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# Variation in *Toxoplasma gondii* seroprevalence: effects of site, sex, species and behaviour between insular and mainland macropods

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## Abstract

***Context.*** Feral cats threaten wildlife conservation through a range of direct and indirect effects. However, most studies that have evaluated the impacts of feral cats on species of conservation significance have focussed on direct impacts such as predation; few studies have considered the indirect impacts of cat-borne disease. *Toxoplasma gondii*, a cat-borne parasite, causes both acute and latent disease in a range of wildlife species, and macropods are particularly susceptible. Kangaroo Island is Australia's third largest island and supports a high density of feral cats and high seroprevalence of *T. gondii* in multiple species, relative to the mainland. This suggests that Kangaroo Island has a high environmental contamination with the parasite and a high risk of infection for other species.

***Aims.*** We aimed to describe *T. gondii* seroprevalence in culled and road-killed macropods, so as to assess the effects of island versus mainland location, sex, species and behaviour.

***Methods.*** Macropod sera were tested for *T. gondii* IgG antibodies using a commercially available modified agglutination test.

***Key results.*** The seroprevalence of *T. gondii* in culled western grey kangaroos (*Macropus fuliginosus*) was significantly higher on the island (20%, 11/54 positive) than on the mainland (0%, 0/61 positive). There was no difference in *T. gondii* seroprevalence between culled and road-killed (21%, 21/102 positive) kangaroos from the island. The seroprevalence of *T. gondii* was significantly higher in female (32%, 12/38 positive) than in male (13%, 8/60 positive) kangaroos, but we observed no sex effect in tammar wallabies (*Macropus eugenii*), and no effect of species.

***Conclusions.*** The higher *T. gondii* seroprevalence in insular macropods supports previous reports of higher *T. gondii* exposure in other Kangaroo Island fauna. The lack of difference in *T. gondii* seroprevalence between culled and road-killed kangaroos suggests that *T. gondii*-positive animals are not more vulnerable to road mortality, in contrast to that suggested previously.

***Implications.*** Our findings suggest greater potential adverse conservation impacts owing to toxoplasmosis on the island than on the mainland. In light of a recent study demonstrating higher cat abundance on the island than on the mainland, the higher observed *T. gondii* seroprevalence in insular macropods is likely to be a consequence of higher cat density.

**Additional keywords:** carcass, feline, *Felis catus*, latent, marsupial, toxoplasmosis.

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## Introduction

Feral cats (*Felis catus*) threaten wildlife conservation globally through predation, resource competition, disruption of migration and seed-dispersal pathways, induced behavioural changes, hybridisation and disease (Medina *et al*. 2014). For example, predation by feral cats has been implicated as the primary cause of failure of the reintroduction of multiple species of macropod (Hardman *et al*. 2016), and competition for food by feral cats is

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reported to reduce the reproductive success of predatory seabirds (Courchamp *et al*. 2003). However, when evaluating the mechanisms by which feral cats threaten species of conservation significance, the majority of studies have focussed on the direct and seemingly most apparent impacts of cats, such as predation, and, to a lesser extent, competition (Medina *et al*. 2014). By comparison, few studies have considered the impacts of cat-borne disease when evaluating threats to wildlife.

Toxoplasmosis, the disease caused by the parasite *Toxoplasma gondii*, causes significant health problems in wildlife worldwide. For example, *T. gondii* infection can influence rodent behaviour (Vyas *et al*. 2007), cause mortality in lagomorphs (Sedlák *et al*. 2000) and new world monkeys (Dietz *et al*. 1997), and cause respiratory, neurological and gastrointestinal problems and mortality in Australian marsupials (Canfield *et al*. 1990). Macropods are particularly susceptible to *T. gondii* infection and, consequently, toxoplasmosis, with disease having been documented in both captive and free-ranging populations. For example, wild Tasmanian pademelons (*Thylogale billardierii*) and Bennett's wallabies (*Macropus rufogriseus rufogriseus*) develop severe blindness and incoordination (Obendorf and Munday 1983), and tammar wallabies (*M. eugenii*) experimentally infected with *T. gondii* experience acute mortality (Lynch *et al*. 1993; Reddacliff *et al*. 1993). However, in some other species, the majority of *T. gondii* infections are asymptomatic (Dubey 2016*a*); therefore, it is possible that many infections in macropods may produce no clinical signs.

