Veterinary Parasitology xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Veterinary Parasitology

journal homepage: www.elsevier.com/locate/vetpar

Research paper

# Prevalence, genetic diversity and potential clinical impact of blood-borne and enteric protozoan parasites in native mammals from northern Australia

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ARTICLE INFO

*Keywords:*
Protozoan parasites
Molecular typing
Marsupials
Wildlife
Epidemiology

ABSTRACT

A molecular survey was conducted to provide baseline information on the prevalence, genetic diversity and potential clinical impacts of blood-borne and enteric protozoans in native wild mammals from the Northern Territory (NT). A total of 209 blood and 167 faecal samples were collected from four target species; the northern brown bandicoot (*Isoodon macrourus*), common brushtail possum (*Trichosurus vulpecula*), northern quoll (*Dasyurus hallucatus*) and brush-tailed rabbit-rat (*Conilurus penicillatus*). Blood samples were screened by PCR at the 18S rRNA gene for trypanosomes, piroplasms and haemogregarines, with faecal samples tested for *Cryptosporidium* spp. at the 18S rRNA locus, and for *Giardia* spp. at the glutamate dehydrogenase (*gdh*) and 18S rRNA loci. The potential clinical impact was investigated by associating clinical, haematological and biochemical parameters with presence or absence of infection. Overall, 22.5% (95% CI: 17.0–28.8%) of the animals tested were positive for haemoprotozoans. Trypanosomes were found in 26.6% (95% CI: 18.7–35.7%) of the bandicoots and were identified as *Trypanosoma vegrandis* G6, except for one unique genotype, most similar to *T. vegrandis* G3 (genetic distance = 7%). The prevalence of trypanosomes in possums was 23.7% (95% CI: 11.4–40.2%), and the genotypes identified clustered within the *T. noyesi* clade. The presence of *Babesia* sp. and *Hepatozoon* sp. was confirmed in bandicoots only, both at a prevalence of 9.7% (95% CI: 2.7–9.2%). The total prevalence of intestinal protozoan parasites observed was relatively low (3%; 95% CI: 1.0–6.9%). No evidence of clinical disease associated with protozoan parasitic infection was observed, however bandicoots positive for *Trypanosoma* exhibited a significantly lower packed cell volume (PCV) compared to negative bandicoots ($p = 0.046$). To the authors' knowledge, this is the first research conducted in the NT to characterise protozoan parasites in threatened native mammals using both molecular and morphological tools; and to assess the potential clinical impacts of these agents. The absence of clear signs of major morbidity in infected animals seems to exclude a direct association between infections with these agents and possible population decline events in northern Australian native mammals. However until the cause(s) of population decline are ascertained for each individual mammal species, further studies are required. The outcome of the present investigation may be used to inform wildlife conservation and zoonotic disease programs.

## 1. Introduction

Evidence from biodiversity surveys across the tropical north or "Top End" of the Northern Territory (NT) in Australia strongly suggests that many small to medium sized mammal species are in rapid and broad-scale decline, even in protected areas such as Kakadu National Park (Woinarski et al., 2010; Woinarski et al., 2011; Ziembicki et al., 2015). The declines in both diversity and abundance have probably been occurring for over 20 years, and infectious disease may be a potential contributor, acting in synergy with other factors such as introduced predators and changed fire regimes (Woinarski et al., 2015; Ziembicki et al., 2015).

Relatively few molecular epidemiological surveys have been conducted on protozoan parasites in Australian threatened wildlife species,

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http://dx.doi.org/10.1016/j.vetpar.2017.04.007
Received 11 January 2017; Received in revised form 15 March 2017; Accepted 3 April 2017

