REVIEW ARTICLE

Epidemiology and Laboratory Diagnosis of Paragonimiasis

T. ShantikumarSingh[1], Forhad Akhtar Zaman[2] & Hiromu Sugiyama[3]

[1]Professor & Head, Department of Microbiology, [2]Professor, Department of Community Medicine, SMIMS, 5th mile, Tadong, Gangtok-737 102, India.

[3]Chief, Laboratory of Helminthology, Department of Parasitology, National Institute of Infectious Diseases, Tokyo, Japan.

Abstract:

Paragonimiasis is one of the most neglected tropical food borne parasitic diseases despite the fact that nearly 293 people are at risk of infection and about 23 million people are infected in the world. Recently, new cases and new foci of paragonimiasis have been detected from North America, Korea, Japan, Lao PDR, Taiwan, Indonesia, the Philippines, Vietnam, India, Nepal, Bangladesh, Sri Lanka, and Myanmar. About 50 Paragonimus species have been described of which 15 are known to infect humans. Recently, the occurrence of P. heterotremus, P. huet'ungensis, P. skrjabini, P. macrorchis, and P. miyazakiimanipurinus n. sub spp and P. pseudo heterotremus have been described from India and Thailand, respectively. The parasites utilize fresh water snails as the first intermediate molluscan hosts and crabs and crayfish as second intermediate crustacean hosts and wild mammals and humans as definitive hosts. Humans acquire the infection by ingestion of raw or undercooked fresh water crustacean hosts that harbor infective larval stage. Pulmonary paragonimiasis is the commonest clinical form which is very often misdiagnosed as pulmonary tuberculosis and lung cancer due to similar clinical and radiological features. Low awareness is the most important reason for the disease being overlooked. Whereas, the microscopy demonstration of Paragonimus eggs is the mainstay of laboratory diagnosis, various specific and highly sensitive serological tests have been developed in countries where it is endemic. Although nonspecific, high blood eosinophilia and raised erythrocyte sedimentation rate are usually associated with paragonimiasis. Praziquantel or triclabendazole or bithionol are equally effective in the treatment of the disease. Primary control strategies should include Information, Education and Communication (IEC) and Behavioral Change Communication (BCC) activities on safe food practices, improved sanitation, and awareness program about paragonimiasis.

Key Words: Paragonimiasis, Neglected Tropical Disease, Review, Epidemiology, Diagnosis.

Introduction:

Paragonimiasis also known as lung fluke infection is one of the most important but neglected food borne parasitic zoonosis caused by trematode species of the genus *Paragonimus*. The disease is widely distributed in many parts of Asia, Africa, and Americas and endemic in China, Japan, Korea, Vietnam, Indonesia, Taiwan, Thailand, Philippines, Belgium Congo, Nigeria, Cameroon, Peru, Ecuador, Colombia, Venezuela and Mexico [1]. Humans acquired the infection by ingestion of raw or undercooked second intermediate fresh water crustaceans hosts harboring the infective larval stage or paratenic host containing the juvenile stage of the parasite. The most common clinical form is the pulmonary paragonimiasis which is almost always mistaken for tuberculosis. The infection can also occur in sites other than lungs. Recently, WHO has considered paragonimiasis as one of the most neglected tropical diseases [2].

Address for correspondence:
Dr . T. Shantikumar Singh
Professor,
Department of Microbiology,
Sikkim Manipal Institute of Medical Sciences,
Tadong, Gangtok-737 102, India.
Email: **shantikumar_singh@rediffmail.com**

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WHO was paying attention to a parasitic disease which has been struggling hard for its recognition as one of the important food borne parasitic infections for the past several decades.

In terms of disability adjusted life years (DALYs) paragonimiasis surpasses opisthorchiasis, fascioliasis, and intestinal diastome infection combined [3]. In the recent years since 1980s new endemic areas have been discovered in India [4,5,6,7].

North America [8] and Nepal (personal communication), however, it appears that public health authorities has not paid much attention to this disease. The most important problem with paragonimiasis is the confusion with diseases having similar symptoms and delay in the diagnosis causing significant impact on the health and economy of the patient and family. If aware it can be diagnosed with simple laboratory tests and effectively treated with praziquantel or bithionol, 2, 2'-thiobis [4,6-dichlorophenol] or triclabendazole {5-chloro-6 (2,3-dichlorophenoxy)-2 methyl ethiobenzimidazole}. This review is intended to give an account of the epidemiology and diagnosis of paragonimiasis and bring in awareness about *Paragonimus* and paragonimiasis with special reference to Indian subcontinent where it has emerged as public health problem, recently.