Felids are the only known definitive host of *T. gondii*. After consuming infected prey, cats shed the oocyst stage in faeces (Hutchison *et al*. 1971). Oocysts are environmentally resistant and remain viable under favourable conditions for 18 months or longer (Yilmaz and Hopkins 1972; Frenkel *et al*. 1975). Any warm-blooded animal can act as an intermediate host and becomes infected by consuming food, water or soil contaminated with oocysts (Aramini *et al*. 1999; Hill and Dubey 2002). Within the intermediate host's digestive tract, oocysts transform into tachyzoites, which rapidly divide within the host cells, causing them to rupture and release tachyzoites to infect other host cells (Dubey 2016*b*). Tachyzoites disseminate via blood and lymph, and their rapid multiplication and spread during the early stages of infection may cause tissue necrosis, organ failure, and death in severe cases (acute toxoplasmosis). In less severe cases, the extensive multiplication of tachyzoites is suppressed by the host's immune system, causing the parasite to transform into microscopic cysts in muscle and neurological tissues that contain bradyzoites. Bradyzoites remain dormant in the tissues for life (latent toxoplasmosis); the lifecycle of *T. gondii* is then restarted when a naïve felid consumes bradyzoites in the tissues of an infected intermediate host.

Latent toxoplasmosis may cause behavioural abnormalities in some intermediate host species. For example, in rodents, the innate fear of feline pheromones becomes an attraction in latently infected animals (Vyas *et al*. 2007). Behavioural abnormalities due to latent toxoplasmosis have also been speculated to explain differences in the seroprevalence of *T. gondii* between road-killed and culled macropods by reducing their reaction time (Hollings *et al*. 2013).

Kangaroo Island, in southern Australia, supports several threatened and endemic wildlife species, but it also has a high relative abundance of feral cats (14.6 cats site$^{-1}$) compared with the adjacent mainland (1.39 cats site$^{-1}$; Taggart *et al*. 2019). The seroprevalence of *T. gondii* in feral cats (*Felis catus*) and sheep (*Ovis aries*) on the island is substantially higher than that on the Australian mainland (O'Donoghue *et al*. 1987; Fancourt and Jackson 2014; P. L. Taggart, M. M. McAllister, D. Rutley, and C. G. B. Caraguel, unpubl. data, 2019). This suggests a comparatively high level of environmental contamination with *T. gondii* on the island and a high risk of infection to other species. However, in some ecosystems, a high seroprevalence of *T. gondii* has been recorded in some species, but not others sampled from the same location (Dubey *et al*. 2006; Murata *et al*. 2018). This highlights the need to investigate *T. gondii* seroprevalence separately for different wildlife species on Kangaroo Island and the adjacent mainland, particularly for species that could be expected to suffer from toxoplasmosis.

We conducted a *T. gondii* serosurvey in macropods on Kangaroo Island and a climatically similar location on the adjacent mainland. Western grey kangaroo (*M. fuliginosus*) from both locations was tested for *T. gondii* exposure in conjunction with culling for population control. Road-killed kangaroos and tammar wallabies (animals killed by collision with motor vehicles along roads) were also tested opportunistically to compare *T. gondii* seroprevalence with that of animals killed by culling, to investigate the potential for behavioural differences in macropods with latent toxoplasmosis. On the basis of studies in cats and sheep (Fancourt and Jackson 2014; O'Donoghue *et al*. 1987), we anticipated *T. gondii* seroprevalence to be two to three times higher in kangaroos on the island than on the adjacent mainland.

## Materials and methods

We followed the CONSISE guidelines for the reporting of seroepidemiologic studies outlined in Horby *et al*. (2017).

### *Study regions*

Kangaroo Island (–35.8015$^{0}$, 137.9752$^{0}$) is located 13.5 km south-west of the Fleurieu Peninsula (–35.5886$^{0}$, 138.1986$^{0}$), the closest region of mainland South Australia. The study areas will be referred to here as 'island' and 'mainland' respectively. Both the island and mainland sites experience similar Mediterranean climates (Jenkins 1985; Schwerdtfeger 2002) and comprise similar land uses and vegetation types, predominately cropping and pasture land interspersed with patches of native vegetation (largely low *Eucalyptus* spp. woodlands). However, relative cat abundance on the island is known to be substantially higher than that on the mainland (Taggart *et al*. 2019), which could be expected to influence *T. gondii* seroprevalence in intermediate hosts (de Wit *et al*. 2019).

