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Infection of Haematozoan Parasites Found in Birds of NWFP (Pakistan)

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Abstract: A total blood films of 489 birds were made from different spots of NWFP (Pakistan) belonging to Falconiformes, Galliformes, columbiformes, Psithaciformes, Piciformes and Passeiformes were examined, *Plasmodium relictum* was found in sparrows, incidence was 23%. *P. gallinaceum* (7.27%) and *P. Juxtanucleare* (0.91%) was found in chickens. *P. elongatum* was carried by partridges (2.9%) *Haemoproteus columbae* was found in Pigeons (12.2%) in sparrows (17%) and in Myna (12.50). Two unidentified microfilariae were recovered form sparrows and Myna. Eagles, Doves, Parrots, woodpeckers, Crows swallows and Ducks were also examined for parasitic infection but found refractory.

Key words: *Plasmodium elongatum, Plasmodium relictum, Plasmodium juxtanucleare, Plasmodium gallinaceum, Haemoproteus columbae*

INTRODUCTION

It is a well established fact that haematozoa cause several disease which affect the death rate of the wild life and also disturb the function of the haemopoietic system, prevalence of blood parasites in avian population has been widely recorded in the literature over the past 110 years. Interest in the naturally occurring avian malaria began since the classical work of Grassi and Feletti[1]. This significant work was followed by a series of reports on occurrence of avian malaria in nature as observed by many earlier workers. While-more isolated *Plasmodium relictum* from an sparrow in New York. Huff[2] first described the species *P. elongatum* from a canary and sparrow from USA.

The second comprehensive review on avian malaria was published by Huff[3]. Hartman[4] observed three species of Bird Malaria many other workers Givannola[5], Coatney[6], Bhattia[7], Fallis[8], Becker[9], Garnham and Black well[10] have examined the prevalence of malaria and Haemosporidia in birds. Anderson[11] studied the *Ornithofilaria* sp. from domestic Duck and Microfilaria in water fowl Sarwar[12] on some spirurid and Filariid Nematodes of Birds in Pakistan. Mohiuddin[13] observe *Microfilaria turdoidis* sp. n from the Jungle Babbler of West Pakistan. Lapage[14], Admn *et al.*[15] and Baker[16] studied on blood parasites of birds and mammals. Shahabuddin and Kaslow[17], Joseph *et al.*[18] worked on chitinases of the Avian Malaria parasite *P. gallinaceum* a class of enzymes necessary for parasite.

The present study was carried out to observe the blood parasites of naturally occurring birds of NWFP as a result of this investigation five species of malaria parasites (plasmodium), one specie of Haemoproteus and two unidentified species of microfilaria were observed in 83 birds belonging to 15 species.

MATERIALS AND METHODS

A total of 489 blood smears of different species of birds (i.e. 15 species of birds) were collected form different areas of NWFP in the month of June and July 2002.

Birds were shot with an air gun, blood was taken directly from the heart however in live birds the blood smears were made by pricking and taking a drop of blood from brachial vein of avian hosts.

The blood smears were made on the clean sterilized slides. Slides were cleaned with 70% alcohol. Mostly these blood films were made which after fixing with methyl alcohol were stained with Giemsa. Stain (BDH) at a concentration of 2 drops of stain in one cc of buffered distilled water (pH 7.2). Stained blood smears were examined under oil immersion (x100). A thorough examination of each slide was made from 20/30 fields.

The diagrams of the parasites were drawn by the help of camera lucida and the measurement of parasites infected and normal RBC were taken by ocular micrometer on an calibrated microscope.

RESULTS AND DISCUSSION

A total of 83 out of 489 birds have shown the infection with haematozoa having a percentage of infection approximately 16.97. The density of infection in heavily infected birds ranged from two to four parasites per x100, objective field. In majority of birds a single plasmodium species appeared.

The main criteria for identification of species were based on diagnostic characters described by Garnhum[110].