Epidemiology

Problem Statement

In 1995, it was estimated that about 20.7 million people are affected by paragonimiasis worldwide [9]. More recent estimate (2012) indicated that 293 million people are at risk and about 23 million people in 48 countries are infected [2,10]. Over the years several new cases of paragonimiasis have been reported from India, China, Thailand, Vietnam, Nepal, Myanmar, Sri Lanka, Bangladesh, North and South America, and Africa [11]. We consider the above figure as underestimate due to the fact that not all the patients sought medical attention and not all cases are published in the journals.

Americas: In Ecuador the highest prevalence of human paragonimiasis was reported in in 2011 and is still considered a public health problem. Official data recorded from 1978-2007 indicated an annual incidence of 85.5% cases throughout the 19 provinces, with an estimated 17.2% of the population at risk of infection with a total case burden of 0.5 million and still this prevalence was considered lower than the expected as a result of underreporting because the patients belonged to poor families in rural areas where health services were lacking 12 Till 2009, a total of 16 cases of indigenous paragonimiasis caused by *P. kellicotti* have been reported in the United States, with the largest number from Missouri [8].

Asia: In China, the total nationwide prevalence of paragonimiasis was 1.71% from 2001 to 200413 and over 20 million are infected and 195 million are at risk [14]. In Japan, where it was almost eradicated following health education, improved food hygiene and socio-economic standards, a total of 152 cases has been reported from 1998 to 2002 [15]. Uchiyama et al reported that both children and adults were infected and there was no significant difference in male and female ratio [16]. Korea reported a prevalence of more than 1.5 million cases till 1997 [17]. The estimated prevalence of paragonimiasis in the Philippines in 1984 was between 0.7 to 9.96% [18]. Paragonimiasis is currently very rare in Taiwan [19] only sporadic reports of paragonimiasis occur in Taiwan from elderly populations that immigrated from China [20]. According to a study in Thailand, there have been at least 10 reports in the literature of 68 cases of pulmonary paragonimiasis in the Thai population [21] Khoo (1957) have described nine human cases of pulmonary paragonimiasis in Singapore but considered the infection might have acquired from Japan where the patients have eaten raw crabs [22]. In Vietnam, the first case of paragonimiasis was reported in 1906 and from 1906 to 1992, over 30 cases of paragonimiasis were reported [23]. In spite of over 15 years of repeated mass screening, treatment, and education about paragonimiasis, Doanh et al [24] still found patients in some previously reported endemic areas, especially in Sin Ho district of Lai Chau Province of Vietnam, where seroprevalence rate was 12.7%. Recently, in Lao PDR with several small foci of paragonimiasis, nine cases of paragonimiasis of primary persistent pleural effusion were reported [25].

Indian Sub-continent: In India, the first indigenous case of pulmonary paragonimiasis was detected by Singh et al in 1981 [26] followed by the discovery of several cases in the northeast Indian state [27]. A case of pulmonary paragonimiasis was also reported from Maharashtra by Patil et al in 1984 [28]. Recent epidemiological surveys indicated that paragonimiasis is endemic in the northeast India with the prevalence rate ranging from 2% - 36% (average: 6.7%) in Manipur, 52% among children under 15 in Arunachal Pradesh and 50% among school children in Nagaland [5-7].

Recently, Mohamed et al (2012) reported pulmonary paragonimiasis and massive pleural effusions in three Myanmar refugee children at Kaula Lumpur, Malaysia [29] Also, a case of pleuropulmonary paragonimiasis in a 58 year old Burmese (now Myanmar) male immigrant was reported from Colorado Denver, USA [30]. In Nepal, an endemic focus of paragonimiasis was discovered in Gadavari forest near Kathmandu in 2012 (personal communication) in which eight out of ten people who have eaten barbeque of freshwater mountainous crabs became infected with *Paragonimus*. Two of them presented initially as bilateral pleural effusion associated with high eosinophilia (83%) about two weeks after consuming the crabs. Till date, no report of human paragonimiasis from Bangladesh, Bhutan,

Afghanistan, and Pakistan is available in the extensive search of literatures.

Epidemiological Factors

Age & Sex- Paragonimiasis may occur at any age but findings in most of the studies suggests that it is more common among the children (age ≤ 15) [6,7,31,32,33] and in some other studies it was found to be common in the age group of 15 -45 years 5 Most of the studies found no significant difference in male and female ratio [16]. However, some studies showed male predominancefor example in Manipur, India male to female ration was reported as 2.1:1, probably as a result of increased exposure to infection [4]. In an epidemiological survey the possibility of limited access to health care for women as an explanation for the observed difference does not arise, however, it may have some influence on the study in the health care settings.

