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Description of Two New Species of Avain Haemoproteidae -9, *Haemoproteus zhobensis* Sp. Nov. and *Haemoproteus balochiorum* Sp. Nov from Cranes (Gruidae) in Pakistan

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Abstract: Two new species of Haemoproteids, *Haemoproteus zhobensis* (*H. zhobensis)* sp. Nov and *Haemoproteus balochiorm (H. balochiorum)* sp. Nov are described from the cranes *Anthropoides virgo* (L) and *Grus grus lilfordii* (Sharpe) with the incidence of 0.86% and 1.56% respectively, collected from Balochistan and the tribal areas of North West Frantier Province (N.W.F.P).

Key word: Birds · Haemotology · Protozoan · Parasite · Host Species

INTRODUCTION

Although an extensive literature is available on the haematozoon of birds [1], however relatively very little sporadic work has been done on haematozoa from birds, furthermore least studies are available in Asia [2]. There is no specific survey work has been planned to study these parasites in birds and particularly from Balochistan, Pakistan. Nothing has so for been reported on Haematozoa, an area which is fanatically interesting because of its geographic resemblance to Palaeortic Region and concurrent possession of some unique migrant avifauna [2-5].

Relatively few haemoproteids are reported from the avian family Gruidae. The only record from Gruidae is *Haemoproteus antigonis (H. antigonis*) de Mello, 1935 from Anthropoidis virgo from India Bhatia [6]. Pierce [7] described *Haemoproteus balaericae(H. balaericae*) from the crowned crane, *Balearica pavonina* in West Africa. Many unidentified and incomplete described haemoproteids have been reported by various authors in or from Africa [8] nothing has been reported from the rest of the world including Pakistan. Haemoproteids are family specific and on the basis of few recognizable morphological characters the species can be identified upto species level, [9]. Key to the identification of Haemoproteids species in Gruidae [10, 11] is also revised

MATERIALS AND METHODS

A total of 180 cranes, 116 *Anthropoides virgo* (L) and 84 *Grus grus lilfordii* were examined during the trapping/hunting season of cranes in Balochistan and the tribal areas of North West Frantier Province (N.W.F.P) [2-5] Blood smears were prepared , air dried, fixed in 100% ethyl/methyl alcohol and Giemsa's Stain was used. Microscopic examination and screening of blood slides were done using 100X lens. Dimension of the parasites (Macro; and micro gametocytes) were measured as described by Bennett and Campbell, 1972 and result, are represented means +S.D. Micro photographs were taken with NIKON-HFX-11 Photomicroscope with the magnification of 100 objective X 10 eye piece X 5 instrument=5000X. All measurements are in microns. "N" represents number of specimens examined. NDR= nuclear displacement ratio. Permanent slides and photographs are with the senior author, Parasitology section, Zoology Department, Baluchistan University, Quetta. Pakistan.

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RESULTS

Family: Haemoproteidae Doflien, 1961
Genus: Haemoproteus Kruse, 1890.
Type species: *Haemoproteus zhobensis* sp. nov
Type locality: Zhob, Balochistan
Type Host: *Anthropopoides virgo* Linnaeus, 1758 (Demoiselle Crane)

Description: Acrogametocyte (Fig.1and Table 1, 3) N=7 Matured gametocytes sausage–shaped; not extending over host cell nucleus. Outline entire not amoeboid. Host Cell nucleus markedly replaced laterally; parasite attached with nucleus; cytoplasm finely granular deep blue with Giemsa's stain; pigment granules prominent scattered randomly throughout cytoplasm usually fused with one another. Parasite not reaching ends of host cell. Pigment granules ranging from12-16 per parasite and averaging 14.00(0.00) parasite measured 10.6 (0.43) in length and 3.8 (0.2) in width and 40.90 (1 .23) in area. Nucleus diffused and not seen. Hypertrophy not marked. The host cell nucleus markedly dislodged laterally; NDR = 0.30 (0 .01).

Microgametocyte: (Fig. 2) Sausage-shaped, cytoplasm pale blue with Giemsa's stain NDR = 0.40; other characters as for macrogametocytes.

Incidence of Infection: Out of 116 Cranes only once crane harboured this parasite it. It was present with mixed infection of *Puasmodium elongatum* and *Puasmodium relictum*.The incidence was 0.86%.

Intensity of infection: Only few matured gametocytes were observed. The intensity was 1/20000 RBC.

Remarks: It is the only sausage-shape haemoproteid described so from Gruidae. *H. antigonis* is slightly halteridial but larger in size occupying about 80% of the host cell, while this species is smaller in size sausage-shape and occupies about less than 50% of the host cell with altering the host cell.

