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Trypanosomiasis in an Australian little red flying fox (*Pteropus scapulatus*)

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Abstract

Case report An adult female Australian little red flying fox (*Pteropus scapulatus*) presented with icterus and anaemia. Examination of a blood smear revealed numerous trypanosomes 20.4–30.8 µm long with tapered ends. Necropsy and histological findings were consistent with trypanosome infection of lymphoid tissue and intravascular haemolysis. Sequence and phylogenetic analysis demonstrated this trypanosome species to be genetically distinct and most similar to *Trypanosoma minasense* and *Trypanosoma rangeli* (with a genetic distance of 1% at the 18S rRNA locus for both).

Conclusion To the authors' knowledge this is the first report of a trypanosome infection associated with clinical disease in bats.

Keywords: anaemia; bats; flying foxes; haemolysis; trypanosomes

Bats are currently the subject of increased research because of their demonstrated or suspected role as natural reservoirs for agents of human disease, including Hendra virus, Nipah virus, rabies virus, Australian bat lyssavirus, SARS-like coronavirus, Ebola virus and Marburg virus.[1] Bats (Order Chiroptera) consist of microbats and megabats. The latter are fructivorous and include flying foxes (genus *Pteropus*).

This report describes clinical disease consistent with trypanosomiasis in an Australian little red flying fox (*Pteropus scapulatus*; suborder Megachiroptera), caused by a novel trypanosome.

Case report

An adult female little red flying fox presented to the Australia Zoo Wildlife Hospital in April 2014 after being found on the ground at Redcliffe in south-east Queensland, Australia. At the time of rescue it was quiet though responsive, but had deteriorated overnight. On presentation to the wildlife hospital the bat was comatose with pronounced yellow discolouration of the sclera and oral mucous membranes. It weighed 250 g. Radiographs revealed generalised consolidation of the left lung lobes.

An EDTA venous blood sample (0.5 mL) was collected from the cephalic vein. The packed cell volume determined by centrifugation using a microhaematocrit tube was 0.15 L/L. Plasma protein determined by refractometry was 50 g/L. Although there appear to be no published reports of reference ranges for *P. scapulatus*, the packed cell volume was markedly low compared with reported reference ranges in other *Pteropus* species.[2] Light microscopic examination of a blood smear stained with Diff Quik (Dade Behring Diagnostics, NSW, Aust) revealed numerous slender extracellular flagellates with tapered ends, morphologically consistent with a trypanosome (Figure 1). A central nucleus and a terminal small, round, deeply staining internal structure consistent with a kinetoplast were also observed. Digital images of the trypanosomes in blood films were used to measure key morphological features.[3, 4] Total length (length of body measured along the mid-line including free flagellum) was 20.4–30.8 μm and width (maximum width measured at the level of the nucleus, including undulating membrane) was 1.3–2.3 μm .

In view of the anaemia and rapid deterioration in clinical status, the flying fox was euthanased by intravenous injection of pentobarbital. Necropsy examination revealed it was in good body condition but had generalised icterus (Figure 2), as well as patchy haemorrhages in the lungs and pectoral and abdominal muscles. A range of tissues (lymph nodes, spleen, kidney, lung, liver, heart, adrenal, pancreas, stomach, intestine, brain) was collected in neutral buffered 10% formalin, processed routinely for histopathology and stained with haematoxylin and eosin.

Histological examination revealed depletion of lymphoid tissue in lymph nodes and spleen with replacement by multiple foci of histiocytic inflammation (Figure 3A). The foci of inflammation contained numerous 2–4- μ m round to oval, apparently intracellular, organisms consistent with protozoa with a distinct basophilic nucleus and a kinetoplast. In the kidneys there was moderate acute haemoglobinuric nephrosis (Figure 3B). These findings were consistent with trypanosome infection and intravascular haemolysis. Additional histopathological findings included a mild acute interstitial pneumonia characterised by infiltration of alveolar septae by mononuclear cells and in the liver a mild increase in sinusoidal leucocytes and prominence of Kupffer cells.

