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Toxoplasma gondii seroprevalence in the endangered Australian sea lion (*Neophoca cinerea*)

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Toxoplasma gondii is a ubiquitous parasite increasingly detected in marine mammals and suspected to contribute to limited recovery of endangered populations. This study reports on the exposure of the Australian sea lion (*Neophoca cinerea*) to this protozoon using archived adult and pup sera from three island colonies in South Australia. Modified agglutination testing (MAT) detected a seroprevalence of 30.4% (95% CI 13.2-52.9; n = 23) and high antibody titers (512 to > 2048 IU/ml) in adult females (median age 9.5 y, range 5.5-14.5 y) at Dangerous Reef, a felid-free island. Antibodies weren't detected in any surveyed pup (97.5% CI 0.0-2.0%, n = 184) at two felid-free islands (Dangerous Reef: n = 21; Olive Island: n = 65), nor at Kangaroo Island (n = 98), which has a high-density feral cat population. Kangaroo Island pups of known age were 7-104 d old, while standard length and bodyweight comparison suggested younger and older pup cohorts at Dangerous Reef and Olive Island, respectively. This study provides the first quantification of disease risk in this endangered species from *T. gondii* exposure. The absence of detectable pup seroconversion supports the lack of detectable congenital transmission, maternal antibody persistence or early post-natal infection in the sampled cohort yet to commence foraging. An extended serosurvey of *N. cinerea* colonies is recommended to confirm the hypothesis of predominantly forage-associated exposure to *T. gondii* in this species. Findings have implications for parasitic disease risk in wildlife inhabiting Australia's islands and for the feral cat control program on Kangaroo Island.

KEYWORDS

antibody, Australian sea lion, *Neophoca cinerea*, seal, pinniped, serology, *toxoplasma gondii*

Introduction

Toxoplasma gondii is an emerging pathogen in marine mammals, with substantial worldwide seroprevalence in pinniped (seal and sea lion), cetacean (dolphin and whale) and mustelid (sea otter) species, (reviewed in [Dubey et al., 2020](#)) and documented mortalities in critically endangered populations ([Roe et al., 2013](#)). Understanding of the parasite's potential to modulate marine mammal population size and geographical range comes from surveys of European and North American cetaceans and marine mustelids ([Ahmadpour et al., 2022](#)). In the threatened southern sea otter (*Enhydra lutris nereis*), for instance, *T. gondii*-associated encephalitis was considered a primary or contributing cause of more than a quarter of deaths (1998-2001: n = 105), predominantly in adults ([Kreuder et al., 2003](#)). In one spatial cluster, it was the primary cause of half of all deaths (n = 16). A longer-term study showed nearly two-thirds of sea otters (1998-2012: n = 542) were infected with *T. gondii* and the parasite contributed to at least one in ten deaths, while infectious disease more generally was the leading cause of death in this threatened species ([Miller et al., 2020](#)).

In pinnipeds, and specifically otariids (eared seals), *T. gondii* infection and clinical toxoplasmosis are reported in free-ranging populations worldwide ([Jensen et al., 2012](#); [Carlson-Bremer et al., 2015](#); [Sepulveda et al., 2015](#); [Michael et al., 2016](#)). Dependent on serological method and selected cut-off value, seroprevalence can exceed 50% ([Jensen et al., 2012](#)). In comparison, surveying for *T. gondii* exposure in Australian otariid species is limited to Australian fur seals (*Arctocephalus pusillus doriferus*) in Bass Strait, where in 2007-2008 an absence of *T. gondii* antibodies were detected in adult females of unspecified age (n = 104) ([Lynch et al., 2011](#)). Reporting of sporadic cases of disseminated toxoplasmosis is also rare: the most recent in Australia was a subadult male long-nosed fur seal (*Arctocephalus forsteri*) that hauled out on the east coast in 2012 ([Donahoe et al., 2014](#)). For the endangered Australian sea lion (*Neophoca cinerea*), toxoplasmosis is listed as a potential threat to the species' recovery ([Threatened Species Scientific Committee, 2020](#)), based on observations in other marine mammals and documented disease susceptibility ([Kabay, 1996](#)). To date there has not been any serological survey for *T. gondii* (or other infectious pathogen) exposure reported in this declining species. This is of particular importance given the species' relatively low genetic diversity potentially associated with an increased susceptibility to infectious disease ([Bilgmann et al., 2021](#)), and a vulnerable population structure of approximately 80 effectively closed sub-populations resulting from extreme female natal site fidelity ([Campbell et al., 2008](#)). Consequently, disease-induced mortality outbreaks pose the

greatest risk to the species' resilience based on modelling of future population decline ([Bilgmann et al., 2021](#)).