Family : plasmodidae
Genus : plasmodium

***Plasmodium relictum*:** Trophozoites were rounded measuring 2.08 x 2.86 micron. Schizonts were some what rounded or irregular in shape size: 7x4-7 micron. Gametocytes were rounded or irregular measuring 6.95x5.5-6.85 micron. Microgametocytes were more rounded and have pale cytoplasm with central diffused nucleus, around which scattered black pigment granules of irregular shape. Macrogametocytes have coarser cytoplasm with much pigment in round black dots. Schizonts and gametocytes displaced the host cell nucleus (Fig. 1). Single infection of *P. relictum* was observed in five species of avian host having an incidence of 36.38 (Table 1).

Mixed infection of *P. relictum* with *P. elongatum* was observed in four species of avian host having an incidence of 50.7, *P. relictum* with *Haemoproteus columbae* have an incidence 1.27 (Table 2).

***Plasmodium elongatum*:** Trophozoites were oval or elongated with ragged out line, no pigment granules. Schizonts were spherical have 6-10 elongated merozoites with pointed end. Gametocytes were elongated with

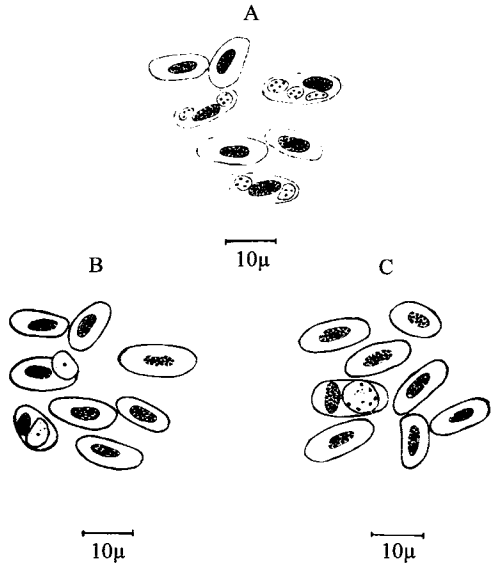

Fig. 1: *Plasmodium relictum*
A. Schizonts, B. Microgametocyte,
C. Macrogametocyte

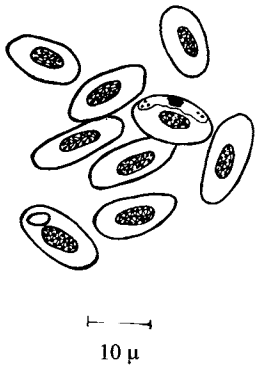

Fig. 2: *Plasmodium elongatum*
Microgametocyte

irregular internal margin. Microgametocytes were long, do not curve much round the host cell nucleus, diffused nucleus (Fig. 2). 1-2 large rounded granules lie at both or one ends measuring 8.34-9.73×1.39-2.0 micron. Macrogametocyte differ in staining reaction, pigment similar but consist up to 20 dark grains.

Single infection of *P. elongatum* was found in six different species of avian host having 28.7% (Table 1). *P. elongatum* found mixed infection with *P. relictum*. Mixed infection of *P. elongatum* with *P. relictum* found in four different host and has an incidence of 50.7, *P. elongatum* also found mixed with *P. circumflexum* with an incidence of 100 (Table 2).

***Plasmodium circumflexum*:** The most stricking diagnostic features were the growing gametocytes forming broken rings around the nuclei of infected erythrocytes. Pigments were few coarse and granular. The *P. circumflexum* was found in two different species of avian host. Incidence of single infection of *P. circumflexum* was 50 (Table 1) while incidence of mixed infection of *P. circumflexum* with *P. elongatum* was 100 (Table 2).

Plasmodium juxtanucleare: Parasite very minute in size and embedded in the nucleus of host cell (Fig. 3). Gametocytes were oval, microgametocyte have larger and diffused nucleus while macrogametocyte have small compact. Pigment was in the form of dark brown black granules, 1-4 in numbers, *P. juxtanuclear* was found only in one chick and incidence of infection was 0.9 (Table 1).

