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# Studies on Mammalian Haematozoan Parasites of NWFP Pakistan

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**Abstract:** Blood smears of 424 mammals were collected from different areas of NWFP belonging to Chiroptera, Legomorpha, Rodentia, Carnivora, Ruminantia were examined. Bats were found infected with *Polychromophilus melanipherus* incidence was 31.7%. *Babesia canis* was observed in Jackal 100% *Anaplasma ovis* was found in cattle. A crithidia stage of *Trypanosoma melaphagium* was recorded from sheep. Rabbits, rats, mice, guine pigs, cat were also examined but found refractory.

**Key words:**Polychromophilus melanipherus, Babesia canis, Anaplasma ovis, Anaplasma marginale, Crithidia

### INTRODUCTION

The haematozoa causes several diseases which affect the death rate of the wild life and also disturb the function of haemopoietic system of mammals. This significant work was followed by a series of reports. Olson<sup>[1]</sup> studied the biology and life cycle of Animal parasites. Garnham<sup>[2-5]</sup> carried out an extensive survey and discovered several instances of malarial infection among the mammals. Lapage<sup>[6]</sup> discussed the parasites of veterinary importance. Mohiuddin<sup>[7]</sup> studies parasitic protozoa. Admn *et al.*<sup>[8]</sup> described the importance of Medical and veterinary protozoan. Hore<sup>[9]</sup> reported the crithidial stage of *Trypanosoma melophagium* distributed in the Central Asia, Northern India, Japan, Australia, New Zealand, Hawaii, Europe, North and South America.

Levin<sup>[10]</sup> discussed the protozoan parasites of domestic animals. Iliyas and Ansari<sup>[11]</sup>, Faust *et al.*<sup>[12]</sup> gave attention on the importance of community medicine and clinical parasitology.

Haider<sup>[13]</sup> reported the *Plasmodium* malarial infection in *Anthropithecus troglodytes* in Karachi. Haider and Bilqees<sup>[14]</sup> studies on Plasmodium infection in some mammals in Karachi. Anderson<sup>[15]</sup> reserach on blood parasites of mammals and discovered specie of *Hepatozoon* in New Guinea spiny bandicoot (*Echymipera kalubu*), trypanosome infection were found in three genera of rodent hosts. de Roode *et al.*<sup>[16]</sup> and Reece *et al.*<sup>[17]</sup> research on Rodent malaria Parasites (*Plasmodium chabaudi*). Borssy<sup>[18]</sup>, Jones *et al.*<sup>[19,20]</sup> investigated on the malaria of penguins. Haematological survey on two species of sea Turtles in Arabian Sea were

reported by Alkindi and Mahmoud<sup>[21]</sup>. Basham *et al.*<sup>[22]</sup> studied the effect of thiocyanate on the blood and muscles of camels (*Camelus dromedaries*). Talat<sup>[23]</sup> observed different species of plasmodium in birds.

The present investigation was carried out to observe the blood parasites of mammals in NWFP Pakistan.

## MATERIALS AND METHODS

Blood films of 424 different mammals were collected from different areas of NWFP in June and July 2003. The blood of goats, Sheep and cattle were collected from the slaughter house while the blood of wild mice taken from the tail vein and pricking ear pinna in case of rabbits and guinae pigs.

The blood smears were made on clean sterilized slides, which were cleaned with 70% alcohol. Mostly thin blood films were made which after fixing with methyle 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 a calibrated microscope.

# RESULTS AND DISCUSSION

Among 424 mammals 59 i.e. 13.91% have shown the infection with haematozoa. Five species of haematozoa

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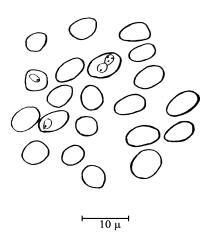


Fig. 1: Babesia canis (Trophozoites)

Table 1: Incidence of infection among mammals

		No. of positive		Percentage among
Mammalian	Species of	Total No.	Infection	total
host	parasite	of samples	(%)	mammals
Bat	Polychromophilus	6/19	31.7	1.41
	melunipherus			
Flying squirrel	-	0/4	0	-
Cat	-	0/1	0	-
Jackal	Babesia canis	1/1	100	0.23
Rabbit	-	0/17	0	-
Rat	-	0/9	0	-
Mice	-	0/38	0	-
Guinae Pig	-	0/8	0	-
Sheep	Anaplasma ovis	19/136	13.2	4.47
	Trypanosoma	1/136	0.73	0.23
	melophagium (crithidiastage)			
Goat	Anaplasma ovis	7/73	9.59	1.64
Cow	Anaplasma marginale	8/53	15.00	1.88
Buffalo	Anaplasma marginale	17/65	26.00	4.00

were found in the blood of mammals, among these four species have been diagnosed. The main criteria for identification of species were based on diagnostic characters described by Garnhum<sup>[6]</sup>.

Family: Babesiidae Genus: *Babesia* 

**Babesia canis:** Trophozoites (Fig. 1) were piriform measuring 4-5  $\mu$  long or amoeboid i.e 2-4  $\mu$  in diameter. Trophozoites generally contained a vacuole. Multiple infections was commonly observed paired forms attached to each other by their narrower ends.

Blood slide of one jackal was observed and found positive. Incidence of infection was 100% (Table 1).