Domestic (*Felis catus*) and wild (including feral) felids are the only definitive hosts of *T. gondii*, an intracellular and zoonotic apicomplexan protozoan parasite capable of infecting all warm-blooded animals. Across these species, the mammals and birds, parasite transmission is related either to foraging – ingestion of oocysts in contaminated food or water, or of tissue cysts in prey species – or to transplacental transmission from recently infected mother to offspring ([Dubey, 2022](#)). In the marine mammals, the former can contribute directly to illness or death from localized or disseminated infection, reported for instance in the California sea lion (*Zalophus californianus*) ([Carlson-Bremer et al., 2015](#)), Mediterranean monk seal (*Monachus monachus*) ([Mazzariol et al., 2021](#)) and multiple other seal species ([van de Velde et al., 2016](#); [Reisfeld et al., 2019](#)). Additionally, infection can increase the risk of death from other causes such as predation and conspecific trauma, as observed in the southern sea otter ([Kreuder et al., 2003](#)) and New Zealand sea lion (*Phocarctos hookeri*) ([Roe et al., 2017](#)). Transplacental transmission, placental inflammation and disseminated congenital fetal infection, resulting in early or late-term abortion or stillbirth have also been documented in multiple species, among them Risso's dolphin (*Grampus griseus*) ([Resendes et al., 2002](#)), the California sea lion ([Carlson-Bremer et al., 2015](#)) and the southern sea otter ([Shapiro et al., 2016](#)).

The infection of marine mammals indicates contamination of coastal and marine environments with oocysts shed in cat feces, by terrestrial water run-off or from ocean sewage discharge ([Miller et al., 2002](#); [Fayer et al., 2004](#)) ([Figure 1](#)). For species frequenting coastal habitats, such as pinnipeds, infection could also result from direct contamination of haul out sites by cat feces. In any case, infection is most likely to result from oocyst (versus tissue cyst) ingestion, given the cold-blooded nature of their prey species (e.g., fish and marine invertebrates) precludes susceptibility to active *T. gondii* infection. Oocysts are environmentally resistant, capable of sporulation (becoming infectious) and temperature-dependent maintenance of infectivity in sea water for at least 6-24 months ([Lindsay et al., 2003](#); [Lindsay and Dubey, 2009](#)). Increased exposure of marine mammals is likely facilitated by parasite carriage and maintenance of viability in cold-blooded prey species acting as phoretic hosts – including filter-feeding bivalve mollusks (e.g., blue (*Mytilus edulis*) ([Bigot-Clivot et al., 2022](#)) and green-lipped (*Perna canaliculus*) mussels ([Coupe et al., 2018](#))) and fish species ([Marino et al., 2019](#)), the latter already a suggested source of infection for coastal and pelagic seabirds ([Poulle et al., 2021](#); [Campbell et al., 2022](#)). Independent of prey species, the initial entry of the parasite into the marine food chain is likely enabled by aggregation in gelatinous marine polymers and by adhesion

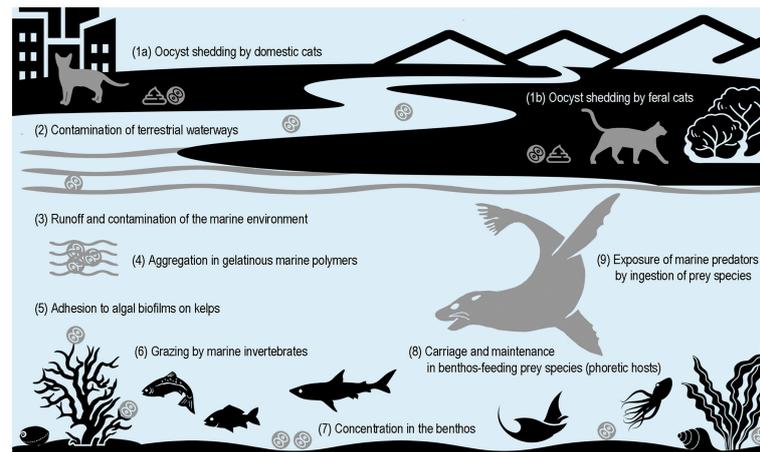


FIGURE 1

Proposed pathways for exposure of marine mammals to *Toxoplasma gondii* oocysts shed by terrestrial-bound definitive felid hosts.

to algal biofilms on kelps grazed by marine invertebrates (Shapiro et al., 2014).

