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Blood Parasites of Mammals from Papua New Guinea

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ABSTRACT: Thin blood smears were collected from 126 mammals representing four genera of marsupials and six genera of murid rodents. A species of *Hepatozoon* was discovered in the New Guinea spiny bandicoot (*Echymipera kalubu*), trypanosome infections were found in three genera of rodent hosts and the prevalence of a rickettsial parasite of the genus *Grahamella* was recorded in rodents from the genera *Rattus* and *Melomys*. Dried blood samples also were taken and screened serologically for antibodies to arenavirus infection but with negative results.

Key words: Bandicoot, murid rodents, trypanosomes, *Herpetosoma* spp., *Hepatozoon* sp., *Grahamella* sp., arenavirus, immunofluorescent antibody test (IFAT), survey.

The blood parasites and diseases of mammals from Papua New Guinea (PNG) have received scant attention in the literature. In a review Ewers (1973) listed only two protozoan parasites from the blood of PNG's mammal fauna. Here I describe the parasites found in the course of work on the ecology of mammals in the Western Highland Province of PNG in 1985 (Anderson et al., 1988; Berry et al., 1987).

Mammals were live trapped at two sites: (1) in lowland and lower montane forest at the Baiyer River Sanctuary (144.10°E, 5.35°S; 1,200 m above sea level) and (2) in montane and moss forest around the village of Rogut (144.15°E, 5.50°S; 2,200 m above sea level) in the Tulman Valley near Mt. Hagen. At Rogut collections were supplemented with animals supplied by villagers.

Thin blood smears were prepared in the field using peripheral blood from the tip of the tail; these were air dried and fixed with absolute methanol within 2 hr of collection. The slides were stored in the dark and stained with Giemsa within 3 mo of collection. Slides were examined under oil immersion at 1,000× magnification for 10 min and observed parasites were measured using a calibrated ocular micrometer (Bausch and Lomb, Rochester, New York 14692, USA). The intensity of parasitemias were measured by surveying an estimated 10,000 erythrocytes. The results of the survey are summarized in Table 1.

Collection of blood samples for serology was carried out as follows. Blood from each animal was absorbed onto glass fiber discs (Whatman grade GF/B, Whatman International Ltd., Maidstone, England), air dried and stored at 4 C. Later, at the London School of Hygiene and Tropical Medicine (LSHTM, London, England), blood proteins were eluted from the glass papers into 400 μl PBS-A and screened for arenavirus group antibodies using an indirect immunofluorescent antibody test (IFAT) (Howard, 1986). Previous results indicate that IFAT is a sensitive assay for detecting arenaviral antibodies (Howard, 1986). Sera were pooled in groups of 10 and incubated with acetone fixed Vero Clone 1 cells infected with lymphocytic choriomeningitis virus (LCMV). The presence of arenavirus specific antibody was detected using a secondary anti-mouse antibody-FITC conjugate or Protein A-FITC conjugate (Organon Teknika Corporation, Cappel Division, Durham, North Carolina 27704, USA). For all tests LCM virus antibodies at a dilution of 1:100 were used as positive controls and showed bright fluorescence.

Gamonts of a hemogregarine of the genus *Hepatozoon* were found in the erythrocytes of 41% of *E. kalubu* from Baiyer River (Table 1). There was no significant difference in the prevalence of infection between sexes ($\chi^2 = 1.768$, $P > 0.05$). A similar parasite was found in one *E. kalubu* from Rogut which suggests that this *Hepatozoon* sp. is widespread in populations of this bandicoot in the area around Mt. Hagen. The intensity of parasitemias (defined as the number of parasites per 100 erythrocytes) were low ranging from 0.01%

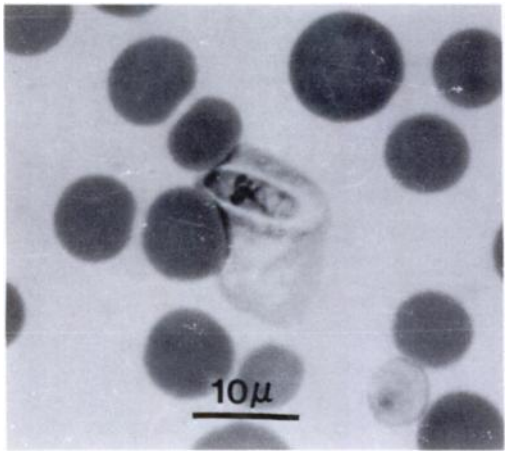

FIGURE 1. Gamont of *Hepatozoon* sp., a parasite of *Echymipera kalubu*, in a distorted erythrocyte.

to 0.44% (mean = 0.079%; median = 0.02%). Ten parasites were measured. The gamonts were rod shaped (7 to 8 μm long × 2.5 to 3.0 μm wide). The nuclei, which measured up to 3.0 μm across and had a dark beaded appearance, varied in their position within the cell (Fig. 1). Normal erythrocytes had diameters of 6.5 to 7.5 μm but infected cells were highly distorted measuring up to 20 μm across and staining pale pink. In five smears extracellular gamonts, measuring 9 to 10 μm × 3.0 μm, were also observed. It is possible that this parasite was *H. peramelis* which has been found in two bandicoot species (Mackerras, 1959) in Australia but is previously unreported from the genus *Echymipera* or from any other bandicoots from PNG. The parasites found in this study morphologically resembled those described by Mackerras (1959) although they were marginally smaller. However *Hepatozoon* spp. are highly host restricted and further studies of the parasite from *E. kalubu* may show it to be taxonomically distinct from *H. peramelis*.