***Plasmodium gallinaceum*:** Young parasite was oval lying at one pole or laterally in R B C. Trophozoites were irregular but oval (Fig. 4). Pigment localized at one extremity. Pigmets were black with golden luster.

Table 1: Incidence of haematozoan infection in birds of NWFP (Pakistan)

Avain host	Locality	Sp. of Parasite	Positive total	%
Coturnix Coturnix (quail)	D.I. Khan	*P. elongatum*	2/64	3.10
Saxicola Torquata rubicola (Chat)	Bannu	*P. relictum*	1/3	33.30
		P . elongatum	1/3	3.33
Red sparrow	"	*P. relictum*	2/2	100.00
Upupa epops epops (Had hud)	"	*P. relictum*	1/3	33.30
Pyononotus cafer intermedius (Bul bul)	Peshawar City	*P. relictum*	2/15	13.30
		P . elongatum	1/15	6.60
Brown spotted owl	"	*P. circumflexum*	2/4	50.00
Acridotherus Tristis (Myna)	"	*P . elongatum Haemoproteus*	2/16	12.50
		columbae	1/16	6.20
		Microfilaria	3/16	18.70
Passer domesticus	"	*P . elongatum*	28/158	17.40
		P. relictum Haemoproteus	3/158	2.00
		columbae	9/158	5.70
		Microfilaria	4/158	2.50
Mallard	"	*P . elongatum*	1/1	100.00
Columba livia	"	*Haemoprotaus*	11/90	12.20
Gallus domastica	"	*P. juxtanuclear*	1/110	0.90
		P. gallinaceum	8/110	7.20
Parrots	"	Nil	0/4	0.00
Anas sp. (Duck)	"	"	0/9	0.00
Carvus sp. (House crow)	"	"	0/7	0.00
Aguila sp. (Eagle)	"	"	0/3	0.00

Table 2: Mixed infection of haematozaon parasites found in birds of NWFP (Pakistan)

Avain host	Locality	Mixed infection	Positive total	%
Coturnix Coturnix (quail)	D.I. Khan	*Plasmodium elongatum* with *P. relictum*	1/64	1.56
Saxicola Torquata rubicola (Chat)	Bannu	*P. elongatum* with *P. circumflexum*	1/1	100.00
Domoiselle Crane (Koony)	"	*P. elongatum* with *P. relictum*	1/1	100.00
Starling	Peshawar City	*P. elongatum* with *P. relictum*	1/1	100.00
Passer domesticus	"	*P. elongatum* with *P. relictum*	2/158	1.27
		P. relictum with *Haemoproteus columbae*	1/158	1.27
		Haemoproteus columbae with *Microfilaria*	1/158	0.64

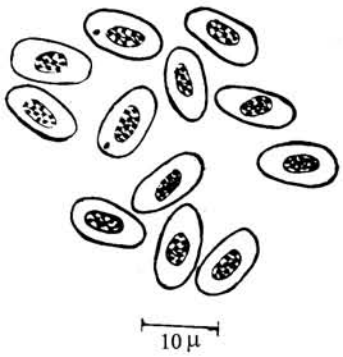

Fig. 3: *Plasmodium juxtanucleare*
Trophozoites

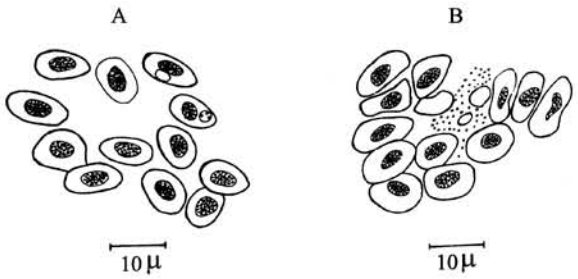

Fig. 4: *Plasmodium gallinaceum*
A: a. Trophoziote b. Schizont
B: Merozoite
Family: Haemoproteidae
Genus: Haemoproteus

Schizonts were rounded, irregular, displaced host cell nucleus, produce 8-30 merozoites. Shape of gametocyte varies according to the position of the host cell nucleus. Macrogametocyte stained grayish blue and nucleus in the form of irregular mass. Black pigment granules were heavier but less numerous than in microgametocyte, scattered through out the cytoplasm. Percentage of infection was 7.2 (Table 1).