It was also compared with newly described species *H. balochiorum* (Fig 3).The difference were noted in shape and size of granules, covered area, number of pigment granules and NDR. One the basis of above remarks it was considered as new species and the name *Haemoproteus zhobensis* was proposed for it on the basis of locality from where the host of this new species was collected (Table 1).

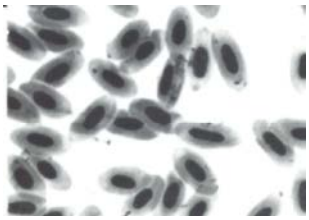

Fig. 1: Macrogametocyte of H.zhobensis sp.nov.

Fig. 2: Microgametocyte of H.zhobensis sp.nov.

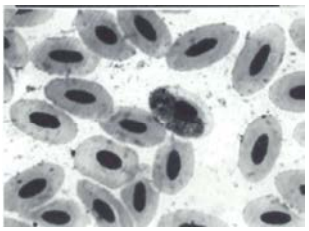

Fig. 3: Macrogametocyte of H. zhobensis sp.nov.

Type species: *Haemoproteus balochiorm* sp, nov,
Type locality: Zhob, (Balochistan)
Type Host:*Grus grus lilfordii* Sharpe, 1894 (Common crane)

Description: Macrogametocyte(Fig. 3,4 Table -2,3)N=10 Maturedgametocytes sausage-shaped; outline entire not amoeboid; parasite lateral to host cell nucleus; host cell nucleus slightly displaced laterally; parasite not extending oversurface of host cell nucleus; cytoplasm course,. staining deep blue with Giemsa's stain; pigment granules prominent not rounded; rod shaped; randomly distributed in cytoplasm; ranging from 10-14 and averaging 12.2 (0.13) per parasite; parasite nucleus median, .large, broadly ovoid; averaging 3.35(0.03) in length 2.55 (0.11) in width and 8.72 (0.59) in area; staining pale to deep pink with Giemsa's stain.

Parasite measures 15.2 (0.07) in length, 5.65(0.05) in width and 8.10 (1.73) in area; constituting about50% host parasite complex; host cell nucleus slightly displaced laterally; nucleus displacement ratio (NDR) = 0.45.Host cell hypertrophied 9.15% in length, 13.37% in width and 25.36 in area.

Table 1: Dimensions of the *Haemoproteus zhobensis* sp. n. All measurements based on 7 parasites and normal erythrocytes from one host of the same species. Figures in parenthesis are standard deviation. All measurements are in microns.

	Erythrocyte			Parasite				
	Length	Width	Area	Length	Width	Area	No. of granules	NDR
Parasitized	18.8 (0.28)	6.8 (0.22)	92.22 (2.05)	10.6 (0.43)	3.8 (0.2)	40.90 (0.123)	18.00 -	0.30 -
Non-parasitized	13.8 (0.18)	6.6 (0.22)	91.5 (4.58)	-	-	-	-	-

Table 2: Showing morphometric measurements of *Haemogroteus balochiorum* sp.n. All measurement based on 10 parasites and normal and parasitized erythrocytes from 5 hosts of the same species. Figures in parenthesis are standard deviation. All measurements are in microns.

		Parasitized BRC		Parasite	
Measurements	Normal RBC	Macrogametocy	Microgametocyte	Macrogametoc	Microgametocyte
Length	13.9 (0.19)	15.2 (0.07)	15.13 (0.08)	15.2 (0.07)	14.13 (0.40)
Width	7.55 (0.03)	8.56 (0.13)	8.63 (0.05)	5.56(0.05)	4.68 (0.07)
Area	105.04(0.65)	131.68 (0.55)	130.29 (1.26)	86.10 (1.73)	71.08(1.52)
Nucleus					
Length	5.78(0.21)	5.05(0.02)	5.38 (0.04)	3.35(0.03)	-
Width	2.01 (0.002)	1.86 (0.04)	1.93(0.03)	2.55(0.11)	-
Area	11.60 (0.15)	9.47 (0.24)	10.37 (0.11)	8.72(0.59)	-
No. of granules	-	12.2(0.13)	12.00(0.00)	12.2(0.03)	-
NDR	1.0	0.45	0.62	0.45	0.62
N	40	5	40		5
% hyprotrophy in					
Length	-	9.15	8.84	-	-
Width	-	13.37	14.30	-	-
Area	-	25.36	24.04	-	-

