Transactions of the Royal Society of S. Aust. (2005), **129**(1), 49-52.

HEPATOZOON TACHYGLOSSI SP. NOV. (HAEMOGREGARINIDAE), A PROTOZOAN PARASITE FROM THE BLOOD OF A SHORT-BEAKED ECHIDNA, *TACHYGLOSSUS ACULEATUS*

by P. CLARK[1], P. HOLZ[2] & D. M. SPRATT[3]

Summary

CLARK, T., HOLZ, P. & SPRATT, D. M. (2005) *Hepatozoon tachyglossi* sp. nov. (Haemogregarinidae), a protozoan parasite from the blood of a short-beaked echidna, *Tachyglossus aculeatus*. *Trans. R. Soc. S. Aust.* **129**(1), 49-52, 31 May, 2005.

Hepatozoon tachyglossi sp. nov. is described from monocytes in the peripheral blood of a debilitated short-beaked echidna from the Healesville region of Victoria. Of the *Hepatozoon* Miller, 1908 species known to occur in Australian native mammals, all of those in marsupials occur in erythrocytes and only H. muris from introduced and native rodents occurs in monocytes. *H. tachyglossi* is distinguished from *H. muris* by its larger size and the lack of a capsule.

KEY WORDS: *Hepatozoon tachyglossi*, new species, echidna, *Tachyglossus aculeatus*.

Introduction

The blood of the short-beaked echidna, *Tachyglossus aculeatus* (Shaw & Nodder, 1792), has yielded relatively few haemoparasites. Those identified have been restricted to the Piroplasmidae and include *Babesia tachyglossi* (Backhouse & Bolliger, 1959) and *Theileria tachyglossi* (Priestly, 1915) (Backhouse & Bolliger 1957, 1959; Mackerras 1959). We report a novel species of *Hepatozoon* from monocytes in the peripheral blood of a short-beaked echidna from the Healesville region of Victoria and compare the species with other members of the genus previously described from Australian native mammals.

Clinical history and methods

A male, juvenile, short-beaked echidna was presented to the veterinary service of Healesville Sanctuary. The animal had several injuries including a damaged tongue and a fractured humerus. During captive management and treatment, the animal developed severe dyspnoea and a mucopurulent nasal discharge. It was euthanased after a poor response to treatment with antimicrobial drugs and supportive therapy.

A sample of blood was collected ante-mortem from the bill sinus. The morphology of haematological cells in a blood film, stained with Wright's and Giemsa stains, was assessed by light microscopy. Tissue samples of bone marrow, liver and spleen, were collected post-mortem and fixed in 10 percent buffered formalin. The tissues were processed using standard histological methods; sections were stained with haematoxylin and eosin stains and examined using light microscopy. The organisms and their host cells were digitally photographed. A stage micrometer was photographed at the same magnification and used to insert scale bars to all micrographs. The micrometer was also used for all measurements which are presented in microns as the mean ± standard deviation followed by the range in parentheses.

Results

Intracellular organisms were observed within leukocytes in the peripheral blood (Fig. 1). These were observed in 24/50 monocytes but were not observed in any granulocytes.

Hepatozoon tachyglossi sp. nov.
(FIG. 1)

Host
Tachyglossus aculeatus.

Location
Healesville, Victoria.

Type
Slide in South Australian Museum No. 28751.

Typically the organisms were oval to elongate in shape but were quite pleomorphic with some pyriform and round forms observed. The organisms were 9.9 ± 1.4 (7.8-12.4) in length and 4.7 ± 0.7 (3.8-5.9) in width (n = 18). Most organisms had an eccentric, subterminal nucleus and some exhibited a

[1] School of Clinical Sciences, Division of Veterinary and Biomedical Sciences, Murdoch University, South St, Murdoch, Western Australia, 6150.
[2] Healesville Sanctuary, P.O. Box 248, Healesville, VIC, 3777.
[3] CSIRO Sustainable Ecosystems, GPO Box 284, Canberra, ACT, 2601