Modes Of Transmission and Risk Factors:

Generally, people acquire infection by eating raw or undercooked fresh water crabs and or crayfish or shrimps containing the infective larval stage of *Paragonimus*. Therefore, infection is common in countries where people consume raw or inadequately cooked crustaceans. There are a variety of dishes prepared locally as per the tradition and culture, practice and preference of the people in the community. In northeast India, raw or smoked or fried or improperly cooked crabs are consumed as a delicacy, dietary supplement of protein, and as folk medicine to enhance immunity. A popular Manipuri dish, "Waikhuametpa" or "Waikhu Singju" (waikhu meaning crab) wherein raw or smoked crabs are chopped and crushed into smaller pieces to which salt, chilies, garlic, onion, ginger and coriander are added. The recipe is usually consumed with alcoholic beverage. In the Philippines, "Kilano" a raw crab dish with citrus fruit juice and coconut milk are taken with alcoholic beverage. In Korea, a preparation called "Kejang" a raw crustacean dishes are eaten with soya bean sauce [17]. A Japanese dish "Oboro-jiru" wherein raw crab juice is eaten with bean paste soup and in China "drunken crab" (live crabs soaked in wine overnight) is considered a delicacy [34,35]. Women in Korea and in Cameroon in Africa uses raw crayfish juice for the treatment of measles as folk medicine and for boosting fertility respectively [17 ,36,37].

In the northeast India, some communities use raw crab juice per oral as folk medicine for the treatment of fever, allergy, asthma, malnutrition and skin diseases. Sometimes, raw crushed crabs is applied topically to treat skin wounds and certain allergic conditions. In this case percutaneous transmission is possible as the metacercariae may excyst and migrate through subcutaneous tissue to cause infection. Infection can also occur when metacercariae are accidentally ingested through contaminated utensils, hands and fingers during the preparation of dishes like "Waikhumahithongba" a local Manipuri dish similar to "Sinigang" or "Kinagang" in the Philippines.

Another mode of transmission although rare is the consumption of raw or insufficiently cooked meat of paratenic hosts in which the parasite remains as juvenile worm. The juvenile worm if ingested by a definitive host can develop into adult. Pigs and white mice in China, wild boar in Japan and rats in the Philippines serve as paratenic hosts of *Paragonimus* [38,39,40].

***Paragonimus* species**

About 50 species including synonyms of the genus *Paragonimus* have been described [41] and 15 are known to cause infection in humans [42]. Among them, *P. westermani* is the most widely distributed in Asia. Recently, in Thailand, *P. pseudoheterotremus*, was described and proposed as a nominal new species but not specifically distinct from *P. heterotremus* [43] and this species has been described as causing human infection [44]. With this, the number of *Paragonimus* species in Thailand has gone up to eight species, namely, *P. heterotremus*, *P. westermani*, *P. siamensis*, *P. bangkokensis*, *P. harinasutai*, *P. paishuihoensis*, *P. macrorchis* and *P. pseudoheterotremus*.

P. pseudoheterotremus, on the basis of DNA sequences formed a sister group with *P. heterotremus* from northeast India [45]. In Sri Lanka, the occurrence of four species, namely, *P. westermani, P. siamensis, P. compactus* [46] and *P. macrorchis* [47] were reported. Iwagami et al (2007) based on the results of molecular phylogenetic study suggested that *P. westermani* species in SriLanka is an ancient divergence remarkably different from *P. westermani* occurring in southeast Asia and East Asia [48]. In Vietnam, occurrence of seven *Paragonimus* species namely, *P. heterotremus, P. westermani, P. skrjabini, P. vietnamensis, P. proliferus, P. bangkokensis* and *P. harinasutai* have been described. Of these, *P. heterotremus* has been found as the only human pathogen [49].

In India, the occurrence of seven species namely, *P. compactus, P. westermani, P. heterotremus, P. huet'ungensis, P. skrjabini, P. macrorchis,* and *P. miyazakiimanipurinus* n. sub spp. have been described till now. Currently *P. heterotremus*, and *P. westermani* are confirmed as human pathogens [50].

In Americas, two distinct species: *P. kellicotti* and *P. mexicanus* have been described exclusively in North America and in Central and South America, respectively. The role of *P. caliensis* in human paragonimiasis is still unknown [8].

Life cycle

The parasites utilize a wide variety of fresh water snails and crustaceans (crabs, crayfish and shrimps) as first and second intermediate hosts, respectively and complete the life cycle in permissive wild mammals of the Canidae and Felidae family and humans as definitive hosts (Figure 3).

