



HAL
open science

Exposure of pelagic seabirds to *Toxoplasma gondii* in the Western Indian Ocean points to an open sea dispersal of this terrestrial parasite

Marie-Lazarine Poulle, Matthieu Le Corre, Matthieu Bastien, Elsa Gedda, Chris Feare, Audrey Jaeger, Christine Larose, Nirmal Shah, Nina Voogt, Byron Göpper, et al.

► To cite this version:

Marie-Lazarine Poulle, Matthieu Le Corre, Matthieu Bastien, Elsa Gedda, Chris Feare, et al.. Exposure of pelagic seabirds to *Toxoplasma gondii* in the Western Indian Ocean points to an open sea dispersal of this terrestrial parasite. PLoS ONE, 2021, 16 (8), pp.e0255664. 10.1371/journal.pone.0255664 . hal-03390473

HAL Id: hal-03390473

<https://hal.univ-reims.fr/hal-03390473v1>

Submitted on 21 Oct 2021

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution 4.0 International License

RESEARCH ARTICLE

Exposure of pelagic seabirds to *Toxoplasma gondii* in the Western Indian Ocean points to an open sea dispersal of this terrestrial parasite

Marie-Lazarine Poulle^{1,2*}, Matthieu Le Corre³, Matthieu Bastien^{1,3,4}, Elsa Gedda¹, Chris Feare⁵, Audrey Jaeger³, Christine Larose⁵, Nirmal Shah⁶, Nina Voogt⁷, Byron Göpper⁷, Erwan Lagadec⁴, Gérard Rocamora^{8,9}, Régine Geers^{1,10}, Dominique Aubert^{1,10}, Isabelle Villena^{1,10}, Camille Lebarbenchon⁴

1 Epidémiologie et Circulation des Parasites dans les Environnements (ESCAPE), EA 7510, CAP SANTE, Université de Reims Champagne Ardenne, Reims, France, **2** CERFE, Université de Reims Champagne-Ardenne, Boult-aux-Bois, France, **3** UMR Ecologie marine tropicale des océans Pacifique et Indien (ENTROPIE), CNRS IRD, IFREMER, Université de Nouvelle-Calédonie, Université de la Réunion, Saint Denis, La Réunion, France, **4** Université de La Réunion, UMR Processus Infectieux en Milieu Insulaire Tropical (PIMIT), INSERM 1187, CNRS 9192, IRD 249, Saint Denis, La Réunion, France, **5** WildWings Bird Management, Haslemere, Surrey, United Kingdom, **6** Center for Environment and Education, Nature Seychelles, Roche Caïman, Mahé, Seychelles, **7** Cousine Island, Seychelles, **8** Island Biodiversity and Conservation Centre, University of Seychelles, Anse Royale, Seychelles, **9** Island Conservation Society, Mahé, Seychelles, **10** Laboratoire de Parasitologie-Mycologie, Centre National de Référence de la Toxoplasmose, Centre de Ressources Biologiques *Toxoplasma*, CHU Reims, Reims, France

* marie-lazarine.poulle@univ-reims.fr

Abstract

Toxoplasma gondii is a protozoan parasite that uses felids as definitive hosts and warm-blooded animals as intermediate hosts. While the dispersal of *T. gondii* infectious oocysts from land to coastal waters has been well documented, transmission routes to pelagic species remain puzzling. We used the modified agglutination test (MAT titre ≥ 10) to detect antibodies against *T. gondii* in sera collected from 1014 pelagic seabirds belonging to 10 species. Sampling was carried out on eight islands of the Western Indian Ocean: Reunion and Juan de Nova (colonized by cats), Cousin, Cousine, Aride, Bird, Europa and Tromelin islands (cat-free). Antibodies against *T. gondii* were found in all islands and all species but the great frigatebird. The overall seroprevalence was 16.8% [95% CI: 14.5%-19.1%] but significantly varied according to species, islands and age-classes. The low antibody levels (MAT titres = 10 or 25) detected in one shearwater and three red-footed booby chicks most likely resulted from maternal antibody transfer. In adults, exposure to soils contaminated by locally deposited oocysts may explain the detection of antibodies in both wedge-tailed shearwaters on Reunion Island and sooty terns on Juan de Nova. However, 144 adults breeding on cat-free islands also tested positive. In the Seychelles, there was a significant decrease in *T. gondii* prevalence associated with greater distances to cat populations for species that sometimes rest on the shore, i.e. terns and noddies. This suggests that oocysts carried by marine currents could be deposited on shore tens of kilometres from their initial deposition point and that the number of deposited oocysts decreases with distance from the