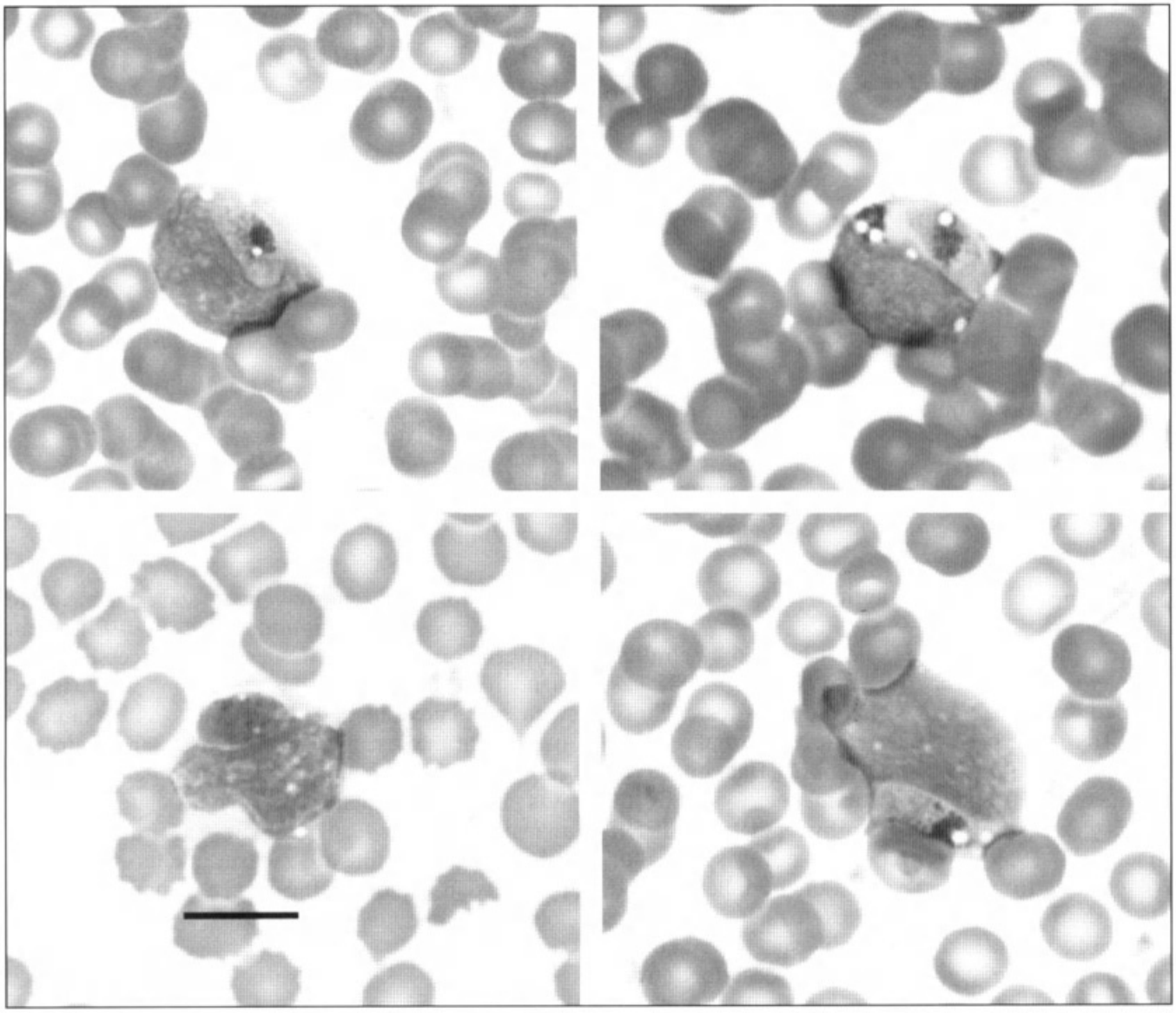

Fig. 1. Examples of *Hepatozoon tachyglossi* sp. nov. within monocytes from the peripheral blood of a short-beaked echidna. Bar = 10 µm.

small amount of punctate, dark brown – black pigment in the cytoplasm. No distinct capsule was observed and there was only a subtle difference in colour between the cytoplasm of the organism and the cytoplasm of the cell. Only one organism was evident per cell; in some cases this caused displacement of the host cell nucleus. No consistent changes in cell morphology were evident. No extracellular organisms were noted. Examination of histological sections of bone marrow, liver and spleen did not reveal schizonts.

