus microplus*, *Haemaphysalis bispinosa*, *Hyalomma anatolicum anatolicum*
16.	Haora	*Boophilus microplus*, *Haemaphysalis bispinosa*, *Hyalomma anatolicum anatolicum*
17.	Maldah	*Haemaphysalis bispinosa*, *Hyalomma anatolicum anatolicum*

Distribution of Tick Fauna

The genera and species of ticks in different districts of West Bengal are shown in Table-II and Figure-1 (Sanyal and De, 2001). These indicate that ticks are known from all the districts in the State. The district Darjiling occupies highest position among the other districts and the district alone represents 77.8% of genera and 56.3% of species known from West Bengal.



The second highest position (40.8%) is occupied by the Jalpaiguri district. The other districts in order of number of totalspecies are Calcutta (34.4%), Medinipur (21.9%), North 24 Parganas and Puruliya (each 18.8%), Hugli (15.6%), Bankura and

Barddhaman (each 12.6%), Birbhum, Haora, Koch Bihar, Nadia, South 24 Parganas and West Dinajpur (each 9.4%) and Maldah and Murshidabad (each 6.3%). The poor distribution and abundance of ticks in many districts may be due to the fact that no intensive survey for study of ticks in different districts is done due to inaccessibility and administrative reasons.

Zoogeographical distribution of ticks found in West Bengal

The figure-I shows the zoogeographical relationship of ticks so far known from West Bengal. It shows that maximum similarity in distribution of species exists between West Bengal and Palaeractic region (25%). The other zoogeographical regions in order of similarity are Ethiopion (12.5%), Pacific (9.4%). Nearctic and Neotropical (each 6.3%). The Figure-I also indicates that 62.5% of tick species known from West Bengal are found to occur only in the oriental region.

It is also noted from the available data that *Amblyomma supinoi* is recorded from the only Indian state West Bengal. The other interesting observation by Sanyal and De, 2001, reports that there is no tick species in West Bengal which is endemic to India.

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