OPEN ACCESS

Citation: Poulle M-L, Le Corre M, Bastien M, Gedda E, Feare C, Jaeger A, et al. (2021) Exposure of pelagic seabirds to *Toxoplasma gondii* in the Western Indian Ocean points to an open sea dispersal of this terrestrial parasite. PLoS ONE 16(8): e0255664. <https://doi.org/10.1371/journal.pone.0255664>

Editor: Vitor Hugo Rodrigues Paiva, MARE – Marine and Environmental Sciences Centre, PORTUGAL

Received: December 11, 2020

Accepted: June 25, 2021

Published: August 18, 2021

Copyright: © 2021 Poulle et al. This is an open access article distributed under the terms of the [Creative Commons Attribution License](https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Data Availability Statement: All relevant data are within the paper and its [Supporting Information](#) files.

Funding: CL was supported by a "Chaire Mixte Institut National de la Santé et de la Recherche Médicale (INSERM) – Université de La Réunion". The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing interests: The authors have declared that no competing interest exist.

nearest cat population. The consumption of fishes from the families Mullidae, Carangidae, Clupeidae and Engraulidae, previously described as *T. gondii* oocyst-carriers (i.e. paratenic hosts), could also explain the exposure of terns, noddies, boobies and tropicbirds to *T. gondii*. Our detection of antibodies against *T. gondii* in seabirds that fish in the high sea, have no contact with locally contaminated soils but frequent the shores and/or consume paratenic hosts supports the hypothesis of an open-sea dispersal of *T. gondii* oocysts by oceanic currents and/or fish.

Introduction

The land-to-sea transport of the free infective forms of zoonotic protozoa (oocysts or cyst), dispersed with the faeces of humans, pets and farm animals has a growing negative impact on public health and marine life [1, 2]. While several studies have been carried out on faecal contamination of the coastal environment with *Cryptosporidium*, *Giardia* and *Toxoplasma* [3–5], less attention has been paid to the open ocean, resulting in a critical lack of information on the transmission routes of protozoan parasites to pelagic species. This gap is particularly problematic for *Toxoplasma gondii* because this apicomplexan parasite is currently emerging as an important pathogen in aquatic systems [6–8]. *Toxoplasma gondii* is responsible for toxoplasmosis, one of the most common parasitic infections of warm-blooded animals, including humans [9]. The finding of acute toxoplasmosis and the detection of antibodies against *T. gondii* in marine mammals in the Eastern, Central and Western Pacific [10], the Canadian Arctic [11], the Northeastern and Western Atlantic [10, 12], the Philippine archipelago [13] and the Mediterranean Sea [14] suggests a worldwide contamination of marine habitats.

The environmental contamination with *T. gondii* necessarily comes from felids since domestic cat, *Felis catus*, and wild felids are the only known definitive hosts in which the sexual multiplication of *T. gondii* occurs, resulting in the faecal shedding of oocysts into the environment [15]. These oocysts are highly resistant and can remain infective in soils for months [16–18]. All warm-blooded animals can be intermediate host for *T. gondii* [9]. Once the oocysts have been ingested by a mammal or a bird, the development of *T. gondii* continues until the formation of infecting tissue cysts [19]. These cysts can persist lifelong in the host and IgG antibodies probably do the same [9, 20]. The prevalence of antibodies to *T. gondii* is therefore generally higher in adult than in juvenile populations, both in wild birds [21] and in wild and domestic mammals [22, 23] due to a longer period of exposure which increases the likelihood of infection.