DNA was extracted from blood using a MasterPure™ DNA purification kit (EPICENTRE Biotechnologies, WI, USA). An approximately 900-base pair fragment of the 18S rRNA gene was amplified using a nested PCR.[5] The positive PCR product was sequenced using an ABI Prism Terminator Cycle Sequencing kit (Applied Biosystems, CA, USA) on a 3730 DNA analyser (Applied Biosystems). The nucleotide sequences generated for the 18S rDNA locus were imported into Geneious R7 and combined with sequences from a number of related trypanosomatids from GenBank Sequences. Alignments were obtained by MUSCLE.[6]

After selection of the most appropriate evolutionary model by MEGA 6, the evolutionary history was inferred by the maximum likelihood method implemented in the PhyML program (v3.0), on the Phylogeny.fr platform.[5,7] Reliability for internal branch was assessed using the bootstrapping method (500 bootstrap replicates). Genetic distances were generated in MEGA 6 based on the Tamura-Nei algorithm.

Phylogenetic analysis of a 730-base pair fragment of the 18S rRNA locus demonstrated this trypanosome species to be genetically distinct and most similar to *Trypanosoma minasense* and *Trypanosoma rangeli* (with a genetic distance of 1% at the 18S rRNA locus for both).

Discussion

Trypanosomes are flagellated unicellular blood parasites transmitted by arthropod vectors. Some trypanosome species cause severe disease in humans and animals, while others produce either mild or unapparent infection, especially in mammalian reservoir hosts.[8] Mechanisms contributing to disease include systemic inflammation and anaemia caused by the intravascular organisms, and interstitial inflammation caused by organisms residing in perivascular locations.[8] The trypanosome host–parasite relationship is complex and varies with the strain and species of trypanosome, host species and, in some situations, the husbandry or environmental conditions.[8] In addition, trypanosome species that are usually non-pathogenic may occur in very large numbers in the blood of animals suffering from a primary, and usually immunosuppressive, disease.[8]

Trypanosomes have been reported in a variety of Australian native mammals, including koalas (*Phascolarctos cinereus*), Western grey kangaroos (*Macropus fuliginosus*), bandicoots (*Isodon macrourus*; *I. obesulus*) and some species of bats (*Pteropus alecto*; *Hipposideros ater*), usually in the absence of clinical disease or lesions, although there is some evidence to suggest association with illness in native rats (*Hydromys chrysogaster*; *Rattus fuscipes*), woylies/brushtailed bettongs (*Bettongia penicillata*) and koalas.[4, 5, 9-12]

To the authors' knowledge this is the first report of trypanosome infection associated with clinical disease in bats and involved a novel trypanosome. The finding of numerous organisms in the blood and lymphoid tissues of this bat, together with icterus and haemoglobinuric nephrosis, is consistent with trypanosomiasis as the cause of the haemolytic anaemia and clinically significant illness. The mild interstitial pneumonia and hepatic sinusoidal leucocytosis may reflect a systemic inflammatory process caused by trypanosomiasis.

Trypanosome species have been previously described in Australian bats and flying foxes in the absence of clinical disease, including *T. pteropi* in the black flying fox (*Pteropus alecto*) and *T. hipposideri* in dusky leaf-nosed (horseshoe) bat (*Hipposideros ater*).^[4] *Trypanosoma pteropi* has been described as having a slender body (total length 18–20 µm; width 2–4 µm), an underdeveloped undulating membrane and a long, free flagellum.^[4] *Trypanosoma hipposideri* is described as very small and slender (total length 10.5–13 µm; width 1.5–2 µm), with a large kinetoplast located near the posterior end and a delicate short, free flagellum at the anterior end. The trypanosome identified in the present study (total length 20.4–30.8 µm; width 1.3–2.3 µm) was longer than both *T. pteropi* and *T. hipposideri*. Unfortunately, genetic sequences for *T. pteropi* and *T. hipposideri* are not available and therefore cannot be compared with the novel trypanosome identified in the present study. Recently, a high prevalence of *T. vegrandis* was also identified in both microbat and megabat species in Australia.^[13] The clinical effect of *T. vegrandis* in these bats has not been determined. In the present case, the organism causing clinical trypanosomiasis was genetically distinct from organisms for which sequence data is available. It was most closely related to *T. minasense* and *T. rangeli*, both of which infect a variety of mammalian species across a wide geographical area in Central and South America but are considered non-pathogenic in mammalian hosts.^[14,15] Further studies are required to determine the species status of this novel trypanosome.