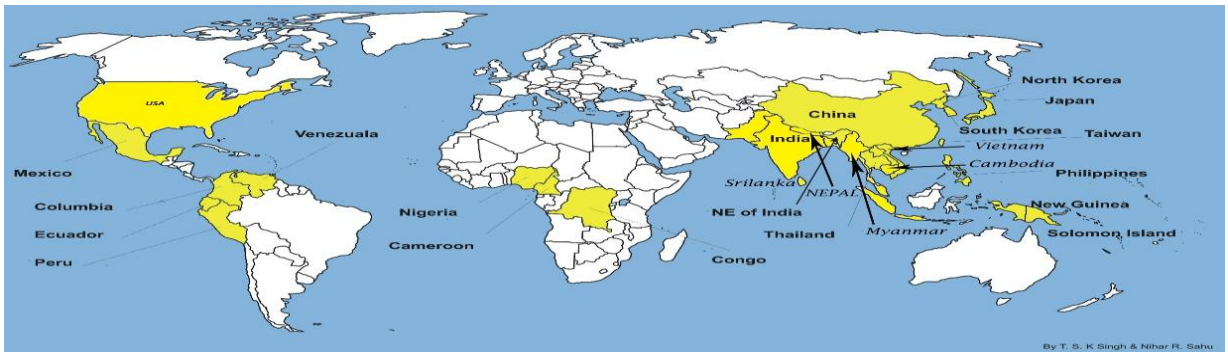

***Figure 1. Geographical distribution of paragonimiasis in the world*.**
[Yellow color indicates countries where paragonimiasis is endemic]

Figure 2a: ***Figure 2b:***
Fig- 2 Crab recipes

Figure 2a: Hand pounding of raw fresh water crabs for preparation of' "Waikhu Singju" common in northeast India.
Figure 2b: Fried fresh water crabs, Moreh market, India bordering Myanmar.

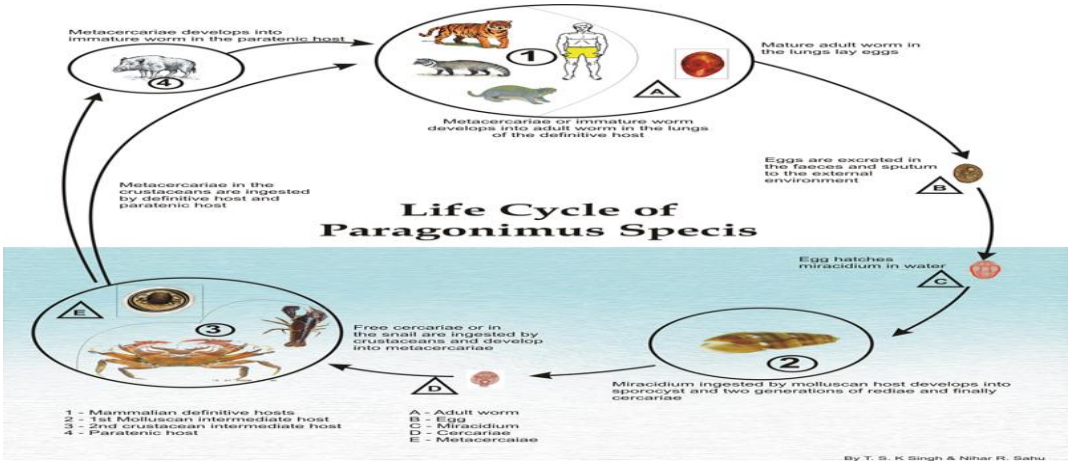

Figure 3: Life Cycle of Paragonimiasis Species

The life cycle begins with the eggs discharged by the adult worms in the lungs of definitive hosts via feces or sputum to the external environment. The eggs enter fresh water usually mountain streams or brackish water. The eggs require 2 to 3 weeks' time in water to complete embryonation and hatch ciliated miracidia [51]. The ciliated miracidium swims about in water to infect a permissive first intermediate snail hosts where it develops into a mother sporocyst and then asexual reproduction ensues through first and second generation rediae to cercariae. The time taken for the development of miracidium to cercariae within the snail host is 9 to 13 weeks [52,53]. The cercariae within 24 hours of release from the snail host must infect a permissive crustacean host [54]. The crustaceans are infected either by free cercariae directly penetrating its tissues or by ingestion of the infected snail host [55]. In the crustacean the cercariae develop into metacercariae within several weeks [50]. Generally, the metacercariae localize in the hepatopancreas, gills, intestine, skeleton muscles and sometimes, in the heart and pericardium of the crustacean, however, certain species may have predilection for certain tissue or organ for example, the metacercariae of *P. kellicotti* for the heart and pericardium and *P. caliensis* for hepatopancreas [56]. In India, *P. skrjabini* localize also in the pericardium and hepatopancreas. A single crab may be infected with metacercariae of more than one *Paragonimus*species simultaneously. It is presumed that once infected crustacean may remain infective