The Australian sea lion inhabits the shores and islands of Australia's southern and south-western coast (Gales et al., 1994), much of which is arid coastline of relatively low human population density. Australia's abundant feral cat population, estimated to be 2.1–6.3 million animals dispersed across 99.8% of the continent (including the arid regions) (Legge et al., 2017) is a likely source of environmental contamination by *T. gondii* oocysts. Furthermore, while some Australian sea lion colonies exist on felid-free offshore islands (e.g., Olive Island and Dangerous Reef, South Australia), others (e.g., Seal Bay, Kangaroo Island, South Australia) are exposed to a feral cat density estimated to be 10 times greater than that of the adjacent mainland (Taggart et al., 2019a). For colonies on feral cat-inhabited islands there are potential health implications for pups (and adults) from increased exposure risk, given pups are mainly terrestrial until their first molt at 4–5 months of age, and are not weaned until 15–18 months of age. However, pups commence exploration of the adult foraging range by 6 months of age and obtain hunting skills at least 8 months prior to weaning (Lowther and Goldsworthy, 2012) at which age, their foraging-associated exposure risk (ingestion to *T. gondii* oocysts present in prey species) is likely to be similar to that of an adult animal.

This study aimed to provide the first estimate of *T. gondii* exposure in *N. cinerea*, and to infer route of infection in neonatal animals. We hypothesized that adult seroprevalence would parallel rates reported in other otariids, and that seroconversion would not be detected in young, pre-weaned pups yet to commence foraging. The latter investigation would also enable assessment of the persistence of any transplacental or colostrum-derived maternal antibodies.

Materials and methods

Study colonies

Archived sera from adult female Australian sea lions and pups were collected from Dangerous Reef (-34.817, 136.217), a small, uninhabited, and felid-free rocky island in the Spencer Gulf, South Australia (Figure 2), located 17.5 km from Lincoln National Park on the east coast of the Eyre Peninsula and 33 km from the coastal town of Port Lincoln (population 16,400). Pups were also sampled on Olive Island (-32.717, 133.983), a small, uninhabited, and felid-free island located 5 km from Cape Bauer on the west coast of the Eyre Peninsula and 25 km from the small regional coastal town of Streaky Bay (population 1,400), and at Seal Bay (-35.995, 137.317) on the south coast of Kangaroo Island, Australia's third largest (4,405 km²) inhabited island (population 4,700). Kangaroo Island, located 13.5 km from the southern tip of the Fleurieu Peninsula and 112 km southwest of the state capital city of Adelaide (regional population 1,359,760), has a high-density feral cat population (Taggart et al., 2019a).

Sample collection and age estimation

Australian sea lion pup samples were collected with approval of South Australian Department of Environment and Water (Permit/License Number: MR00073-2-R; Scientific Research Permit Number: A25088-12) and the study protocol was approved by The University of Sydney Animal Ethics Committee (Protocol Number 2014/726). Adult Australian sea lion samples were collected with approval of the South Australian Department for Environment and Heritage (DEH) (Permit Number U24630) and the study protocol was approved

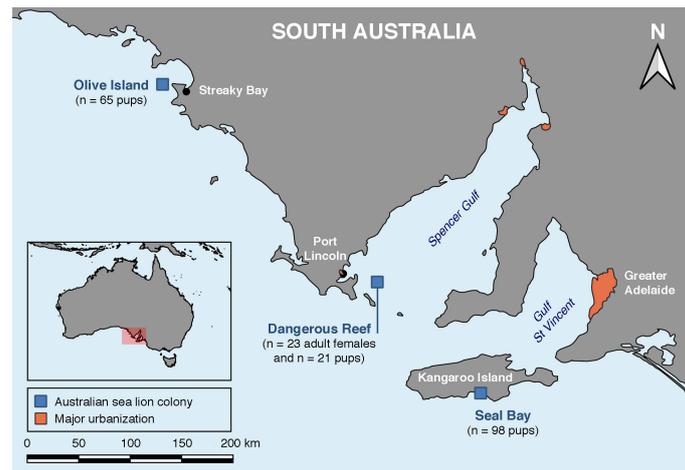


FIGURE 2

Location of the three Australian sea lion (*Neophoca cinerea*) colonies sampled for the serological survey of *T. gondii* antibody presence in adult females and pups.