Discussion

The organism described is morphologically similar to a species of *Hepatozoon* Miller, 1908, the only coccidian genus that inhabits the blood of mammals. Desser (1990) highlighted the taxonomic confusion arising from the problem of differentiating species of *Hepatozoon* from that of *Haemogregarina* Danilewsky, 1885 on the basis of gamonts in the blood of the vertebrate host. Differentiation was based primarily on the size of the oocysts and the presence or absence of sporocysts in the invertebrate definitive host. Desser (1990) tested and confirmed the hypothesis of Landau *et al.* (1972) that tiny cystic stages (cystozoites) in the liver and lungs represent a common feature in all species of *Hepatozoon* and, as a consequence, differentiation of the genera *Hepatozoon* and *Haemogregarina* based solely on the stages in the vertebrate host is possible. These findings further support the evidence of Landau *et al.* (1972) that transmission of species of *Hepatozoon* may be by predation, as well as by ingestion of the infected arthropod vectors.

The genus has not been reported previously in

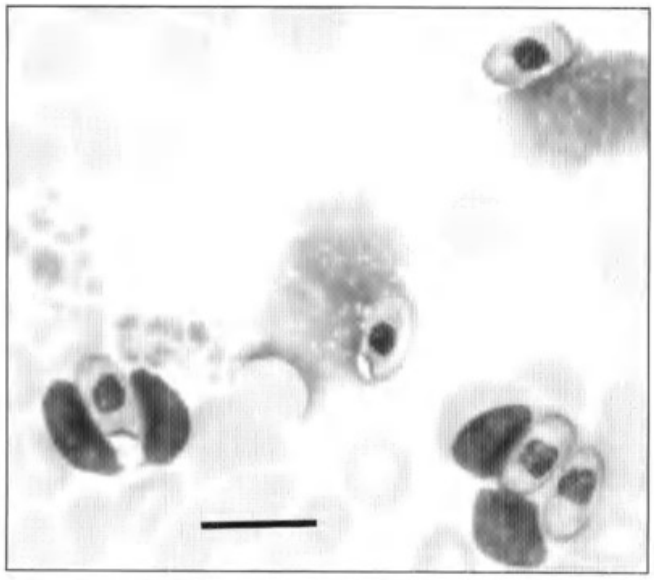

Fig. 2. Examples of *Hepatozoon muris* from the blood of *Rattus lutreolus*. Bar = 10 µm.

monotremes. However, *H. peramelis* (Welsh & Dalyell, 1909), *H. dasyuroides* Mackerras, 1959, *H. dasyuri* (Welsh, Dalyell & Burfitt, 1909), *H. petauri* (Welsh & Barling, 1909) and *H. pseudocheiri* Mackerras, 1959, have been described from Australian marsupials (see Mackerras, 1959). In addition, O'Donoghue and Adlard (2000) reported several unidentified species of *Hepatozoon* from Australian marsupials. All reports have recorded the parasite within erythrocytes or 'free' in the blood (Mackerras 1959; Speare *et al.* 1984; Bettiol *et al.* 1996). Also, *H. muris* (Balfour, 1906) has been recognised in both the introduced *Rattus norvegicus* (Berkenhout, 1769) and *R. rattus* (Linneaus, 1758), and the native rodents, *R. fuscipes* (Waterhouse, 1839) and *R. sordidus* (Gould, 1858) (Mackerras 1959; O'Donoghue & Adlard 2000). *Hepatozoon muris*, in contrast to the species reported in marsupials, infects leukocytes (Soulsby 1982).

The organisms in the current study were oval to elongate in shape, as are many species of *Hepatozoon*, and were similar in size to *H. peramelis* (9.0 – 10.0 µm by 3.0 – 3.5 µm; Welsh & Dalyell 1909) and slightly larger than *H. petauri* (7.5 – 8.0 µm by 3.5 – 4.0 µm; Mackerras 1959), *H. pseudocheiri* (8.0 – 13.0 µm by 1.5-3.0 µm; Mackerras 1959), and *Hepatozoon sp* (8.7 ± 0.2 µm by 2.1 ± 0.4 µm, Bettiol *et al.* 1996). In contrast, *H. dasyuroides* (12 – 13 µm by 1 – 2 µm; Mackerras 1959) and *H. dasyuri* (12 µm by 4 µm; Welsh Dalyell & Burfitt 1909, 1910) are longer, narrower parasites.