throughout life or until eaten by a permissive natural definitive host or human essential for the completion of life cycle. Following ingestion the metacercariaeex cyst larvae in the small intestine of the definitive host. The factors which cause metacercarial excystation in the intestine are not completely studied, but external cues present within the mammalian gastrointestinal tract have been described to signal excystation [57] and so also the role of bile salts and cysteine proteinases in excystation of metacercariae of *P. ohirai* [58]. Nevertheless, external environment factors outside crustacean hosts, temperature, oxygen tension, pH, etc. may significantly influence the excystment of metacercariae as they can excyst in the saline stored at room temperature or even during microscopy examination. The excysted juvenile worm penetrates intestinal wall and enters abdominal cavity in 3 to 6 hours [59]. Depending on the infecting species and host, the route of migration may vary, some species like *P. heterotremus* migrate from the peritoneal cavity to enter thoracic cavity via penetration of diaphragm. Some other species may have a "wandering phase", hence there can be a variety of ectopic infections (e.g. the liver, abdominal wall, omentum, and brain). However, most larvae return to the abdominal cavity after 5-7 days, thereafter, migrate through the diaphragm into the pleural cavity [60]. We also have observed several large cysts containing mature adult worms in the peritoneum and omentum of experimental albino rats infected with *P. skrjabini*metacercariae. In the pleural cavity, the juvenile worms wander for few days to find their suitable partners and on meeting enter into the lung parenchyma to form worm cyst where they mature into adults and start laying eggs, 5-6 weeks after the infection. However, some may remain in pleural cavity adhered to parietal or visceral pleura with or without cyst formation. The adult worms discharge eggs to the environment through sputum and or feces for further continuation of the life cycle.

Clinical manifestations: Clinically, paragonimiasis can be categorized into three main forms: pulmonary, pleuropulmonary and extra pulmonary.

Pulmonary paragonimiasis is the commonest form occurring in 76-90% of cases [4,27]. A higher rate of infection (94%) was reported in Korea by Jeon et al [61]. Major presenting symptoms comprised pain, difficult breathing, and coughing up rusty brown or blood-stained sputum or recurrent hemoptysis. Generally, pulmonary infection has high morbidity and low mortality unless complicated with infection in vital organs such as heart, and brain. Most importantly, the condition is very often misdiagnosed and treated as pulmonary tuberculosis and lung cancer.

Pleuropulmonary paragonimiasis occurs in approximately 6% of cases in which both lung parenchymal and pleural lesions are produced by the migratory worms. The clinical symptoms comprised pleuritic chest pain, fever, difficult breathing, and coughing up blood stained sputum or recurrent hemoptysis. A migratory paragonimiasis between the lungs and pleura accompanied by a benign arachnoid cyst was reported in a Chinese boy [62].

Extra pulmonary paragonimiasis occurs due to aberrant migrations of juvenile worms. Extrapulmonary infection commonly occur in pleurae, brain, skin, intra-abdominal organs, and genitalia, although any organ and tissue may be involved. Pleural effusion is fairly common and very often confused with tubercular or malignant causes. The effusion may be bilateral or unilateral, minimal to moderate, sometimes massive and persistent and rarely encysted [63]. The massive effusion without parenchymal lesion is rare [64]. Cerebral paragonimiasis is the most common extra pulmonary infection. This may be associated with serious complications and patient may die due to hemorrhage. The condition is usually associated with pulmonary or pleuropulmonary paragonimiasis. Cerebral paragonimiasis can be mistaken for tuberculoma or tubercular meningitis, tumor or other fungal or parasitic infections.

Cutaneous paragonimiasis is second most common after cerebral occurring in 16% of children [27]. This is usually presented as a painless slowly migrating subcutaneous nodule but in a few tender and non migratory. The diagnosis is often difficult or delayed until investigated appropriately [65]. Patient may also have subcutaneous swelling that might be diagnosed prior to the expression of a pleural effusion [66].

Paragonimiasis involving heart is very rare but a serious condition. Singh TS et al [27] have described a case of pleuropulmonary paragonimiasis involving heart in a child who developed congestive heart failure and died despite praziquantel therapy. There is limited documentation of colonoscopy findings of intestinal paragonimiasis [67]. Findings in cases of abdominal involvement may also include palpable masses [68]. The worms can also reach other tissues such as striated muscles which may lead to rupture of the cyst and inflammatory reaction presenting like allergic reaction [69]. In ocular infection clinical signs including impaired visual acuity because of optic atrophy, papilledema, and hemianopsia have been described [68]. Scrotal paragonimiasis may mimic epididymitis or an incarcerated hernia [68]. Extra-pulmonary manifestation of arthritic in nature is rare, clinical presentation may be extensive recurrent pruritic urticarial subcutaneous induration, permanent asymmetrical pauciarthritis associated with joint swelling [70]. Lymphadenopathy is apparently an uncommon finding in patients with paragonimiasis.