by La Trobe University Animal Ethics Committee (Permit AEC00/42(L)). Adult southern sea lion samples were collected with approval of the Falkland Islands Government (Research Permit R16/2012). Adult female samples ($n = 23$) from Dangerous Reef were collected from 17 September to 21 November 2003, for a demographic study of the female breeding population (McIntosh, 2007). All but one female had recently given birth (pup age range 4–60 d). Dental aging was available for 20 animals (median age 9.5 y, range 5.5–14.5 y). Additional morphometric data were bodyweight (median 84.5 kg, range 65.5–103.5 kg) and standard length (median 159 cm, range 142–169 cm). Blood samples were collected into clot activation tubes and the separated serum stored in liquid nitrogen (-196°C) in the field before longer-term freezing (-80°C). Pup samples were collected as previously described (Lindsay et al., 2021), for health monitoring and hookworm disease investigations. Pup morphometric data included bodyweight (kg) and linear standard length (cm), both proxies for pup age. At Seal Bay, known pup age based on observation of birth or detection of the pup as a newborn, was recorded when available and repeat sampling was performed on a subset of pups. Known-age pups provided a reference to determine indicative pup ages for other cohorts. Simple linear regressions of the age proxies of standard length and bodyweight were made between six cohorts, one each from Dangerous Reef and Olive Island, and four from Seal Bay (initial and repeat samples from pups of known age, initial and repeat samples from pups of unknown age). A Bonferroni correction was used to adjust for multiple comparisons between pairs of the above cohorts.

Sample collection dates for Dangerous Reef ($n = 21$), Olive Island ($n = 65$) and Seal Bay ($n = 126$) were 21 July to 29 August 2017, 15 to 19 September 2017, and 5 March to 8 May 2018,

respectively. Serum was separated within 12 h of blood collection after centrifuging plain blood tubes for 2 min at 13,700 g (StatSpin MP, StatSpin Technologies, Norwood, USA). Samples were stored in the field in liquid nitrogen (Dangerous Reef and Olive Island) or frozen at -20°C (Seal Bay), before longer term freezing at -80°C .

Serological testing and analysis

A commercially available modified agglutination test (MAT) (Toxo-Screen DA[®], Biomerieux, Marcy-l'Étoile, France) was used to screen sera for *T. gondii* tachyzoite immunoglobulin G (IgG) antibodies at 1:40 and 1:4,000 dilutions, as per manufacturer directions. Samples (freeze-thaw cycles: adults (1), pups (≤ 3)) were analyzed between 11 July and 27 September 2018 at the Veterinary Diagnostic Laboratory, The University of Adelaide. Non-specific agglutination was suppressed by including 2-mercaptoethanol (0.2 mol/L) in the dilution buffer. Manufacturer supplied positive and negative goat control sera and phosphate-buffered saline control were included with each MAT test plate. Quantitative assessment of antibody titer was determined for positive sera using sequential doubling of sample dilution (from a 1:40 baseline and commencing at 1:640). Antibody titer was converted to IU/ml as per the manufacturer's instructions (equivalent to one-tenth of the reciprocal of the highest dilution giving a positive reaction – a higher titer indicating a higher level of antibody).

When seropositive adult females were detected, the association of demographic factors (age, bodyweight, and standard length) with seropositivity was explored using simple logistic regressions. Binomial exact 95% confidence intervals were reported for estimated seroprevalences in this study and

calculated for comparative studies using published data. Seroprevalence analyses were conducted using version 17.0 of the Stata software package (StataCorp LLC., College Station, Texas, USA). For pup groups that tested all seronegative, a one-sided 97.5% confidence interval was reported. For these cohorts, the survey sensitivity (SSe) was calculated *post-hoc* according to the achieved sample size using the online AusVet EpiTools calculator (Sergeant, 2018a). Assumptions were representative sampling, 100% diagnostic test sensitivity and specificity, as well as a 5% design seroprevalence (minimum assumed seroprevalence in an exposed animal group). The population size was considered finite according to referenced pup production estimates for each colony. Calculation of the SSe used a modified hypergeometric exact method and was then used to estimate the probability of pup population freedom from *T. gondii* exposure (Sergeant, 2018b). Assumptions for probability of freedom estimations were 100% diagnostic (and therefore survey) specificity, as well as a non-informative 50% pre-survey probability of freedom from exposure.