***Haemoproteus columbae*:** Only gametocytes found in RBC these were elongated and sausageshaped, partially encircle host cell nucleus. Contained variable number of dark brown pigment granules, small in macrogametocytes and bigger in microgametocytes (Fig. 5). Macrogametocyte had deep blue cytoplasm about 14 small dark brown pigment granules, compact nucleus, size: 9.73-13.9 x 2.78-5.56 micron. Microgametocytes were less Halteribium – shaped differed in coloration, huge pigment granules, (no 6-8) grouped at two extremities. *H. columbea* was found in three different species of avian host. Incidence of single infection was 8 (Table 1) and

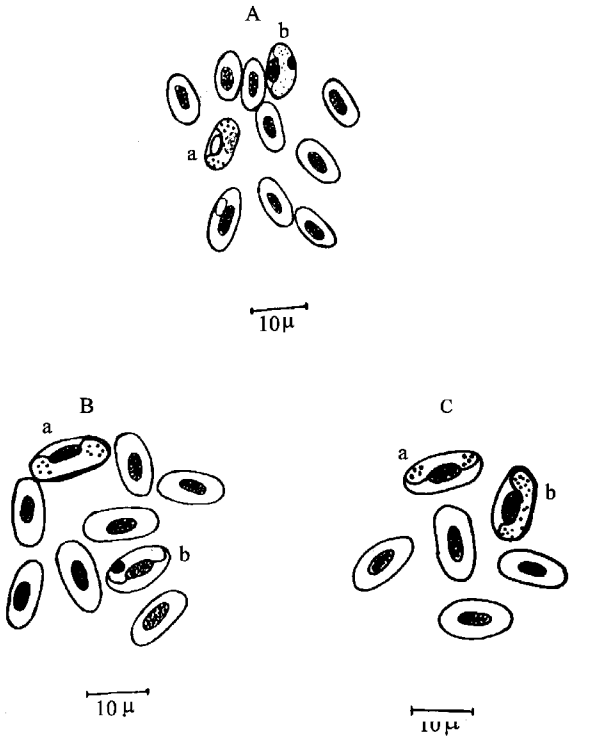

Fig. 5: *Haemoproteus columbae*
A: Gametocytes (in Pigeon's blood)
B: Gametocytes (in Sparrow's blood)
C: Gametocyte (in Myna's blood)
Family: Dipetalonematidae
Sub family: Splendidofilailinae
Genus: Ornithofilaria *Ornithofilaria* sp.

incidence of mixed infection with *P. relictum* was 1.27 and with microfilaria was 0.64 (Table 2)

There are two unidentified species of microfilaria found in the blood slides of Myna and sparrow. The microfilaria which was found in Myna blood belongs to the genus Ornithofilaria.

(I) Microfilaria found in Mynas blood film (Fig. 6). Earth worm like in shape, anterior end rounded and ended abruptly in a pointed tail, No sheath. Cuticle smooth with striation and no cuticular basses. Incidence of infection was 18.7 (Table 1)

(ii) Microfilaria found in sparrows' blood film (Fig. 7). Long, unsheathed, rounded at anterior end, body made up of oval curves, nuclear column in two rows (left and right) nuclear absent at anterior tip and not seems to continue up to posterior tip. Striations also found in some specimens. Incidence of single infection was 2.5 (Table 1) while Incidence of mixed infection with *Haemoproteus columbae* was 0.64 (Table 2)