Laboratory diagnosis

Microscopy demonstration of *Paragonimus*eggs in the clinical samples is the gold standard of laboratory diagnosis of paragonimiasis. However,

the test is less sensitive compared to immunological and molecular techniques. The eggs can be detected in the sputum, fecal specimens, pleural fluid, CSF and biopsy tissue or cyst or at necropsy. Juvenile worm or adult may be found in the biopsy material and rarely, adult worm may be expectorated in the sputum [71]. Over the years, highly sensitive and specific serological tests have been developed but the availability is limited within the country where they are manufactured. Radiological and other imaging techniques are extremely useful tools but adjunctive for the diagnosis of paragonimiasis. CBC as a routine test provides valuable information such as eosinophilia, leukocytosis and raised ESR although nonspecific are usually associated with acute and subacute paragonimiasis.

Sputum microscopy: Early morning sputum samples are ideal for microscopy detection of parasite eggs as the expectoration of the overnight secretion that have accumulated in the respiratory tract is expected to contain more eggs than any other samples collected during day time. The rusty brown or bloody or blood-stained sputum usually contains numerous *Paragonimus* eggs and Charcot-Leyden crystals. The *Paragonimus*eggs are characteristically oval shaped golden brown, and operculated measuring 80 to 90µm x 50 to 55 µm in size. The sensitivity of microscopy is generally low as single sputum specimen has a sensitivity between 30 and 46% [4,34,61,72]. Singh et al found *Paragonimus*ova in the sputum specimens of 55.6 to 72% of pulmonary paragonimiasis cases [4]. A positivity rate varying from 6.5 to 34.6% in endemic areas and 0% in non-endemic areas was reported by Yokogawa et al [73] *Singh TN,* et al found ova positive sputum in 20.9% and 4.1% of pleuropulmonary paragonimiasis in children and adults, respectively [74]. The finding, however, is unusual because sensitivity of sputum examination is expected to be higher in adults than in children who generally swallow sputum. It is recommended that at least three consecutive morning samples should be examined before declaring a negative test. It is a good laboratory practice to examine the sample first by direct wet mount and if negative by sodium hydroxide concentration. Charcot Leyden crystals (CLC) and eosinophils are usually present in the sputum even in *Paragonimus* eggs negative sample [4,75]. Therefore, positive CLC is strongly suggestive of pulmonary parasitic infection requiring extensive search for *Paragonimus* eggs in multiple sputum samples and serological tests for paragonimiasis.

Pleural fluid: *Paragonimus* ova can be detected in the centrifuged deposit of pleural fluid in about 10% of pleural effusion cases [76]. Examination of repeat samples will increase the positivity rate. In the absence of demonstrable egg, pleural fluid analysis showing glucose content less than10mg/dl, lactose dehydrogenase greater than 1000 I.U. /liter, high-protein value, low pH and eosinophilia is strongly indicative of pleuropulmonary paragonimiasis [77].

Stool: Stool examination for *Paragonimus* egg is recommended in children who usually swallow sputum and in patients whose sputum samples are egg negative. Ideally, two to three stool samples collected at consecutive days should be examined by formalin-ether sedimentation or AMS III concentration technique; recent study demonstrated that the latter technique led more accurate results [78]. Studies have shown that the sensitivity of a single stool examination varied from 11 to 15% [72,79]. Singh et al, reported a sensitivity of 25.6% of which 60% were children aged ≤ 10 years on examination of three repeat stool samples [4].

Biopsy: Excision biopsy serves both diagnostic and therapeutic purposes. Adult or immature worm may be found in a carefully dissected nodular or cystic lesion unless the worm has migrated. Microscopy examination of the exudates will show inflammatory cells, eosinophils, *Paragonimus* egg and Charcot-Leyden crystals. Histopathological slide will reveal fibro collagenous tissue, inflammatory cells, eosinophils, ova and in some cases sections of the worms.

Serologic Tests:

Serological tests are important for the diagnosis of pulmonary, ectopic and early infection (prepatent period) paragonimiasis where eggs are not demonstrable by microscopy and for differential diagnosis. Some of these tests may be used by clinicians and researchers for evaluating the therapeutic responses to specific chemotherapy. It is the only test to establish diagnosis of paragonimiasis presented with eosinophilia due to while investigating eosinophilia of unknown etiology. The serological tests which have been developed and fully evaluated for the diagnosis of paragonimiasis are intradermal test (ID), complement fixation test (CFT), immunodiffusion, indirect hemagglutination test (IHA), enzyme-linked immunosorbent assay (ELISA), dot-ELISA, dot-immunogold filtration assay (DIGFA) and Western blot. Newer tests that have been developed in the recent years but not fully evaluated are Immunochromatography test (ICT) and immunoblotting technique.