### *Culled kangaroo sampling on island and mainland*

Kangaroos were sampled in conjunction with cull programs on two separate landholdings (2.4 km$^2$ each) on the Dudley Peninsula, Kangaroo Island, and a single landholding (44.5 km$^2$) at the tip of the Fleurieu Peninsula on mainland South Australia. Approximately 10 kangaroos were sampled on

the mainland, and then immediately on the island (1–2 days later), at approximate 2-month intervals for a period of 12 months. We estimated the age of kangaroos as adult, young-at-foot or pouch young. Although information was obtained on kangaroo sex, this information could not be linked back to individual serum samples (sex information was collected 1 day after serum collection).

### *Road-killed macropod sampling on island*

Road-killed kangaroos and wallabies were predominately sampled (83%) along the Hog Bay Road where vehicle traffic is greatest. As the collectors regularly drove the same stretch of road, the majority of blood samples were collected and frozen whole within 24 h of the animal's death. We recorded the sex and species of sampled carcasses, before dragging them away from the road to ensure they were not sampled twice. All road-killed macropods were considered adults as we could not differentiate between adults and young-at-foot post-death.

### *Blood collection and processing*

We collected blood from culled macropods within minutes after death. Blood was collected via cardiac puncture where possible. For road-killed animals, where the thoracic cavity was damaged or blood was coagulated, we opened the thoracic cavity and collected blood or haemolysed fluids from within the cavity or via direct cardiac puncture. Blood samples from road-killed macropods were usually very haemolysed or diluted by other bodily fluids.

Blood samples from culled kangaroos were allowed to clot at ambient temperature, centrifuged at 4000*g* for 5 min and the extracted sera were stored at –20°C until serological testing. Whole blood from road-killed macropods were first stored at –20°C for up to 3 months after collection, before being defrosted, centrifuged, and sera being collected and stored until serological testing as described above. All serum samples were frozen for periods of 1–6 months and underwent two freeze–thaw cycles before serological testing. Long-term storage of frozen sera or whole blood does not significantly modify the interpretation of *T. gondii* serologies (Fancourt *et al*. 2014; Dard *et al*. 2017).

### *Toxoplasma gondii antibody test*

Sera were tested for *T. gondii* IgG antibodies by using a commercially available modified agglutination test (MAT; Toxo-Screen DA, bioMe'rieux, Marcy-l'Etoile, France), following the manufacturer's guidelines. The MAT is based on the direct agglutination of fixed *T. gondii* tachyzoites of the RH strain, with sera pre-treated with 2-mercaptoethanol to neutralise IgM antibodies (Desmonts and Remington 1980; Dubey and Desmonts 1987). Immunoglobulin G antibodies typically take greater than 1 week to develop, but, once developed, persist for the remainder of the animal's life; consequently, this test may give false negative results during the first week of infection (Dubey and Crutchley 2008). It is not known how quickly IgG antibodies degrade in carcasses exposed to ambient environmental conditions. Sera were screened at 1 : 40 and 1 : 4000 dilutions, and classified as positive if agglutination occurred at either dilution. A dilution was classified as positive when agglutination of *Toxoplasma* formed a mat covering about half of the well base. In each assay, we included a positive kangaroo control, the positive and negative goat controls provided by the manufacturer, and a negative phosphate-buffered saline (PBS, pH 7.2) control. The kangaroo positive control was sourced from a captive western grey kangaroo showing neurological signs (head tilt) consistent with toxoplasmosis and that tested positive to *T. gondii* using the MAT in a different laboratory at a dilution of 1: 64 000.

The diagnostic sensitivity and specificity of the MAT in western grey kangaroos and tammar wallabies is unknown, but is expected to be high on the basis of the test performance in other species (Mainar-Jaime and Barberan 2007). Diagnostic-test misclassification in our study was expected to be non-differential, potentially reducing our power to detect between-group differences in *T. gondii* seroprevalence (Dohoo *et al*. 2009). We are not aware of any serologic cross-reactivity with the MAT or the direct agglutination test (Dubey 2016*b*; Gondim *et al*. 2017).