Results

Seven adult female Australian sea lion serum samples ($n = 23$) collected from Dangerous Reef were seropositive for *T. gondii* (30.4%; 95% CI 13.2–52.9%). All animals screening positive were positive at the 1:4,000 dilution and the result was confirmed in all cases by subsequent titration. Antibody titers of 512 ($n = 3$), 1024 ($n = 2$) and >2048 IU/ml ($n = 2$) were

determined. There was no significant association between age (OR 0.95 [0.67–1.36]), bodyweight (OR 0.96 [0.88–1.05]) or standard length (OR 1.06 [0.92–1.23]), and *T. gondii* seropositivity.

None of the Australian sea lion pup serum samples ($n = 212$ samples from 184 animals) collected from Dangerous Reef, Olive Island (both felid-free), and Kangaroo Island (felid-inhabited), were seropositive (0.0%; 97.5% CI 0.0–2.0% for pooled initial sampling; Table 1). None of the pups sampled from any colony was likely to have commenced foraging, based on subjective assessment of age (R. Gray, pers comm). One-third ($n = 33$) of the 98 pups initially sampled from Seal Bay were of known age (median age 26 d, age range 7–73 d). From these 98 pups there were 28 pups subsequently resampled approximately four weeks later, and half ($n = 15$) of these pups were of known age (median age 62 d, age range 34–104 d). Multiple pair-wise comparisons of age proxies (bodyweight and standard length; Table 1) found that using either parameter: 1. at initial sampling of both Seal Bay cohorts, the pups of unknown age were significantly larger (older) than the pups of known age (Bonferroni corrected $P < 0.05$; Supplementary Figure 1); 2. at their initial sampling, the Seal Bay pups of unknown age did not differ significantly from the Seal Bay pups of known age at their subsequent resampling ($P > 0.05$); 3. the Dangerous Reef pups did not differ significantly from the Seal Bay pups of known age at their initial sampling (Bonferroni corrected $P > 0.05$); 4. the Olive Island pups did not differ significantly from the Seal Bay pups of known age at their subsequent resampling ($P > 0.05$).

TABLE 1 Results of a serosurvey of Australian sea lion (*Neophoca cinerea*) pups for *T. gondii* antibodies sampled at three South Australian colonies, using a modified agglutination test (MAT).

Capture order	Location (population size)	Sampling period	Pups sampled	Bodyweight (kg) Median (range)	Standard length (cm) Median (range)	Seroprevalence (97.5% CI) [#]	Survey sensitivity	Probability of freedom
First	Seal Bay (232 pups) ¹	5 March–14 April 2018	98	11.4 (6.6–24.0)	73.0 (61.0–96.0)	0.0% (0–3.6%)	99.9%	99.9%
	Olive Island (101 pups) ²	15 September–19 September 2017	65	15.6 (7.4–26.0)	80.0 (67.0–94.0)	0.0% (0–5.5%)	99.8%	99.8%
	Dangerous Reef (338 pups) ²	21 July–29 August 2017	21	10.0 (6.4–11.9)	68.5 (62.0–77.0)	0.0% (0–16.1%)	66.4%	74.8%
	Combined (671 pups)	21 July 2017–14 April 2018	184	12.6 (6.4–26.0)	75.0 (61.0–96.0)	0.0% (0–2.0%)	100.0%	100.0%
Second	Seal Bay (232 pups) ¹	7 April–8 May 2018	28	13.4 (8.4–19.0)	76.3 (70.0–88.0)	0.0% (0–12.3%)	78.6%	82.4%

CI, confidence interval. [#]One-sided, 97.5% confidence interval report following 100% negative results. ¹ 2018 estimate based on cumulative births (Goldsworthy et al., 2019). ² Calculated as the mean of the estimated pup production reported for seasons prior to and following the sampling season (Goldsworthy et al., 2020).

The probabilities of detecting at least one seropositive pup if the pup cohort had been exposed to *T. gondii* are reported in Table 1. The resultant probabilities of the seasonal pup population freedom from *T. gondii* exposure at Dangerous Reef, Olive Island and Seal Bay (initial and subsequent sampling) were 74.8%, 99.8%, 99.9% and 82.4%, respectively. Pooling all first capture negative results provided 100% probability of the combined pre-weaned seasonal pup population (indicative age range 7-104 d) freedom from *T. gondii* exposure.