Acute toxoplasmosis is rarely reported in terrestrial birds and mammals that have co-evolved with felids and their parasites, but wildlife species recently exposed to *T. gondii* can be severely affected [24, 25]. Fatal toxoplasmosis is notably reported in marsupials and native terrestrial birds in Australia [26, 27] and Hawaii [28] where *T. gondii* was absent until the introduction of the domestic cat. Meningoencephalitis associated with *T. gondii* also results in morbidity and mortality in free-ranging sea otters, *Enhydra lutris* [29], sea lions, *Zalophus californianus* [30] and dolphins [14], especially when associated with poly-parasitism or environmental pollutants [31, 32]. As a result, *T. gondii* is considered a pathogen of concern for several marine mammal species [33].

Recent molecular epidemiology studies provide evidence that freshwater can carry *T. gondii* oocysts from terrestrial to marine coastal habitats [34–36]. The dilution of oocysts to a low concentration in the marine environment is compensated by their ability to survive and to remain infectious for several months in seawater [37], by their filtration and bio-accumulation

in marine bivalves [38, 39] and their capture by planktonic animals that are a major source of food for fish and invertebrates [7, 40]. Oocysts can also adhere to kelp grazed by marine snails, resulting in a high concentration of oocysts in their faecal pellets [41, 42]. In addition, infectious oocysts can be transported in the digestive tract of migratory filter feeding fish [43]. The consumption of marine fishes and invertebrates that carry *T. gondii* oocysts (i.e. paratenic hosts) may therefore be considered as responsible for the contamination of coastal marine predators like sea otters [34, 44, 45], coastal dolphins foraging in Atlantic Ocean bays and Mediterranean coasts [46, 47] or beluga whales and seals from the St. Lawrence stream, Canada [48, 49]. Antibodies against *T. gondii* have also been detected far away from potential contamination sources by cats as in Weddell seals, *Leptonychotes weddellii*, and elephant seals, *Mirounga leonina*, sampled in the Antarctic Peninsula [50, 51], or in pelagic dolphins [14, 46], as well as in pelagic seabirds breeding on felid-free islands [52–54]. In all these cases, the transport of infectious oocysts by marine currents or by fish have been mentioned as the two likely routes of transmission of *T. gondii* to pelagic species but without evidence of the involvement of one and/or the other in the exposure of the species studied.

The present study aims at exploring the variability in exposure to *T. gondii* in ten pelagic seabird species breeding in the Western Indian Ocean in order to elucidate the routes of transmission of this protozoan to “offshore” species. Pelagic seabirds are good models for assessing the relative importance of *T. gondii* transmission routes in pelagic environments since they spend most of their time far at sea, rarely venturing close to land except to breed, and obtain their food and most of their drinking water from fish, squids and other marine invertebrates [55]. Serum samples were obtained from seabirds breeding on eight islands, two of which are colonized by cats and six are felid-free. Based on this sampling, we tested whether the prevalence of *T. gondii* in seabirds varied according to age-class, species, islands and nesting habits. In particular, we expected a lower prevalence on cat-free islands than on islands where birds are exposed to oocysts dispersed by resident cats, and a higher prevalence in ground nesters than in tree-nesters as the latter are assumed to be less exposed to oocyst-contaminated soil. For species that frequent the coastline (i.e. terns and noddies), we expected a higher prevalence on islands close to cat populations than on remote islands, as the latter are assumed to receive lower numbers of oocysts on their shores. Finally, based on the literature on seabird diet, we discussed the relationship between the prevalence of *T. gondii* in seabirds and their consumption of paratenic-host fish.