### *Data analysis*

Seroprevalence among groups of macropods was compared using Fisher's exact tests or chi-square tests using the ($n$–1) adjustment as recommended (Campbell 2007). We tested for seroprevalence differences associated with sex, location (island or mainland), species and behaviour (culled vs roadkill). To test whether kangaroo sex confounded the effect of behaviour, we compared the sex ratio between the road-kill and cull groups using the ($n$–1) Chi-squared test. Confidence intervals for seroprevalence estimates are reported as binomial exact. All statistical analyses were performed in R version 3.5.1 (R Core Team 2018).

## Results

We examined 65 culled kangaroos from the mainland, 67 culled and 102 road-killed kangaroos from the island, and 76 road-killed tammar wallabies from the island (Table 1).

### *Sex effect*

We found a significant difference in *T. gondii* seroprevalence between road-killed male and female kangaroos, but not between road-killed male and female wallabies (Table 1).

### *Island effect*

*Toxoplasma gondii* seroprevalence in culled adult kangaroos from the island was significantly greater than in culled adult kangaroos from the mainland (Table 1).

### *Species effect*

We found no difference in *T. gondii* seroprevalence between road-killed kangaroos and road-killed wallabies (Table 1).

### *Behaviour effect*

We found no difference in *T. gondii* seroprevalence between adult culled and road-killed kangaroos from the island (Table 1), and the sex ratio of these two groups did not differ ($P$ = 0.44), indicating that our behavioural analysis was not confounded by kangaroo sex.

**Table 1. Seroprevalence of *Toxoplasma gondii* in culled and road-killed western grey kangaroos (*Macropus fuliginosus*) and tammar wallabies (*M. eugenii*) from Kangaroo Island and the adjacent Australian mainland by age and sex**

| Sample type | Species | Age | Sex | Mainland | | | Kangaroo Island | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | *N* | Seroprevalence % | 95% CI (lower, upper) | *N* | Seroprevalence % | 95% CI (lower, upper) |
| Culled animal | Kangaroo | Adult | | 61 | 0[C] | 0, 6 | 54 | 20[C, E] | 12, 33 |
| | | Young at foot | | 0 | 0 | 0, 0 | 5 | 0 | 0, 43 |
| | | Pouch young | | 4 | 0 | 0, 49 | 8 | 25 | 7, 59 |
| Road-killed animal | Kangaroo | Adult | All | | | | 102 | 21[D, E] | 149, 29 |
| | | Adult | Male | | | | 60 | 13[A] | 7, 24 |
| | | Adult | Female | | | | 38 | 32[A] | 19, 48 |
| | | Adult | Unknown | | | | 4 | 25 | 1, 7 |
| | Wallaby | Adult | All | | | | 76 | 15[D] | 8, 24 |
| | | Adult | Male | | | | 39 | 15[B] | 7, 30 |
| | | Adult | Female | | | | 31 | 10[B] | 3, 25 |
| | | Adult | Unknown | | | | 6 | 33 | 10, 70 |

[A]Test for sex effect in kangaroos, $P = 0.03$.
[B]Test for sex effect in wallabies, $P = 0.48$.
[C]Test for island effect in kangaroos, $P = <0.001$.
[D]Test for species effect, $P = 0.29$.
[E]Test for behaviour effect, $P = 0.97$.

## Discussion

We found a significantly higher seroprevalence of *T. gondii* in kangaroos from Kangaroo Island than in those from the directly adjacent Australian mainland, as predicted. This finding is consistent with the results of previous studies in cats and sheep, which have identified higher seroprevalence on the island than the mainland (O'Donoghue *et al*. 1987; Fancourt and Jackson 2014; P. L. Taggart, M. M. McAllister, D. Rutley, and C. G. B. Caraguel, unpubl. data, 2019), but the magnitude of difference in *T. gondii* seroprevalence between the island and the mainland was larger than expected. Our finding of no infection on the mainland was unexpected and warrants further investigation. As felids are definitive hosts of *T. gondii*, and the only hosts known to shed the oocyst stage of the parasite in their faeces following infection, we suggest that the high seroprevalence of *T. gondii* in macropods on the island is likely to be driven by the island's high cat abundance compared with the mainland (Taggart *et al*. 2019).