Discussion

Disease surveillance in an extensively distributed otariid population such as the Australian sea lion is logistically challenging. Sampling of subadult and adult animals is difficult, requiring either chemical restraint with its safety risks or the unlikely sampling of recently deceased individuals. Access to archived sera from 23 adult females sampled in 2003 was a unique opportunity to explore *T. gondii* exposure in a mature cohort. Evidence of *T. gondii* seroconversion in approximately a third of adult females suggests that, even 20 years ago in a felid-free island colony (Dangerous Reef), this cohort's exposure to *T. gondii* was frequent although not systematic. Adapting calculations reported by Taggart et al. (2020), the prevalence of prey carrying *T. gondii* could be as low as 0.001% to achieve an adult female seal seroprevalence of 30.4% (assuming a median cumulative exposure time of 9.0 y and a conservative daily consumption of 10 prey: Supplementary data). That equates to one in every 100,000 prey carrying *T. gondii*, recognizing that higher prevalence is likely to be clustered in areas where contamination of the marine environment may be higher. By comparison, none of the colony's pups (n = 21) sampled 14 years later showed evidence of *T. gondii* exposure. Ideally, paired mother and pup serology would have been analyzed, however, this was not logistically or ethically feasible. Cumulatively across the three surveyed colonies (n = 184) the available serological evidence provided us with approximately absolute confidence (rounded to 100%) that no pups from these colonies had seroconverted and therefore were yet to be exposed to *T. gondii*.

The estimated 30.4% seroprevalence in adult Australian sea lions approximates that reported in other otariids, including the 32.9% (binomial exact 95% CI 30.6-35.2%: n = 1,630) reported in free-ranging California sea lions stranding along the central California coast between 1998 and 2009 (Carlson-Bremer et al., 2015; Dubey et al., 2020). That assessment, using a 1:40 cut-off and indirect fluorescent antibody methodology was reduced to 2.8% (binomial exact 95% CI 2.1-3.7%) at a cut-off titer of 1:640, determined from confirmed cases (Carlson-Bremer et al., 2015). A similar cut-off more specific for diseased (versus infected) animals remains to be determined for the Australian sea lion. In a regional context, the

seroprevalence of antibodies to *T. gondii* in New Zealand sea lions using latex agglutination testing and a 1:32 cut-off was found to be 20.0% (binomial exact 95% CI 2.5-55.6%: n = 10) on the Otago Peninsula of the New Zealand South Island, and 8.3% (binomial exact 95% CI 0.2-38.5%: n = 12) on nearby Stewart Island (Michael et al., 2016) – both islands having a feral cat population (Roberts et al., 2021). In comparison, anti-*T. gondii* antibodies were not detected in animals from the remote and felid-free Enderby Island in the subantarctic (one-sided 97.5% CI 0.0-12.3%: n = 28) (Michael et al., 2016) – despite the presence of a feral cat population on the nearby main island of the Auckland Islands (Roberts et al., 2021). The latter finding is consistent with the negative results (one-sided 97.5% CI 0.0-3.5%: n = 104) of the 2007-2008 survey of the Australian fur seal colony (adult females only) on Kanowna Island (-39.1547, 146.3108) in northern Bass Strait (Lynch et al., 2011), and the low 5.3% (binomial exact 95% CI 0.1-26.0%: n = 19) seroprevalence in a 2014 survey of the southern (South American) sea lion colony (*Otaria flavescens*) (adults of both sexes) inhabiting Big Shag Island (-51.39493, -58.31644) on the north coast of the East Falkland Island (Lindsay and Gray, unpublished data). Both surveys used the same MAT methodology as the present study (with a higher 1:64 cutoff in the fur seal survey).