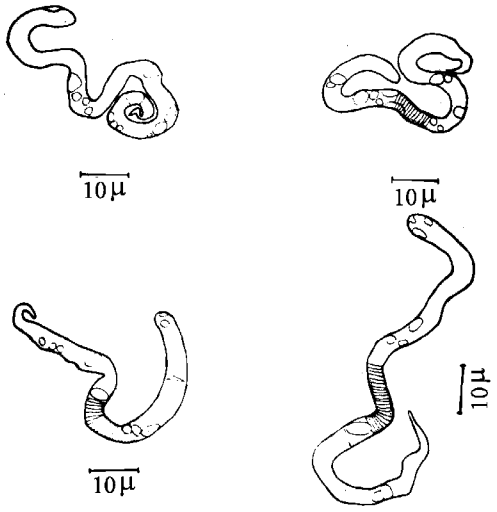

Fig. 6: *Microfilaria* (in Myna's blood)

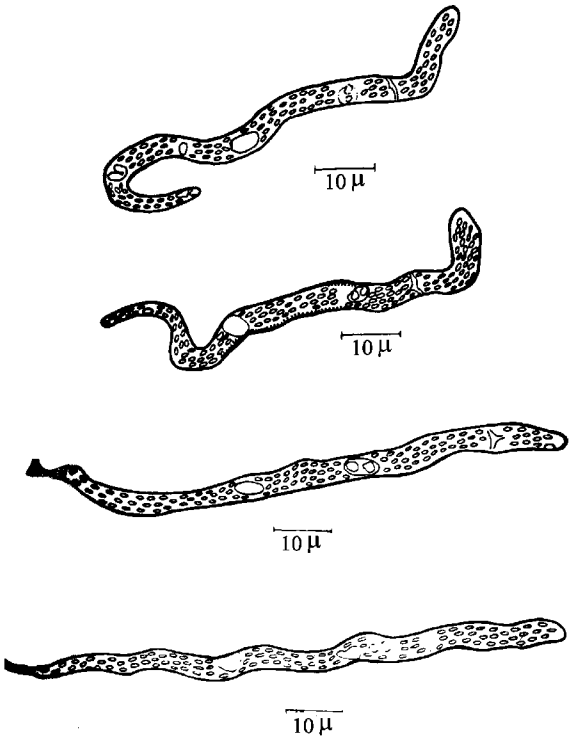

Fig. 7: *Microfilaria* (in sparrow's blood)

Five species of Plasmodium one specie of Haemoproteus and two unidentified species of microfilaris were observed in 83 birds belonging to 15 species. *Plasmodium elongatum* was found in 34 natural avian hosts belonging to six species. *P. elongatum* was found to be more common than *P. relictum.* Infection of *P. relictum* observed in 09 birds of five species.

Natural infection with *P. circumflexum* was very rare. *P. gallinaceum* was found in 8 chicks. While *P. juxtanuclear* was found in only one chick. *Haemoproteus columbae* was found in three different species of birds i.e. pigeon sparrow and Myna. This was the new host record. Coturnix coturnix was the first recorded natural host for *P. elongatum* from D.I. Khan. A mixed infection of *P. elongatum* with *P. relictum* was observed in five birds belonging to four species as shown in Table 2.

A Caspian tern collected from Bannu was the next new host for a mixed infection of *P. elongatum* and *P. circumflexum*, *P. relictum* was also found to be mixed infection with *H. columbae* and *Microfilaria*.

Natural infection of sparrows and a few other birds with *P. relictum* and *P. elongatum* have been reported by many workers from different parts of the world excluding Pakistan. This report on a survey of avian haematozoa in Pakistan is the first contribution to the world record on avian haematozoan infection and their natural host. It is indeed interesting to note during our studies that *P. elongatum* was the most common species found in the natural avian hosts in NWFP. It has been reported as a rare species of plasmodium observed in starlings, song birds, Lapage[14].

This survey may be regarded as a pre-requiazite of further investigations including advance studies on avian malaria in Pakistan.

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