Materials and methods

Ethical approval

Procedures were evaluated and approved by an ethic committee (agreement # A974 001, Comité d'éthique du CYROI # 114; Cyclotron Réunion Océan Indien, Sainte Clotilde, La Réunion, France), and authorized by The French Ministry of Education and Research (reference number APAFIS#3719-2016012110233597v2). Sample collection on Reunion Island, Europa, Juan de Nova and Tromelin was conducted under the approval of the Direction de l'Environnement, de l'Aménagement et du Logement de la Réunion and the Terres Australes and Antarctiques Françaises. Fieldwork and collection of biological material in the Seychelles were approved by the Seychelles Bureau of Standards and the Seychelles Ministry of Environment, Energy and Climate Change.

Study sites and sample collection

Sampling was conducted on eight oceanic islands of the Western Indian Ocean (Fig 1): Reunion Island is part of the Mascarenes Archipelago; Aride, Bird, Cousin and Cousine are

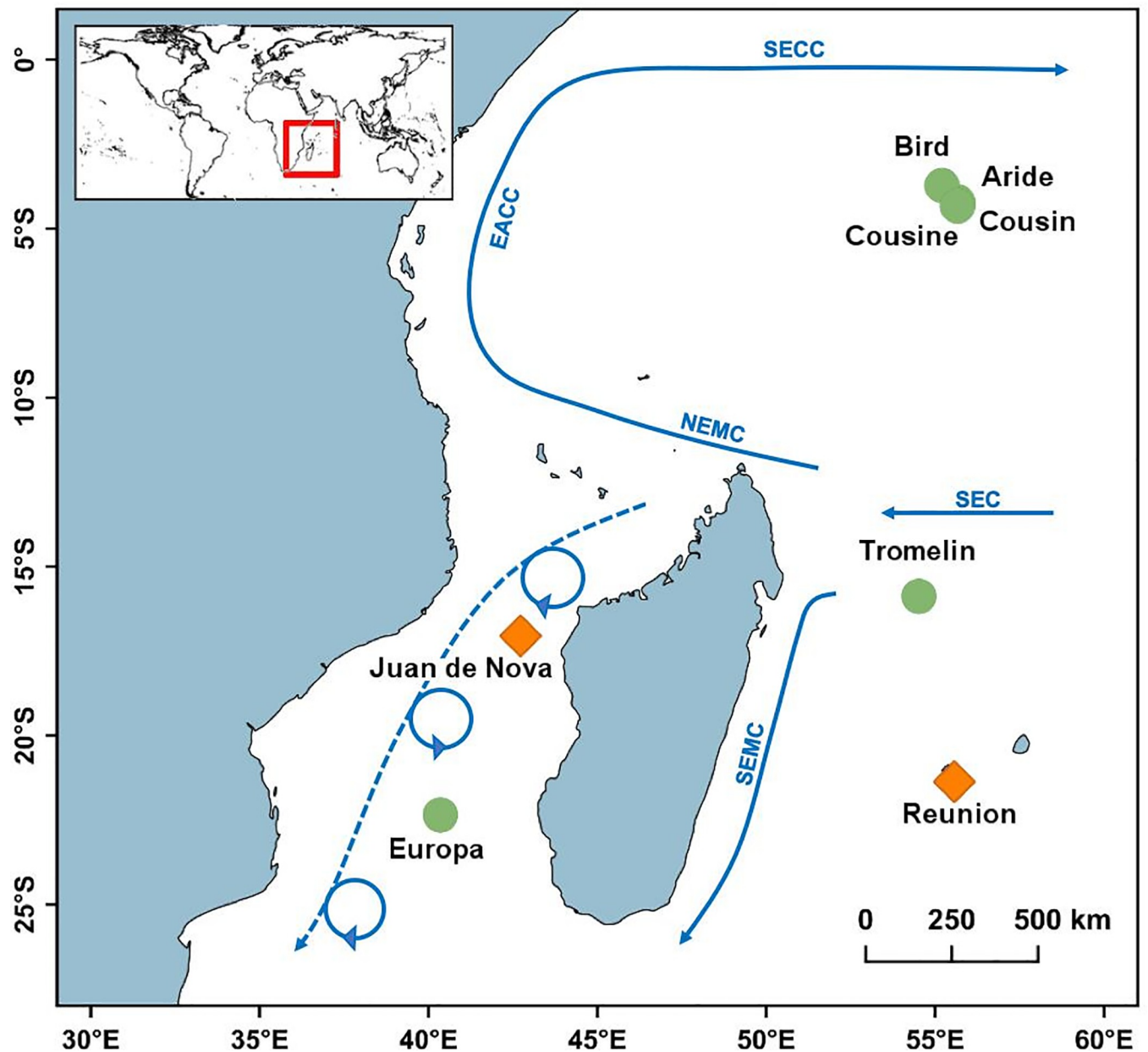


Fig 1. Location of the eight Western Indian Ocean islands where seabird populations were sampled for the detection of *Toxoplasma gondii* antibodies between 2011 and 2015. The orange lozenges correspond to islands inhabited by cats, the green dots to islands free of cats. Blue arrows indicated surface marine currents. SEC = South Equatorial Current, NEMC = North-East Madagascar Current, SEMC = South-East Madagascar Current, EACC = East African Coastal Current, SECC = South Equatorial Counter Current. Dashed arrow in the Mozambique Channel shows eddy circulation. Source: Schott et al. (2009). <https://doi.org/10.1029/2007RG000245> [56].

<https://doi.org/10.1371/journal.pone.0255664.g001>

part of the Seychelles Archipelago; Tromelin lies between the Mascarenes Archipelago and the Seychelles Archipelago; Juan de Nova and Europa are in the Mozambique Channel.

The sampled islands have different histories regarding the presence of cats (Table 1). Cats were likely introduced to Reunion Island in the 17th century and now occupy all habitats on the island [57]. Cats were introduced on Juan de Nova in the 20th century and their population was significantly reduced between 2006 and 2011, but not eradicated at that time [58]. In the

Table 1. Information on the 1014 seabirds sampled in the Western Indian Ocean between 2011 and 2015 whose sera were tested for the detection of *Toxoplasma gondii* antibodies (MAT ≥ 10).