Differences in prevalence by location, particularly for island colonies, likely reflect varying degrees of seal exposure to *T. gondii* through prey – a function of parasite prevalence in prey and the quantity of prey consumed. The latter should result in greater cumulative exposure risk with increasing age, which has been documented in multiple pinnipeds (Lambourn et al., 2001; Carlson-Bremer et al., 2015). The absence of an age correlation with seropositivity in the present study is likely due to the small sample size and relatively narrow age range (median age 9.5 y, range 5.5-14.5 y). The parasite prevalence in prey is likely to vary with the species of prey. The Australian sea lion is principally a benthic (ocean floor) forager of Australian continental shelf waters, its temporally and spatially variable diet including many species of cephalopods (e.g., octopus, cuttlefish and squid), benthic or demersal (near the ocean floor) cartilaginous (e.g., sharks and rays) and ray-finned fish (e.g., leatherjackets, salmon and eels), and crustaceans (e.g., rock lobster and swimming crabs) (Peters et al., 2015; Berry et al., 2017). *Toxoplasma gondii* DNA has been detected in demersal and benthopelagic fish species elsewhere in the world, a Mediterranean basin study finding it in both piscivorous (diet predominantly fish) and omnivorous (diet predominantly small benthic invertebrates or zooplankton) species (Marino et al., 2019). The detection of *T. gondii* DNA in intestinal and gill samples supports ingestion (gut transit) and filter-feeding, respectively, as mechanisms for acquisition and carriage of the parasite. Increased exposure of benthic fish species to the parasite may result from the concentration of oocysts in the benthos, suggested to be facilitated by marine invertebrates (Aguirre et al., 2019). The

benthic foraging behavior of the Australian sea lion is therefore one explanation for its level of exposure to *T. gondii*.

Variation in *T. gondii* prevalence in prey is also likely impacted by other factors determining their exposure to the parasite – for instance, regional cat density (reflecting the degree of urbanization for domestic cats and the population density of feral cats), proximity to terrestrial water run-off (e.g., river delta or estuary), factors determining the volume of terrestrial water run-off (e.g., seasonal rainfall and storm events) and ocean currents (given the organism's prolonged persistence in the marine environment). Unlike males, the foraging range for adult female Australian sea lions is focused on the waters surrounding breeding colonies (Lowther et al., 2011), with inshore and offshore foraging ecotypes identified (Lowther and Goldsworthy, 2011). The waters around Dangerous Reef are impacted by The South Australian current, the easterly flowing current sweeping past some of Australia's less populous and arid southern coastline (Australian Government Bureau of Meteorology, 2022), and by tidal flows exiting the Spencer Gulf and potentially the smaller adjacent and more populous Gulf St Vincent (Australia's Integrated Marine Observing System (IMOS), 2021). A high rate of domestic cat ownership among a predominantly coastally distributed population is therefore a possible source of oocyst contamination of the marine environment around the felid-free island of Dangerous Reef.

Some of Australia's other coastal islands, such as Kangaroo Island, (Taggart et al., 2019a; Algar et al., 2020) have a high feral cat population with a high seroprevalence of *T. gondii* (87-89%) (O'Callaghan et al., 2005). Evidence for these animals substantially contributing to biological environmental contamination comes from the high prevalence of cat-borne parasitic diseases (toxoplasmosis and sarcocystosis) in the island's domestic sheep flocks (Taggart et al., 2019b; Lanyon and O'Handley, 2020). Consequently, a serosurvey of pups from the Seal Bay colony on Kangaroo Island was included in the present study to better understand any direct health implications for the colony's pup population, and as a comparator to the serosurvey results from the two felid-free islands. The absence of exposure evidence for the pup population (and the positive serosurvey of adults from a regional felid-free island) suggest survey of the juvenile and adult cohorts (ideally including mother-pup pairings) is necessary to better understand the age-related level of *T. gondii* exposure in this colony. This could guide risk assessment for epizootic disease outbreak should other factors like comorbidity alter the existing host-parasite balance and inform the scope and effectiveness of the feral cat eradication program recently implemented on the island (Commonwealth of Australia, 2015; Landscape South Australia, 2022). Furthermore, comparison of *T. gondii* genotype from clinical toxoplasmosis cases in seals with that in feral (and domestic) cats may add further to our understanding of disease epidemiology.

While acknowledging the potential for selection and survival bias, an absence of pup seropositivity in the present study excludes detection of early postnatal infection in pre-weaned pups yet to commence prey ingestion, the transplacental vertical transmission of infection from mother to pup, and the transplacental or colostrum transfer of maternal IgG antibodies (or their lack of persistence to sampling age). For seal pups exposed to *T. gondii* oocysts in the postnatal period, seroconversion with MAT-detectable antibodies occurs within three weeks (Gajadhar et al., 2004). The seropositivity of approximately 8-12 m old California sea lion pups (weaning between 6-11 m of age) was attributed to commencement of foraging trips and their initial ingestion of prey (Carlson-Bremer et al., 2015). The transplacental vertical transmission of *T. gondii* to an early or late-stage fetus is less commonly reported but has been detected in marine mammals such as Risso's dolphin (Resendes et al., 2002) and the California sea lion (Carlson-Bremer et al., 2015).