Hepatozoon tachyglossi sp. nov., like *H. muris*, occurs in the monocytes of its host. However, *H. muris* is a smaller (7.0 – 8.0 µm by 3.0 – 3.5 µm; Mackerras, 1959) more ovoid, and typically less morphologically variable parasite, than *H. tachyglossi* (Fig. 2). Additionally, *H. muris* has an eosinophilic capsule that was not evident in *H. tachyglossi*.

Typically, the definitive hosts and vectors of species of *Hepatozoon* are blood feeding arthropods. The mite *Laelaps echidninus* Berlese, 1887 fills this role for *H. muris*. In the current case, ticks identified as *Aponomma concolor* Neumann, 1899, were evident on the echidna but were not examined for sporocysts and could not be proven to be the definitive host.

Although the life history of this species of *Hepatozoon* remains unknown, we consider that specific status is warranted on the basis of morphological features and the occurrence of the parasite in monocytes of the monotreme, *Tachyglossus aculeatus*.

The effect on the host was not determined due to the other pathological processes and the animal's debilitated state may have allowed the organism to proliferate. Further work to identify the prevalence of this organism in short-beaked echidnas, assess any pathogenic effects on the animal and phylogenetic studies to determine its relationship to other species of *Hepatozoon* in Australia need to be undertaken.

Acknowledgements

We thank Dr Peter O'Donoghue for sharing his knowledge of protozoan organisms with us and two anonymous referees whose comments improved the presentation of the manuscript.

References

BACKHOUSE, T. C., & BOLLIGER, A. (1957) A piroplasm of the echidna (*Tachyglossus aculeatus*). *Aust. J. Sci.* **19,** 24-25.

_____ & _____ (1959) *Babesia tachyglossi* n. sp. from the echidna *Tachyglossus aculeatus*. *J. Protozool.* **6,** 320-322.

BETTIOL, S. S., GOLDSMID, J. M., LE, D. D. & DRIESSEN, M. (1996) The first record of a member of the genus *Hepatozoon* in the eastern barred bandicoot (*Perameles gunnii*) in Tasmania. *J.Parasitol.* **82,** 829-830.

DESSER, S. S. (1990) Tissue "cysts" of *Hepatozoon griseisciuri* in the grey squirrel, *Sciurus carolinensis*: the significance of these cysts in species of *Hepatozoon*. *J. Parasitol.* **76,** 257-259.

LANDAU, I., MICHEL, J. C. & CHABAUD, A. G. (1972) Cycle biologique d'*Hepatozoon domerguei*: discussion sur les caractères fondamentaux d'un cycle de Coccidie. *Z. Parasitenk.* **38,** 250-270.

MACKERRAS, M. J. (1959) The Haematozoa of Australian mammals. *Aust. J. Zool.* **7,** 105-135.

O'DONOGHUE, P. J. & ADLARD, R. D. (2000) Catalogue of protozoan parasites recorded in Australia. *Mem. Qld. Mus.* **45,** 1-163.

SOULSBY, E. J. L. (1982) "Helminths, arthropods and protozoa of domesticated animals". 7th ed. (Baillière Tindall, London.)

SPEARE, R., HAFFENDEN, A. T., DANIELS, P. W., THOMAS, A. D., & SEAWRIGHT, C. D. (1984) Diseases of the Herbert River ringtail, *Pseudocheirus herbertensis*, and other North Queensland rainforest possums. pp. 283-302 *In* Smith, A. P. & Hume, I. D. (Eds.) "Possums and Gliders". (Surrey Beatty & Sons, Chipping Norton.)

WELSH, D. A., & DALYELL, E. J. (1909) *Haemogregarina peramelis*: a free haemogregarine of an Australian bandicoot. *J. Path. Bact.* **14**, 547-549.

_____ & BURFITT, M. B. (1909) *Haemogregarina dasyuri*. A preliminary note on an undescribed haemogregarine of the Australian native cat. *Aust. Med. Congr.* for 1908. **2**, 333-337

_____ (1910) *Haemogregarina dasyuri*: a haemogregarine of the Australian native cat. *J. Path. Bact.* **14**, 542-546.