The observed *T. gondii* seroprevalence in western grey kangaroos from the island is consistent with the results of a previous seroprevalence survey in this species in Western Australia; however, our mainland seroprevalence was much lower than that reported in the Western Australian survey. Parameswaran *et al*. (2009*b*) reported a *T. gondii* seroprevalence of 15.5% in 219 free-ranging adult western grey kangaroos from the metropolitan region of Perth in Western Australia. This is comparable to the level of infection observed on Kangaroo Island in our study (20%), but much greater than that observed on the mainland (0%; Table 1). This suggests that environmental contamination with *T. gondii* shows large variation on the Australian mainland, and although the seroprevalence of *T. gondii* on Kangaroo Island appears to be much greater than that on the South Australian mainland, it may be comparable to that in other regions of mainland Australia. Reasons for the comparable *T. gondii* seroprevalence between Kangaroo Island and the metropolitan region of Perth can only be speculated, but could include similarities and or differences in a combination of factors such as cat density and feeding ecology, climate or soil characteristics. Although other studies have assessed *T. gondii* infection in free-ranging western grey kangaroos, only few animals were sampled (Pan *et al*. 2012), precluding any robust comparison.

*Toxoplasma gondii* infection did not seem to be driving higher rates of road-kill in kangaroos as a consequence of behavioural impacts owing to latent toxoplasmosis. We found no difference in the seroprevalence of *T. gondii* between culled and road-killed kangaroos, in contrast to our predictions that seroprevalence would be higher in road-killed animals. These predictions were founded from Hollings *et al*. (2013) who speculated that reduced reaction time in Tasmanian pademelons (*Thylogale billardierii*) because of latent toxoplasmosis may have explained the higher seroprevalence of *T. gondii* that they found in road-killed than in culled animals. Although we are not aware of any evidence in macropods that would support their hypothesis, other studies may provide alternative explanations for their findings. A higher seroprevalence of *T. gondii* may have been observed in road-killed than in culled macropods because of acute disease causing them to become uncoordinated or affecting their vision (Obendorf and Munday 1983). Cat activity may also concentrate along roadsides following the decline of Tasmanian devils (*Sarcophilus harrisii*) that regularly use roads to traverse the landscape (Fancourt *et al*. 2015), subsequently resulting in higher *T. gondii* oocyst contamination; also, Hollings *et al*. (2013) observed higher prevalence of *T. gondii* in macropods along roadsides than in those culled away from roadsides. However, regardless of the mechanism, we foundno evidence to support any hypothesis that might explain a difference in *T. gondii* seroprevalence between road-killed and culled macropods. As we collected substantially more road-kill samples than did Hollings *et al*. (2013), we suggest that their results may have arisen as a result of a low number of road-killed samples.

Our finding of no difference in *T. gondii* seroprevalence between kangaroos and wallabies contradicts what would be expected on the basis of the ecology and physiology of these species. Western grey kangaroos and tammar wallabies are both solely herbivorous and have many similarities in their diet and feeding ecology. However, smaller macropod species, such as wallabies, are generally considered to consume a greater proportion of browse and forbs than do their larger counterparts, the kangaroos, which consume a greater proportion of grass (Wann and Bell 1997; Telfer and Bowman 2006). Accordingly, kangaroos would be expected to spend a greater portion of their time grazing closer to the ground than do wallabies, increasing their relative risk of exposure to *T. gondii* oocysts. Western grey kangaroos are also known to live ~25% longer than do tammar wallabies (Inns 1982; Norbury *et al*. 1988; Ahlert 2002), and, because the risk of *T. gondii* infection increases with age (van der Puije *et al*. 2000; Jones *et al*. 2001), kangaroos would be expected to have a greater average cumulative risk of infection. Some evidence also exists suggesting that wallabies are more susceptible to acute toxoplasmosis than are kangaroos (Johnson *et al*. 1989; Lynch *et al*. 1993; Reddacliff *et al*. 1993); this would be expected to result in a greater mortality of acutely infected wallabies, thereby removing them from the population and leaving a higher proportion of negative wallabies to be sampled. Furthermore, wallabies are known to be within the size range of cat prey items (Fancourt 2015); so, if *T. gondii* infection influences their behaviour, then this could make them more susceptible to cat predation, as has been suggested for rodents and other species (Vyas *et al*. 2007; Poirotte *et al*. 2016). If this occurs, this predation bias would not affect kangaroos because their larger size would exclude them as a possible cat prey item. Although the true seroprevalence of *T. gondii* in kangaroos and wallabies may differ, the lack of difference observed in our study, despite reasonable sample sizes of both species, suggests that the described factors combined do not contribute to a large difference in *T. gondii* seroprevalence. Additional sampling of both species is likely to be required to uncover a small but true difference in *T. gondii* seroprevalence between kangaroos and wallabies in our study.