Conflicts of interest and sources of funding

The authors declare no conflict of interest or specific sources of funding for the work presented here.

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Figure 1. Light photomicrograph of trypanosomes in the blood of Australian little red flying fox (*Pteropus scapulatus*). Note the tapered ends, central nucleus and terminal small kinetoplast (Diff Quik stain).

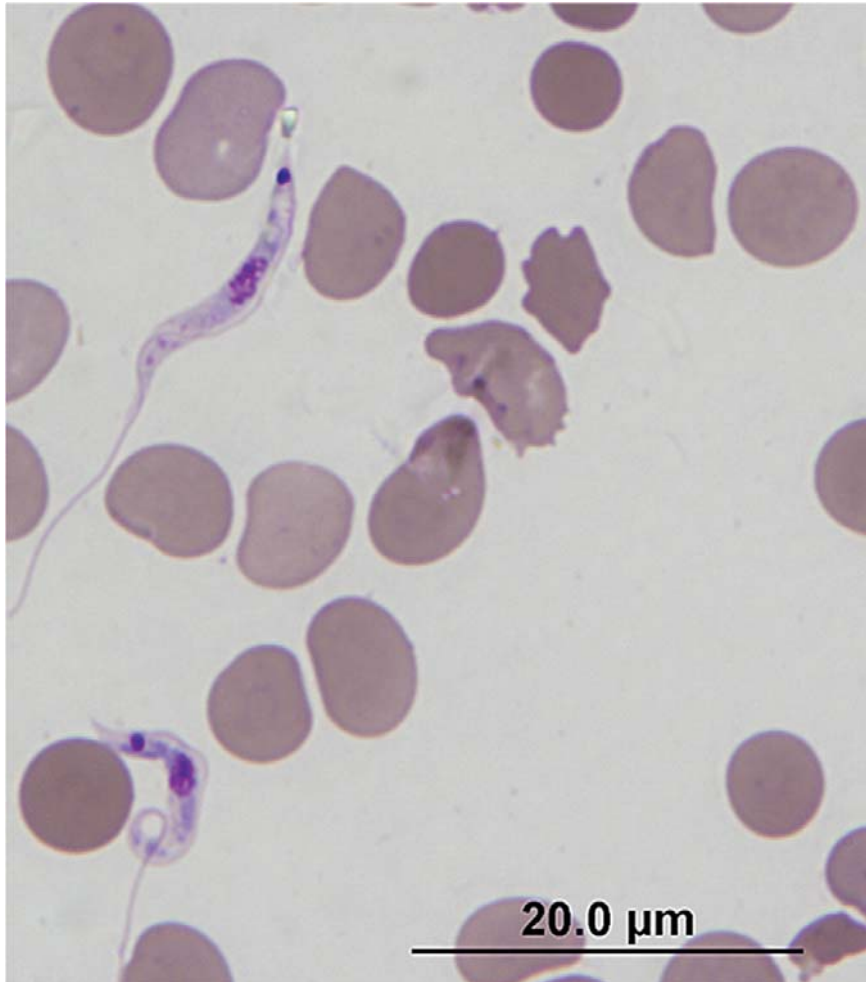


Figure 2. Australian little red flying fox (*Pteropus scapulatus*) showing icteric oral mucous membranes.



Figure 3. Histological sections of (A) lymph node showing depletion of lymphoid tissue (arrows) with replacement by foci of histiocytic inflammation containing protozoa. Inset: within a focus of histiocytic inflammation are numerous round to oval protozoa (arrows) with a distinct basophilic nucleus. (B) Kidney showing haemoglobinuric nephrosis characterised by tubular dilatation, epithelial attenuation and degeneration, and intraluminal haemoglobin casts (H&E; bar = 80 μ m).