Family: Haemoproteidae Genus: *Polychromophilus* 

Polychromophilus melanipherus: Youngest form of gametocytes occupied one-sixth of the diameter of the



Fig. 2: Polychromophilus melanipherus (a) Trophozoite (b) Gametocyte

erythrocyte, they grow in size and tend to assume an oval form with a pigment distributed peripherally. They eventually fill erythrocyte and often still retain a slightly ovoid shape. The envelopes of red blood cell eventually disappear. Mature microgametocyte (Fig. 2) measured about 7-8.34  $\mu$  in diameter while the mean diameter of young gametocyte was 2.78  $\mu$ .

The diameter of mature gametocyte was obviously larger than non-infected corpuscle. Cytoplasm was very pale or grey in colour, and at least half the body of parasite occupied by a pink staining, spongy mass constituting the nucleus. Pigment granules were black and fairly coarse and were distributed over most of the parasite. Macrogametocyte little larger and measured about 8  $\mu$ . Cytoplasm faintly vacuolated and stains bright blue colour, while small nucleus usually lie near edge. Incidence of infection was 31.7% (Table 1).

Family: Anaplasmidae Genus: *Anaplasma* 

Anaplasma marginale: A spherical granule (Fig. 3) which stained bright red varies in size from 0.1-0.5  $\mu$ . It showed no sturcture, only consist of chromatin without any visible cytoplasm. Situated near the edge of RBC. Multiple infection also found. There is no marked change in the size of infected and normal RBC.

A total of 118 blood samples of cattle (53 cows and 65 buffalos) were collected from the slaughter house of Peshawar city out of which 25 (8 cows and 17 buffalos) were found to be infected with *A. marginale* intensity of infection was 15 and 26% in cows and buffalos, respectively (Table 1).

Highly pathogenic cause a great destruction of red blood cells with resultant anemia, high fever, jaundice and digestive troubles.

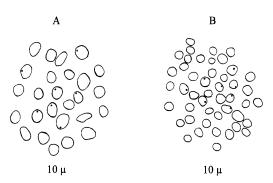


Fig. 3: Anaplasma ovis

(a) In Goat's blood (b) In Sheep's blood

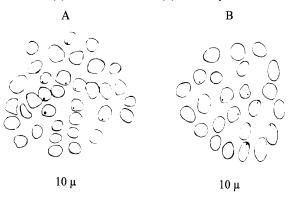


Fig. 4: *Anaplasma marginale*(a) In Cow's blood (b) In Buffalo's blood

# Family: Anaplasmidae Genus: *Anaplasma*

Anaplasma ovis: Spherical granules (Fig. 4) stained bright red which distinguished them from jally bodies and basophilic stippling of the red blood cells. Each of these bodies consists of chromatin only, without any visible cytoplasm. These bodies situated near the margin of RBC. No marked difference between the species that occur in sheep (Fig. 3) and cattle (Fig. 4) out of 209 blood samples of sheep and goats 25 were found to be infected with A. ovis. Incidence of infection among total mammals was 5.6% while among particular hosts; it was 13.2 and 9.59% in sheep and goats, respectively (Table 1). No marked difference between normal and infected RBC. The size of A. ovis ranges from, 0.14-0.20 \( \mu \) A. ovis is highly pathogenic because a great destruction of RBCs. with resultant anemia, high fever, jaundice and digestive troubles.

# Family: Trypanosomatidae Genus: *Trypanosoma*

*Trypanosoma melophagium* (Crithidia stage): Elongated forms (Fig. 5) with short free flagellum, prominent kinetoplast. Range of length was  $9-12 \mu$ , width from the

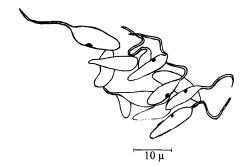


Fig. 5: Trypanosoma melophagium crithidia stage

centre was 2-4  $\mu$ . The length of the flagella ranges from 9-15  $\mu$ . The range of distance between anterior end and kinetoplast was 4-8  $\mu$  stages of parasite observed in the blood of sheep was most probably the crithidial stage of the common sp. of Trypanosomes found in sheep from central Asia and Northern India (Fig. 5). Percentage of infection among total mammals was 0.23 and incidence of infection among particular host was 0.37 (Table 1). This stage of parasite was observed in one blood slide of sheep in only one field of x100. The stage was observed in a rosette form. This finding represent the first recorded natural infection with *Polychromophilus melaniphirus* in Pakistan.

The blood studies of 424 mammals belonging to different areas of NWFP have been examined. Among those only 59 mammals belonging to 12 species were found positive. *Polychromophilus melanipherus* was observed in six natural bats among the mammals. *Babesia canis* was found in one Jackal. *Anaplasma ovis* observed in 19 sheeps and 7 goats. *Anaplasma marginalae* was found in 8 cows and 17 bufallows while the *Trypanosoma melophagium* crithidia stage observed in only one sheep (Table 1).

Young and mature gametocytes of *Polychromophilus melaniphirus* were found in blood slide of bats. These findings represent the first record natural infection with *polychromophilus melanipherus* in Pakistan. *Babesia canis*, *Anaplasma ovis* and *Anaplasma marginalae* were observed only in trophozoite stages. Crithidia stage of Trypanosoma found in the blood of sheep which was most probably the stage of *Trypanosoma melphagium* found in the blood of sheep in central Asia and Northen India.

Natural infection among mammals have been reported by so many researchers from different parts of the world but very little attention was given to natural infection among the mammals in Pakistan. This investigation may be regarded as a pre requiazite of further research work on mammals' infection in Pakistan.

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