Island	Presence/absence of felids	Species	Age-class	No tested	No positive	%	
Aride 4° 12'46"S, 55° 39'53"E	Domestic cats used to be present but eradicated several decades ago. Approximately 9 km away from the nearest island inhabited by cats.	Wedge-tailed shearwater	<i>Ardenna pacifica</i>	Adults	8	0	0
		Sooty tern	<i>Onychoprion fuscatus</i>	Adults	33	12	36.4
Bird 3° 53'S 55° 12'E	Domestic cats used to be present but eradicated several decades ago. Approximately 80 km away from the nearest island inhabited by cats.	Brown noddy	<i>Anous stolidus</i>	Adults	51	9	17.6
		Lesser noddy	<i>Anous tenuirostris</i>	Adults	22	0	0
		Sooty tern	<i>Onychoprion fuscatus</i>	Adults	100	13	13.0
		White-tailed tropicbird	<i>Phaethon lepturus</i>	Adults	13	4	30.8
Cousin 4° 19'S 55° 39'E	Never colonized by felids Approximately 2 km away from the nearest island inhabited by cats	Brown noddy	<i>Anous stolidus</i>	Adults	29	13	44.8
		Lesser noddy	<i>Anous tenuirostris</i>	Adults	22	4	18.2
		Bridled tern	<i>Onychoprion anaethetus</i>	Adults	17	9	52.9
		White-tailed tropicbird	<i>Phaethon lepturus</i>	Adults	18	0	0
		Wedge-tailed shearwater	<i>Ardenna pacifica</i>	Adults	31	1	3.2
Cousine 4° 21'S 55° 38'E	Domestic cats used to be present but eradicated several decades ago. Approximately 5 km away from the nearest island inhabited by cats	Brown noddy	<i>Anous stolidus</i>	Adults	28	8	28.6
		Lesser noddy	<i>Anous tenuirostris</i>	Adults	31	1	3.2
		Wedge-tailed shearwater	<i>Ardenna pacifica</i>	Adults	24	0	0
Juan de Nova 17° 03'S 42° 45'E	Domestic cats introduced in the 20 th . Population reduced between 2006 and 2011 but not eradicated at that time	Sooty tern	<i>Onychoprion fuscatus</i>	Adults	98	20	20.4
				Chicks	57	0	0
Reunion Island 21° 22'S, 55° 34'E	Domestic cats introduced in the 17 th and they occupy all habitats at that time	Wedge-tailed shearwater	<i>Ardenna pacifica</i>	Adults	50	5	10.0
				Chicks	23	1	4.3
Europa 22° 20'S 40° 22'E	Never colonized by felids. Approximately 300 km away from the closest feline population.	Great frigatebird	<i>Fregata minor</i>	Adults	14	0	0
				Chicks	22	0	0
		Red-footed booby	<i>Sula sula</i>	Adults	36	3	8.3
				Chicks	17	2	11.8
		Red-tailed tropicbird	<i>Phaethon rubricauda</i>	Adults	34	2	5.9
		Sooty tern	<i>Onychoprion fuscatus</i>	Adults	138	45	32.6
				Chicks	30	0	0
		White-tailed tropicbird	<i>Phaethon lepturus</i>	Adults	31	6	19.4
Tromelin 15° 53'S 54° 31'E	Never colonized by felids. Approximately 430 km away from the closest feline population.	Red-footed booby	<i>Sula sula</i>	Adults	8	0	0
				Chicks	10	1	10.0
		Masked booby	<i>Sula dactylatra</i>	Adults	5	2	40.0
				Juveniles	14	9	64.3
Total				1014	170	16.8	

<https://doi.org/10.1371/journal.pone.0255664.t001>

Seychelles Archipelago, cats used to be present on Bird [59, 60], Aride [61] and Cousine but were eradicated several decades ago [62]. Cousin, Europa and Tromelin have always been free of cats [62, 63]. In addition, Cousin, Cousine, Aride and Bird are approximately 2 km, 5 km, 9 km and 80 km from Praslin, the nearest cat-inhabited island of the Seychelles archipelago,

while Europa and Tromelin are approximately 300 km and 430 km away from the closest feline population (Table 1).

In total, 1014 individuals belonging to ten seabird species were included in this study (Table 1 and S1 Table). Most samples were collected between 2011 and 2013 as part of a previous study [64] except on Cousin and Cousine where samples were collected in 2015. The sampling strategy was designed to include a maximum number of species on each island. This sampling was adjusted in relation to local geographic, safety and ethical constraints that restrict access to bird colonies, such as in highly mountainous regions (e.g. Reunion Island) or for species highly sensitive to human disturbance (e.g. great frigatebird, *Fregata minor*). Birds were captured with bare hands or hand nets. Individual birds were categorized as chicks (non-flying birds fully dependent on parental feeding), juveniles (sexually immature flying birds), or adults (sexually mature birds, breeding or non-breeding). Whole blood (maximum of 1.0% of body weight) was collected from the medial metatarsal or basilic veins, as appropriate for each species. In the field, blood samples were collected in 2 ml micro-tubes placed in a cooler with ice packs and centrifuged within 12 hours after collection. Sera were transferred in cryogenic tubes and stored at -20°C. Samples were shipped to the laboratory in Reunion Island within a week and held at -20°C until tested.