Table 3: Dimensions of sausage shaped haemoproteids in Gruidae

		Erythrocyte			Parasite					
		Length	Width	Area	Length	Width	Area	No. of granules	NDR	N
Haemoproteus antigonsis	Parasitized	13.5 (0.00)	7.14 (0.05)	96.46 (0.55)	14.4 (0.20)	3.34 (0.05)	48.2 (1.37)	20.4 (0.30)	0.90	10
Host. *Anthopodis virgo*	Non-parasitized	13.8 (0.28)	6.6 (0.22)	91.5 (4.58)	- -	- -	- -	- -		
Haemoproteus zhobensis	Parasitized	13.8 (0.28)	6.8 (0.22)	92.22 (2.05)	10.6 (0.43)	3.8 (0.2)	40.90 (1.23)	14.00 (rounded) 0.40	0.30 (0.01)	7
Host. *A.virgo*	Non-parasitized	13.8 (0.28)	6.8 (0.22)	91.5 (4.58)	- -	- -	- -	- -		
Haemoproteus balochiorum	Parasitized	15.2 (0.07)	8.56 (0.13)	131.68 (0.55)	15.2 (0.07)	5.56 (0.05)	86.10 (1.73)	12.2(rod-like) (0.13)	0.45	10
Host. *Grus grus lilfordii*	Non-parasitized	13.9 (0.19)	7.5 (0.09)	105.04 (0.06)	15.2 (0.09)	5.65 (0.05)	86.10 (1.73)	12.2 (0.13)	0.45	10

Microgametocyte: (Fig. 3, 4, 5 Table-2,3) N=10 Matured gametocytes sausage-shaped; cytoplasm pale pink in colour with Giemsa's stain; parasite nucleus exceptionally diffused and not observed;NDR=0.62.Other characters as for macrogametocytes.

Incidence of Infection: Out of 64 cranes (*G.g. lilfordii*) Examined, only one crane harbored this parasite.Both micro; and macrogametocyte was observed. The incidence was 1.56%.

Intensity of Infection: Moderate infection was observed both Macrogametocyte and microgametocyte were present with the ratio of 1: 3 respectively.The intensity was 1-2/2000 RBC.

Remarks: Very few haemoproteid have been reported from family Gruidae. This is due to less number of canes examined in the world. During the present study 180 cranes were examined for observation of haematozoa; comprising 64 *Grus grus lilfordii* and 116 were

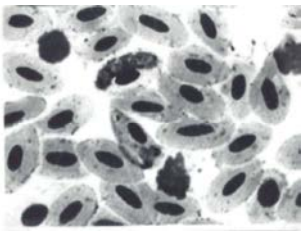

Fig. 4: Macrogametocyte of H.balochiorum sp.nov.

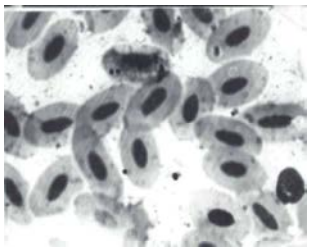

Fig. 5: Microgametocyte of H.balochiorum sp.nov.

Anthropoides virgo. Uptill now it is the highest record of cranes examined for haematozoa in the world, The only sausage-shaped haemoproteid recorded from Gruidae is *Haemoproteus antigonis* which is very slightly halteridial constituting about more than 80% of the host cell. The species under-study is smaller in sizeconstituting about 40-50% of the host parasite complex with very little NDR value and also possesses prominent rod shaped granules in the cytoplasm. It also differ from the newly described sausage shaped *Haemoproteus zhobensis* species in morphometric measurements, shape and size of granules, in NDR and with new host record (Table 2,3). On the basis of above specific characters it is considered as distinct species and the name *Haemoproteus balochiorum* is proposed for it on the basis of first record from Balochistan, Pakistan.

Key to the identification of Haemoproteus species from the family Gruidae (cranes modified after Bennett, 1975)

- Parasite highly amoeboid in outline 2
- Parasite not amoeboid in outline but entire 3

Outline usually broken slightly amoeboid.

- Parasite constitute more than 50% of the host cell-parasite complex with 18-24 (average 20-4) number of granules……*H. laekii* sp.nov.

Outline highly amoeboid, parasite constitute less than 50% of the host cell-parasite complex with number of granules 7.6 (average ……*H.balaericae*.

- Parasite sausage-shaped ……4
- Parasite halteridial……..5
- Parasite broadly sausage shaped, constitute more than 80% of the host cell parasite complex with 18-20 number of rounded granules, host cell nucleus markedly displaced towards periphery ……*H. antigonis*.
- Parasite sausage-shaped, smaller in size, constitute less than 50% of the host cell parasite complex with 12-16 number of granules ……*H. zhobensis* sp.nov.
- Sausage-shaped, constituting more than 50% of the host cell, granules rod-shaped ranging from 10-14 in number……..*H. balochiorum* sp. nov.
- Mature parasites normally totally encircle host cell nucleus, ends of parasites appearing to fuse; host cell nucleus not displaced ……..*H. telfordii*.
- Mature parasite not encircling the host cell nucleus, ends of parasite never fuse, host cell nucleus very little displaced ……*H.anthropoidii* sp. nov.

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