ID: The intradermal test is a simple and highly sensitive test that was popularly used over the past several years in Japan [80,81] China [73] and in India [5] for diagnosis and mass screening in the field. It is an immediate type of hypersensitivity reaction. The test utilizes saline extract or purified protein of adult P. westermani as test antigen. After inoculation of the test antigen the wheal diameters are measured immediately and 15 minutes after the inoculation. A differential wheal diameter of ⩾5mm with erythema and pseudopodia indicates a positive test. Whereas a negative skin test rules out paragonimiasis, a positive test cannot differentiate between the past and the present infection as the test may remain positive as long as 10 to 20 years even after the successful chemotherapy or spontaneous recovery [82]. The sensitivity and specificity of the test can be

up to100% by using purified fractionated antigen [83].

CFT: It has been used in the diagnosis of active infection and to confirm ID positive cases. The test becomes negative within 3 to 9 months after successful treatment [84]. In epidemiological survey, it has been recommended that ID test should be applied first followed by CFT or any other more specific test on individuals who showed positive or doubtful dermal reactions.

Immuno-diffusion: This method was first developed by Biguet et al in 1965 [83]. Double immune diffusion technique (Ouchterloney method), immune electrophoresis and counter current immunoelectrophoresis are reported to be highly sensitive and specific and can be used for speciation by demonstration of specific precipitin bands [84,85,86].

IHA: This is another simple, rapid and sensitive test. In Thailand, the test revealed a sensitivity of 88% in the diagnosis of paragonimiasis heterotrema [87].

ELISA: Quicho et al (1981) first developed the ELISA test for the diagnosis of paragonimiasis in Thailand. 88 Since then ELISA based on different techniques and with different antigen preparations have been developed and evaluated for diagnosis of paragonimiasis [89,90,91,92]. The overall specificity of IgG ELISA using the saline extract of adult worms as an antigen was found to be 97%. A 100% sensitivity and specificity could be obtained in an indirect ELISA using F1 antigen fraction to detect antibody against *P. heterotremus* infection [93]. An enzyme-linked immuneelectro transfer blot has been developed for differential diagnosis between *P. heterotremus* and *P.westermani* infections [94]. The technique involved makes use of 35-kDa antigenic components for the corresponding species. Other ELISA techniques are sandwich ELISA using monoclonal antibodies-based antigen detection assay [95] and multiple dot-ELISA [91]. Generally, ELISA tests are used to detect parasite specific IgG antibodies but the detection of specific IgE antibodies was proven to reduce cross reactions with other trematode infections and detection of parasite specific IgM antibodies was recommended in the diagnosis of infection at the early stage [96, 97]. In India, IgG/IgM ELISA using E/S antigen for diagnosis of paragonimiasis has been developed and is found to be 100% sensitive and specific [98]. It has been stated that analysis of IgG subclass can increase the specificity and sensitivity of the immunological assays for the diagnosis of paragonimiasis [99,100]. The ELISA tests are now most widely used for serological diagnosis of paragonimiasis due to its high sensitivity and specificity. The tests are also applicable to mass screening. However, ELISA tests are more expensive, time-consuming and require costly equipment and experienced persons and all the reagents, antigens, in particular, are not commercially available.

Rapid test: Recently, Dot-Immunogold Filtration Assay (DIGFA) kit was developed in China for anti-*P. westermani* antibody detection. The kit was based on the principle of a membrane-based flow-through immunoassay technique. This kit was prepared using *P. westermani* adult worm soluble antigen and anti human IgG labelled with colloidal gold as color developing agent. Reagents are stable at 4ºC for at least a year. A dot containing diluted human serum or similar is used as a positive control. Serum from patient is added to the nitrocellulose membrane then anti-human antibody or protein A conjugated with colloidal gold is added which forms a colored spot where it reacts with the antigen-antibody complex [1]. It was reported in China to have the sensitivity and specificity up to 99% and 92%, respectively [101]. The DIGFA method is said to be better than ELISA because it is simple and rapid, it does not require any special devices and/or experienced technicians and the results will be obtained within 10 minutes and exhibit comparable sensitivity and specificity [102].

Haematological investigation: Leucocytosis with relative lymphocytosis, eosinophilia and increased ESR were common findings in patients with paragonimiasis. Leucocytosis (WBC: 10,200 to 16,350 per mm3) and eosinophilia (eosiniphils: 650 to 4000 permm3) were found in 62% (24/39) of patients with pulmonary paragonimiasis [4]. Eosinophilia and increased ESR up to 104 mm at the end of 1st hour (Westergren) were consistently found in children with paragonimiasis in Manipur [27]. Eosinophilia has been found more pronounced in acute and subacute paragonimiasis, especially in pleural effusion, cerebral and cutaneous paragonimiasis in children.