Wefound a higher seroprevalence of *T. gondii* in femalethan in male kangaroos, but no effect of sex in wallabies. This difference in *T. gondii* seroprevalence between sexes, and the order of the difference (higher seroprevalence in females than in males), is consistent with the results of studies in sheep and goats (van der Puije *et al*. 2000; Teshale *et al*. 2007), and has even been previously reported in western grey kangaroos (Parameswaran *et al*. 2009*b*). Parameswaran *et al*. (2009*b*) suggested that this difference in *T. gondii* seroprevalence between male and female kangaroos was likely to result from differences in their feeding ecology, where females graze shorter grass than do males (Newsome 1980), increasing their risk of infection. However, Teshale *et al*. (2007) suggested that the difference in *T. gondii* seroprevalence between male and female goats was likely to be due to differences in their average age, and van der Puije *et al*. (2000) suggested the same difference in seroprevalence in sheep and goats was likely to be due to females being more susceptible to infectionthanaremales(Alexanderand Stimson 1988; Ntafis *et al*. 2007; Ahmad *et al*. 2015) and an enhanced immune response in males (Kittas *et al*. 1984). Any of thesealternativeexplanations for the observed difference in *T. gondii* seroprevalence between male and female kangaroos in our study may be plausible, particularly if macropod culling disproportionately removes larger and older male kangaroos from the population.

Like all field and serological studies, our study had inherent limitations and biases. Two limitations, in particular, may have influenced the results of our serological assays. Both culled and road-killed macropod samples were collected on the basis of convenience, and may have misrepresented the seroprevalence of *T. gondii* in the population relative to randomly collected samples. In an attempt to overcome this, the sampling of culled kangaroos was conducted over as large an area as practical, based on active population-control programs at the time of sampling. Although road-kill sampling was restricted to busy roads and routes regularly travelled by sample collectors, *T. gondii* seroprevalence estimates from these samples were equivalent to those from culled samples, suggesting minimal bias owing to non-random sampling. Road-kill sampling and sampling from animal carcasses may additionally underestimate true seroprevalence because the reduced sample quality affects serological results (Tryland *et al*. 2006). However, the majority of road-killed kangaroo blood samples were collected within 24 h of the animal's death, reducing the probability of antibody denaturation. Furthermore, serological results from bloody fluids collected shortly after the animals' death, like those collected from road-killed carcasses in the present study or meat juice in other studies, are known to closely match serological results completed on serum from the same animals (Glor *et al*. 2013). Similarly, serological results from frozen whole blood and serum collected shortly after an animal's death are known to produce equivalent serological results (Fancourt *et al*. 2014). The dilution of blood with other bodily fluids and the gradual degradation of antibodies in carcasses, for example, owing to heat, autolysis, or bacterial putrefaction, will, at some point, have an impact on the accuracy of the serological test, although we do not believe that this has had a large influence on our serological results in road-killed macropods.

In some species, high *T. gondii* seroprevalence is known to, or would be expected to, be associated with a high prevalence of the disease toxoplasmosis (Dubey *et al*. 2012). Accordingly, our study suggests greater potential adverse conservation impacts from toxoplasmosis on the island than that on the mainland. Furthermore, our study was consistent with previous studies, suggesting that Kangaroo Island provides a favourable environment for the proliferation of *T. gondii* relative to the Australian mainland (O'Donoghue *et al*. 1987; Fancourt and Jackson 2014; Taggart *et al*. 2019; P. L. Taggart, M. M. McAllister, D. Rutley, and C. G. B. Caraguel, unpubl. data, 2019). This is of particular concern for other wildlife species on Kangaroo Island because of the known health impacts of *T. gondii* infection (Hillman *et al*. 2016). We suggest that *T. gondii* seroprevalence surveys be performed to assess the infection rate in a greater range of island fauna to help assess disease risk and inform management actions. However, the high level of *T. gondii* infection in kangaroos on the island does not appear to be contributing towards the high rate of road-killed kangaroos on the island. Current evidence suggests that the increased cat abundance on Kangaroo Island compared with the adjacent mainland is likely to be the primary driver of the increased prevalence of *T. gondii* in island fauna.

## Conflicts of interest

The authors declare no conflicts of interest.

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