The adaptation of neonates to the extrauterine environment and their protection from infectious agents is variably reliant on the passive transfer of maternal antibodies across the placenta *in utero*, or in colostrum soon after birth (reviewed in Chucuri et al., 2010). Pinnipeds have an endotheliochorial placenta (Sinha and Erickson, 1974), and typically only 5-10% of maternal IgG antibodies undergo transfer to the fetus in placentas of this type (Tizard, 2018). Acquisition of passive immunity in pinniped pups is likely to be highly dependent on adequate ingestion and absorption of colostrum. The exact duration of persistence of maternal antibodies in pinniped pups is not known, however it is likely to be short, perhaps a few weeks (Harder et al., 1993; Puryear et al., 2021). *Toxoplasma gondii* seropositivity has been detected in a 14 d old grey seal (*Halichoerus grypus*) pup along with seronegativity in two 18-19 d old pups, all three belonging to seropositive females (Measures et al., 2004). Failure to detect maternal antibodies in pups in the present study may therefore reflect limited sampling of pups in the first two weeks of life – partly attributed to protective maternal (and aggressive male mate-guard) attendance for the first 9-10 days after birth. The importance of sampling age is also supported by a seroprevalence study of bearded seals in Norway, which included 65 pre-weaned pups, all of whom tested negative, while two-thirds (n = 15) of adults tested positive (Jensen et al., 2009). This included six seropositive females, all nursing seronegative pups of unspecified age. Recommendations for subsequent *T. gondii* serosurveys of Australian sea lion pups include sampling of stillbirths and perinatal deaths less than two weeks old, and histologic and immunohistochemical examination of placental tissues followed by PCR confirmation of the presence of *T. gondii* DNA. Detection of recent births and collection of well-preserved placental tissues is only feasible at a closely monitored colony such as Seal Bay. The number of observed stillborn animals at this colony is historically low, while perinatal death within the first two

weeks is a more common occurrence, often the result of conspecific trauma, endemic hookworm infection or starvation (R. Gray, pers comm).

Storage time of the adult sera is a potential limitation of the present study, although results are unlikely to have been impacted given their storage at -80°C. Reliable serological measurement of anti-toxoplasma antibodies in human sera has been observed for at least six years in samples stored at -20°C (Dard et al., 2017). A lack of validation in pinniped species of the MAT test is an additional study limitation, although it has been the most used method for serological surveys in these species over the past decade (Dubey et al., 2020), at cut-off values ranging from 1:25 to 1:65. The cut-off value of 1:40 for the present study was selected to best align with previous investigations in these species. MAT has not been validated in other marine mammals either, although in northern sea otters (*Enhydra lutris kenyoni*) for instance, 95% (n = 20) of *T. gondii* isolates in a study of that species were from animals testing seropositive by MAT (Verma et al., 2018). This included one animal seropositive at 1:100 dilution. Comparatively, all seropositive animals in the present study had high titers – positive at >1:4,000 dilution. The MAT methodology has been suggested to overestimate seroprevalence in the presence of sample hemolysis or a high serum lipid content, reducing specificity (Blanchet et al., 2014; van de Velde et al., 2016). Of note was the absence of any false positive results for the pup population, in which grossly detectable lipemia is frequently observed following recent feeding.

The endangered Australian sea lion population has failed to recover to 18th century pre-sealing abundance, despite its protected status. Given the contribution of *T. gondii* to limiting population recovery in other marine mammals and the role of high order predators as sentinels for marine contamination (Bossart, 2011), ongoing serological surveillance is recommended with expansion to include other infectious diseases likely to compromise population resilience.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The animal studies were reviewed and approved by The University of Sydney (Pups - Protocol Number 2014/726) and La Trobe University Animal Ethics Committees (Adults - Permit AEC00/42(L)).

Author contributions

SL and RG designed (with additional input from JS) and executed the study and collected pup samples. SL performed the laboratory testing (with additional input from RO), curated the data, and wrote the initial manuscript draft. SL and CC analyzed the data. All authors contributed to the manuscript and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fmars.2022.965865/full#supplementary-material>

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