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Review

Is *Toxoplasma gondii* a threat to the conservation of free-ranging Australian marsupial populations?

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ABSTRACT

It has often been asserted that Australian marsupial species are particularly susceptible to *Toxoplasma gondii* infection and to clinical toxoplasmosis following infection. This implicates *T. gondii* as a potential threat to marsupial population viability, and contrasts to what is known of *T. gondii* in populations of several other host species. We reviewed the literature, and found a lack of scientifically robust evidence addressing the occurrence of *T. gondii* infection in free-ranging populations of Australian marsupial species, and the impacts of the infection on population health. Key limitations included a lack of studies in free-ranging marsupial populations, study findings susceptible to substantial chance influences, and selection, misclassification and confounding biases. The lack of scientifically robust data available on this topic indicates that assertions that free-ranging populations of Australian marsupials are particularly susceptible to *T. gondii* infection and to toxoplasmosis are premature. The threat of *T. gondii* to the viability of free-ranging marsupial populations should therefore be regarded, at this stage, as a hypothesis.

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1. Introduction

Australia is home to a vast array of endemic marsupial species (superorder Australidelphia). Of an estimated 162 marsupial species present at the time of European settlement in 1788, twelve species have since become extinct, and many others have suffered major contraction of distribution and substantial to severe population declines (Burbidge et al., 2009; Woinarski, 2015). The primary drivers of these population declines are believed to be: anthropogenic habitat loss; habitat destruction associated with climate change, altered fire regimes and the introduction of competing feral herbivores and exotic weeds; and predation by introduced feral species, particularly foxes and cats (Dickman, 1996; Fisher et al., 2003; McKenzie et al., 2007; Burbidge et al., 2009; Saunders et al., 2010).

There has also been speculation as to the role of introduced infectious disease as a contributing factor to population declines of Australian marsupials (e.g., Freeland, 1994; Abbott, 2006; Thompson et al., 2010).

Toxoplasma gondii is arguably the most broadly implicated infection, prompted by reports of cases and outbreaks of severe clinical toxoplasmosis in captive populations of Australian marsupial species. From the published literature, there appears to be a perception that marsupials are particularly susceptible to infection with *T. gondii* and to toxoplasmosis. For example: "Toxoplasmosis is a significant disease of Australian marsupials commonly causing mortality in captive and free-ranging populations…" (Obendorf et al., 1996); "Marsupials and new world monkeys are among the most susceptible animals for developing the clinical disease toxoplasmosis…" (Skerratt et al., 1997); "Australasian marsupials, especially wallabies, are highly susceptible to acute toxoplasmosis" (Dubey and Crutchley, 2008); "Australian marsupials are among the most susceptible hosts for *T. gondii*…" (Parameswaran et al., 2009a); "*T. gondii* is of concern for Australian native marsupials, which appear to be particularly susceptible to acute infection…" (Hollings et al., 2013).

In this paper, we examine the evidence for these assertions. We review estimates of the frequency of *T. gondii* infection in free-ranging Australian marsupial populations, and then review the extent to which the infections are associated with acute toxoplasmosis or with other effects (such as behavioural changes and reduced reproductive success) that may threaten population viability.

1.1. T. gondii infection and toxoplasmosis

T. gondii is a protozoan parasite, which can infect a wide range of endothermic vertebrates. Cats (Felidae) are the definitive host-infected cats shed environmentally resistant oocysts in the faeces. Oocysts become infective in the environment, and if ingested can infect both intermediate hosts (including Australian marsupial species) and other definitive hosts. Following ingestion, sporozoites excyst from oocysts, invade the gut epithelium and transform into tachyzoites. Tachyzoites multiply asexually and may colonise many host tissues, evoking a strong immune response. Tachyzoites differentiate into bradyzoites, which produce tissue cysts that are resistant to the immune response. Bradyzoites may be transmitted to a definitive host, or another intermediate host, upon ingestion of infected tissues. In addition, these hosts may also be infected via vertical transmission from infected mother to foetus/suckling young (Dubey, 1998, 2010).

In most intermediate host species, including people, *T. gondii* infection tends to be subclinical; toxoplasmosis (clinical disease caused by *T. gondii* infection) is usually associated with complicating factors such as immunosuppression (Montoya and Leisenfeld, 2004; Dubey, 2010). Clinical toxoplasmosis may follow recent infection with *T. gondii*, or result from a recrudescent infection. Recrudescence may be prompted by concurrent illness or immunosuppression (Ruskin and Remington, 1976; Lappin et al., 1991; Nicoll et al., 1997).

2. The frequency of *T. gondii* infection in free-ranging populations of Australian marsupial species

No published studies have investigated the incidence of *T. gondii* infection in free-ranging populations of Australian marsupials. Surveys have provided estimates infection prevalence and seroprevalence; these are summarised in Tables 1 and 2.

2.1. T. gondii infection surveys undertaken in free-ranging populations of Australian marsupials

Evidence of *T. gondii* infection has been found in free-ranging populations of red kangaroos (*Macropus rufus*), western grey kangaroos (*Macropus fuliginosus*), common wallaroos (*Marcopus robustus*) and woylies (*Bettongia penicillata*) (Table 1). Findings suggestive of *T. gondii* infection (histopathological evidence without confirmatory testing) have also been obtained from long nosed bandicoots (*Perameles nasuta*), eastern barred bandicoots (*Perameles gunnii*), southern brown bandicoots/quenda (*Isoodon obesulus*), quokka (*Setonix brachyurus*), brushtail possums (*Trichosurus vulpecula*), brush-tailed phascogales (*Phascogale tapoatafa*) and kowari (*Dasyuroides byrnie*) (Table 1).

Prevalence estimates are all limited by uncertain external validity, due to the use of non-proportionate sampling methods: reliance on culled animals, road kill or trapping for study subjects may entail selection bias. Surveys involving small sample sizes have low power to detect the presence of infection, and marked imprecision in prevalence estimates (Table 1). Commonly, the use of diagnostic methodology that is known to be of poor sensitivity and/or specificity in other species leaves apparent prevalence estimates subject to misclassification bias.

In marsupial surveys, the most commonly used diagnostic test has been the mouse bioassay, which lacks sensitivity (Piergili Fioretti, 2004). None of the surveys of Australian marsupials using this technique also used immunohistochemistry or PCR to confirm identification of *T. gondii* bradyzoites. Thus, the specificity of the mouse bioassay may be compromised, as *T. gondii* bradyzoites can appear very similar to those of *Neospora caninum* under light microscopy (Dubey et al., 2009). As with the mouse bioassay, sample inoculation into cell culture (in this case 13-day old chick embryos (Table 1)) lacks sensitivity, often because of laboratory error (Piergili Fioretti, 2004).

Histopathological examination of host tissues, without confirmatory immunohistochemistry or PCR, was used in a number of marsupial surveys (Table 1). However, this is also an insensitive screening tool, particularly in identifying low burden *T. gondii* infections (Piergili Fioretti, 2004). Specificity of these results might also be compromised by misidentification of other protozoan parasites as *T. gondii* (Dubey et al., 2009).

PCR amplification of *T. gondii* DNA in tissue samples is generally considered a sensitive indicator of infection (Burg et al., 1989; Su et al., 2010). PCR has only been used in two surveys of Australian marsupials, which collectively sampled four species (Parameswaran et al., 2010; Pan et al., 2012). A high proportion these animals tested positive for *T. gondii* by this methodology. Though these findings are limited by small sample sizes, they sharply contrast to collective findings of similar species surveyed histopathologically, where infection was rarely identified (Table 1). As the latter studies were based on different species in different