Trypanosomes of the subgenus *Herpetosoma* were found in single specimens of *Melomys rufescens*, *Pogonomelomys ruemmleri* and *Uromys anak* (Table 1). Trypanosome infections have not been reported previously from these three host genera. All three species of these rats belong to the Uromys group of the subfamily Murinae (Tate, 1951), although the taxonomic status of *P. ruemmleri* is at present unclear and it may be transferred to a new genus in the near future. (Menzies and Dennis, 1979).

The trypanosomes from all three rat hosts were *T. lewisi*-like in morphology. Ten trypanosomes from each host species were

TABLE 1. Summary of mammals examined from Baiyer River (BR) and Rogut (R) in Papua New Guinea and blood parasites found.

Host species	Location	Prevalence	Parasite
Echymipera kalubu	BR	16/39 (41)	*Hepatozoon* sp.
	R	1/1 (100)	*Hepatozoon* sp.
Peroryctes raffrayanus	BR	0/1 (0)	
Phalanger gymnotis	R	0/1 (0)	
Murexia longicaudata	BR	0/1 (0)	
Uromys anak	R	1/1 (100)	*Herpetosoma* sp.
Pogonomelomys ruemmleri	R	1/4 (25)	*Herpetosoma* sp.
Melomys rufescens	BR	1/49 (2)	*Herpetosoma* sp.
	BR	6/49 (12)	*Grahamella* sp.
Melomys platyops	BR	1/4 (25)	*Grahamella* sp.
Melomys spp.	R	1/2 (50)	*Grahamella* sp.
Rattus spp.	BR	7/14 (50)	*Grahamella* sp.
Rattus spp.	R	2/4 (50)	*Grahamella* sp.
Mallomys rothschildi	R	0/2 (0)	
Parahydromys asper	BR	0/2 (0)	

[a] Number infected/number examined, (%).

measured and means are presented with one standard error. The trypanosomes in the blood of *M. rufescens* and *P. ruemmleri* were very similar, having overall lengths of 25.93 ± 1.26 μm and 26.21 ± 0.94 μm, and free flagellae measuring 7.16 ± 0.35 μm and 6.30 ± 0.41 μm, respectively. The nuclei were oval (2.3 × 1.4 μm) and situated in the anterior third of the cell; the posterior ends were sharply pointed with round (1 μm diameter), or occasionally oval kinetoplasts situated 2 to 3 μm from the tip. In comparison the parasites from *U. anak* were shorter (24.14 ± 1.12 μm), less slender and more variable in morphology; characteristic of a trypanosome population entering the reproductive phase (Molyneux, 1976). The flagellae of the trypanosomes from *U. anak* were significantly shorter (6.18 ± 0.40 μm) than those from the parasites in *M. rufescens* ($t = 1.765$, $P < 0.05$). No other statistical differences in morphology were found between the three parasite populations. In all three hosts the intensity of parasitemias were <0.1%.

The subgenus *Herpetosoma* contains a large assemblage of species which are host restricted at the generic level in both the mammalian host and the insect vector (Molyneux, 1976), but which cannot be differentiated on the basis of bloodform morphology (Davis, 1952). As a result it is often assumed that *Herpetosoma* sp. from new host genera are themselves new species. However, descriptions of trypanosomes from single blood films are, on their own, an insufficient basis on which to describe new species (Hoare, 1972). In one recent study iso-enzyme analysis was used to differentiate between *Herpetosoma* spp. from six rodent species (Mohamed et al., 1987). The detailed phyllogenies generated by further use of this technique also might shed some light on the evolutionary affinities of host mammals such as *P. ruemmleri*.

Rodents of the genera *Rattus* and *Melomys* from both trapping sites harboured infections of *Grahamella* sp., a common rickettsial parasite of rodent erythrocytes. At Baiyer River 12% of *Melomys rufescens* and 50% of *Rattus* spp. were infected (see Table 1). In both host genera the intensity of parasitemias were low ranging from <0.01% to 0.36% (mean = 0.049%; median = 0.01%).

The serological tests gave no evidence of arenaviral infection in any of the blood samples. Rodents infected with arenavirus have been found in both the New and the Old World and for a number of arenavirus species (e.g., Lassa fever virus, Junin virus) they serve as reservoirs for human infection (Howard, 1986). Evidence of arenaviral infection has not yet been found in mammals from Australasia.

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