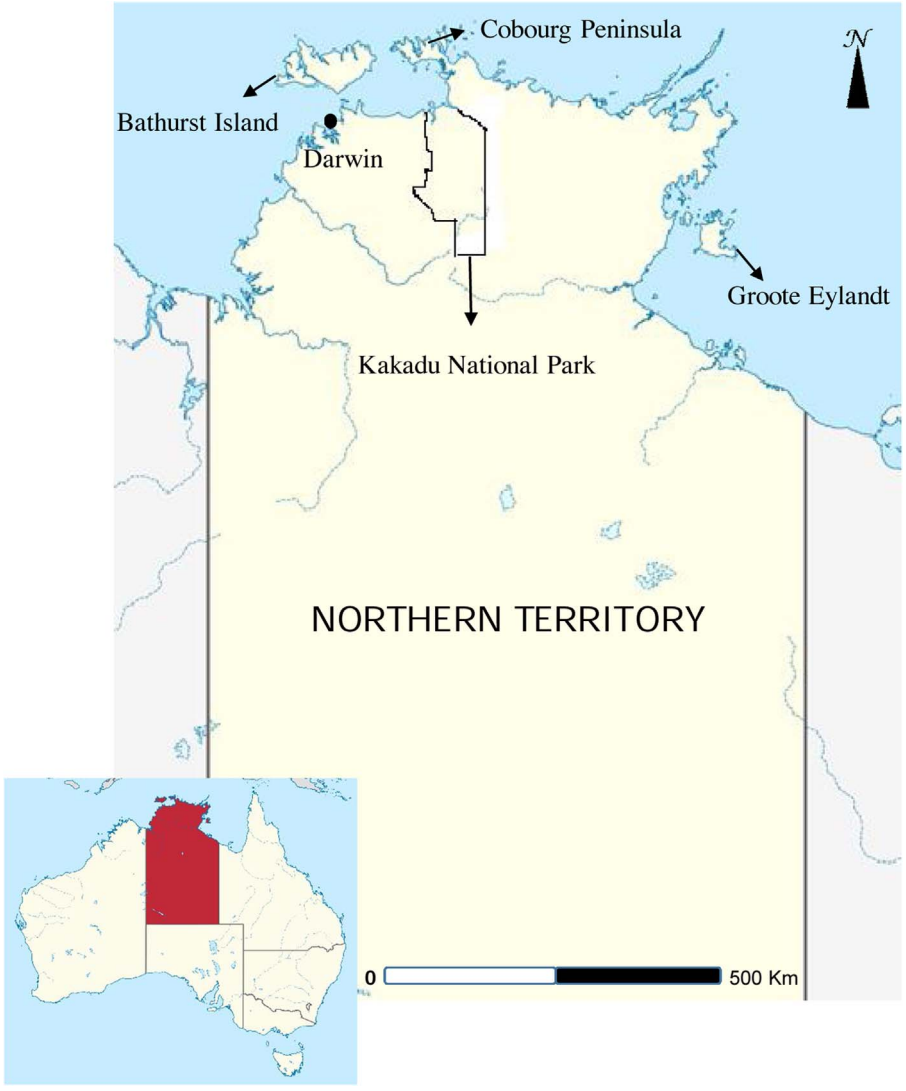


**Fig. 1.** Geographic representation of survey areas within the NT and location of the territory in the Australian continent (insert).

particularly across the NT. Trypanosomes are widespread blood-borne protozoan which can infect a wide range of vertebrates including humans. Ten species of *Trypanosoma* have been described in native mammals in Australia to date (Thompson et al., 2014a; Barbosa et al., 2016a; Botero et al., 2016); there are also reports of the association of *Trypanosoma* spp. with the potential extinction of bulldog rats (*Rattus nativitatis*) and Macleay's rats (*Rattus macleari)* on Christmas Island (Pickering and Norris, 1996; Wyatt et al., 2008); anaemia and increased mortality in koalas (*Phascolarctos cinereus*) (McInnes et al., 2011); woylie or brush tailed bettong (*Bettongia penicillata*) population decline (Botero et al., 2013; Thompson et al., 2014b); and more recently, with severe clinical disease in an Australian little red flying fox (*Pteropus scapulatus*) (Barbosa et al., 2016a; Mackie et al., 2017).

Piroplasms are intraerythrocytic vector-borne protozoans belonging to the order Piroplasmida (phylum Apicomplexa), which include the genera *Theileria*, *Babesia* and *Cytauxzoon.* Australian native mammals are believed to host multiple species of piroplasms, that have previously been identified as members of the genus *Babesia* or *Theileria* (Priestley, 1915; Backhouse and Bolliger, 1959; Mackerras, 1959; Barker et al., 1978; Collins et al., 1986; Bangs and Purnomo, 1996; O'Donoghue and Adlard, 2000; Clark and Spencer, 2007; Lee et al., 2009; Paparini et al., 2012a; Rong et al., 2012; Dawood et al., 2013; Kessell et al., 2014; Donahoe et al., 2015; Paparini et al., 2015). Although infections can be asymptomatic (Clark et al., 2004; Vaughan et al., 2009; Paparini et al., 2012a; Rong et al., 2012; Portas et al., 2014), sporadic exceptions have been reported (Backhouse and Bolliger 1957, 78; Barker et al., 1978; Dawood et al., 2013; Kessell et al., 2014; Donahoe et al., 2015).

Other potential blood-borne pathogens such as *Hepatozoon* spp. have been identified in quolls, possums and bandicoots in Australia (Mackerras, 1959; Bettiol et al., 1996; Wicks et al., 2006). Nevertheless, there is still limited knowledge about the clinical significance of *Hepatozoon* spp. in Australian mammals.

*Cryptosporidium* and *Giardia* are intestinal parasites which can cause diarrhoeal illness in animals and humans worldwide. In Australia, *C. fayeri* and *C. macropodum* are the most common species reported in a range of marsupials; however, kangaroo genotype 1, brushtail possum genotype 1 and zoonotic species such as *C. cuniculus, C. meleagridis*, *C. muris, C. ubiquitum, C. hominis* and *C. parvum* have been reported in the eastern grey kangaroo (*Macropus giganteus*), western grey kangaroo (*Macropus fuliginosus*), southern brown bandicoot (*Isoodon obesulus*), common brushtail possum (*Trichosurus vulpecula*), common wombat (*Vombatus ursinus*) and brush-tailed rock-wallaby (*Petrogale penicillata*) (Koehler et al., 2016a; Zahedi et al., 2016a; Zahedi et al., 2016b). *Cryptosporidium* species including *C. tyzzeri, C. parvum,* mouse genotype II and rat genotypes have also been detected in exotic rodents in Australia such as wild house mice (*Mus domesticus*) and black rats (*Rattus rattus*) (Morgan et al., 1999; Foo et al., 2007; Paparini et al., 2012b). Whilst the majority of *Giardia* found in free-ranging terrestrial