X-rays and other imaging technique: The common radiographic findings are patchy air-space consolidation or opacity with associated pleural reaction or thickening, cystic or cavitary lesions, pleural effusion and nodular opacities which are indistinguishable from tubercular lesions. Computerized axial scan (CAT) and Magnetic resonance imaging (MRI) are very useful for the detection of lesion in the brain and to a great extent specific diagnosis of cerebral paragonimiasis. The most common and characteristic CT or MRI imaging findings are conglomerate, multiple ring-shaped, enhancing lesions with surrounding edema of variable degree. The lesions consist of aggregates of ring like enhancing lesions, resembling 'grape clusters' representing multiple contiguous abscesses like granulomas [103,104]. Each ring is usually smooth and round or oval ranging from few millimeters to more than 3 cm in diameter. The center of the ring shows a density/intensity similar to or slightly higher than that of cerebrospinal fluid on both CT and MRI images of all pulse sequences. The wall of each ring is usually isointense relative to brain parenchyma on T1-weighted MR images and isointense or hypointense on T2 weighted MR

images [104]. It was also noted to mimic granulomatous lesion (tuberculoma) in the some cases [105].

Molecular diagnosis: Application of molecular diagnosis is very limited in clinical practice though it is important for the researchers. DNA Probes are highly sensitive and specific, 1500 bp long, was characterized for *P. heterotremus.* The labeled probe could detect DNA from as few as five eggs with 100% specificity but with low sensitivity [106]. In PCR technique, eggs, either in sputum or in feces are the target for DNA extraction and analysis. The nuclear ribosomal region ITS2 is possible to amplify. A loop mediated isothermal amplification (LAMP) protocol has been established for *P. westermani*. LAMP is said to be far more sensitive than conventional PCR and can possibly be applied for pleural fluid. 102 The approach uses four species- specific primers that recognize six regions in the target DNA. The reaction can be completed in an hour at a single temperature (60ºC) and a simple in tube visualization of successful amplification is possible. No complicated equipment is needed. It could be applied to field identification of metacercariae and eggs, provided a species-specific set of primers are available [102,107].

Prevention and control:

The control of paragonimiasis in animals is not feasible, as the genus of this parasite has widespread distribution in a variety of carnivorous and omnivorous animal hosts. The control of the snail and other crustacean intermediate hosts is also not practicable as it may have unwanted consequences in the ecosystem. However, control of human paragonimiasis is possible to a greater extent through Behavioural Change Communication (BCC) activities which might help people engaged in high risk behaviours to changes their customs and food preparation practices. The success to date in substantially reducing infections in countries that traditionally have high incidences of infection has been directly related to educational efforts. To educate the population at risk in endemic areas on the mode of transmission and life cycle of the parasite is of paramount importance. The future efforts of prevention and control of paragonimiasis will have to employ previously used highly effective educational methods including food hygiene. Proper food preparation strategies should include frequent hand washing when cleaning crustaceans, avoiding contamination of utensils 17 and serving platters with live metacercariae from the infected crustaceans, and boiling or cooking crustaceans to reach an internal temperature of 145°F (63°C) before eating [108]. Advisory for the travelers to endemic areas should include not to eat raw or undercooked fresh water crabs and crayfish. Although vaccination is conceivably possible, it will not likely occur in the near future, given the more pressing need for vaccinations for more prevalent, medically severe, and economically impactful infections [57]. A key but neglected component of prevention and control is research, more specifically the research of everyday human behaviors including their dietary habits.

Conclusion:

Paragonimiasis continued to be a public health problem with the discovery of new endemic foci in North America, and the Asian countries in the recent years. Pulmonary paragonimiasis has often been misdiagnosed and treated as smear negative or MDR pulmonary tuberculosis due to similar clinical and radiological features of the two diseases. A detailed history taking including the dietary habit of consumption of raw or undercooked crustaceans and thorough physical examination are important to appropriate laboratory investigations for a definitive diagnosis. Paragonimiasis can be confirmed by microscopy demonstration of Paragonimus eggs in the sputum smears or specific serological tests. It is recommended that while sputum smears should be screened for both Acid Fast Bacilli (AFB) and the Paragonimus eggs in order to avoid over diagnosis of smear negative pulmonary TB. Control strategies includes Information, Education and Communication (IEC) and Behavioral Change Communication (BCC) activities regarding safe food practices, improved sanitation, and awareness program about paragonimiasis especially among the high risk population.

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