Serological assay

Sera were examined by the Modified agglutination test (MAT) described by Dubey and Desmonts [65]. This serological assay is the most sensitive, specific and used for the detection of IgG antibodies against *T. gondii* in birds [66, 67]. MAT antigen consisted of formalinized tachyzoites produced at the Laboratory of Parasitology, National Centre on Toxoplasmosis, Reims, France. Sera were first screened using 1:6, 1:10 and 1:25 dilutions in phosphate-buffered saline solution (PBS, pH 7.2). Those agglutinating the antigen at one (or more) of these screening dilutions were further tested in a serial 2-fold dilution, to a maximum dilution of 1:12800. Serum samples with agglutination at MAT titre ≥ 10 (i.e. serum dilution $\geq 1:25$) were considered positive for the presence of *T. gondii* antibodies [67, 68]. Samples showing agglutination at further dilution were also mentioned to allow comparisons with literature data based on different dilution thresholds.

Statistical analyses

Pearson Chi square test (χ^2) were used to investigate the effect of the bird species, island, bird age-class (adult *versus* chick), and nest type (tree-nesting *vs* ground-nesting, S1 Table) on the probability of successful detection of *T. gondii* antibodies. Juveniles (N = 14) were excluded from the analysis because of the very low number of sampled birds as compared to chicks (N = 159) and adults (N = 841). Analyses were conducted in R 3.6.3 [69].

Results

Antibodies against *T. gondii* were detected on all islands and all species, except the great frigatebird (Table 1). The overall seroprevalence was 16.8% [95% CI: 14.5%-19.1%]. MAT titres for the 170 seropositive birds ranged from 10 to 400 (Table 2).

The prevalence of *T. gondii* antibodies varied according to bird species ($\chi^2 = 69$, df = 990, $p < 0.001$), islands ($\chi^2 = 17$, df = 992, $p < 0.05$) and bird age class ($\chi^2 = 36$, df = 998, $p < 0.001$). However, differences between bird age classes should be interpreted cautiously because of the low number of chicks as compared to adults, and of the uneven distribution of the sampled chicks for each species (Table 1). The probability of detection of *T. gondii*

Table 2. Number of samples tested positive for *Toxoplasma gondii* antibodies per species, age-class and titre of the Modified Agglutination Test (MAT). In brackets: corresponding dilution.

Species	Age class	MAT titre					
		≥ 10 (1:25)	≥ 25 (1:50)	≥ 50 (1:100)	≥ 100 (1:200)	≥ 200 (1:400)	≥ 400 (1:800)
Bridled tern (<i>Onychoprion anaethetus</i>)	Adult	9	6	0	0	0	0
Sooty tern (<i>Onychoprion fuscatus</i>)	Adult	90	56	31	9	3	1
Brown noddy (<i>Anous stolidus</i>)	Adult	30	13	3	1	0	0
Lesser noddy (<i>Anous tenuirostris</i>)	Adult	5	4	2	0	0	0
White-tailed tropicbird (<i>Phaethon lepturus</i>)	Adult	10	4	0	0	0	0
Red-tailed tropicbird (<i>Phaethon rubricauda</i>)	Adult	2	2	0	0	0	0
Masked booby (<i>Sula dactylatra</i>)	Adult	2	1	0	2	0	0
	Juvenile	9	9	3	0	1	0
Red-footed booby (<i>Sula sula</i>)	Adult	3	1	0	0	0	0
	Chick	3	1	0	2	0	0
Wedge-tailed shearwater <i>Ardenna pacifica</i>	Adult	6	2	2	0	0	0
	Chick	1	1	0	0	0	1
Total		170	93	40	13	4	1

<https://doi.org/10.1371/journal.pone.0255664.t002>

antibodies varied between bird species in both chicks ($\chi^2 = 10.3$, $df = 155$, $p < 0.05$) and adults ($\chi^2 = 66$, $df = 831$, $p < 0.001$; Fig 2).

In adults, *T. gondii* prevalence was 5.3% ± 4.1% in wedge-tailed shearwater, 5.8% ± 7.9% in red-tailed tropicbird, 6.7% ± 5.6% in lesser noddy, 6.8% ± 7.4% in red-footed booby, 24.4% ± 4.4% in sooty tern, 27.7% ± 8.4% in brown noddy, 40% ± 43% in masked booby, and 52.9% ±

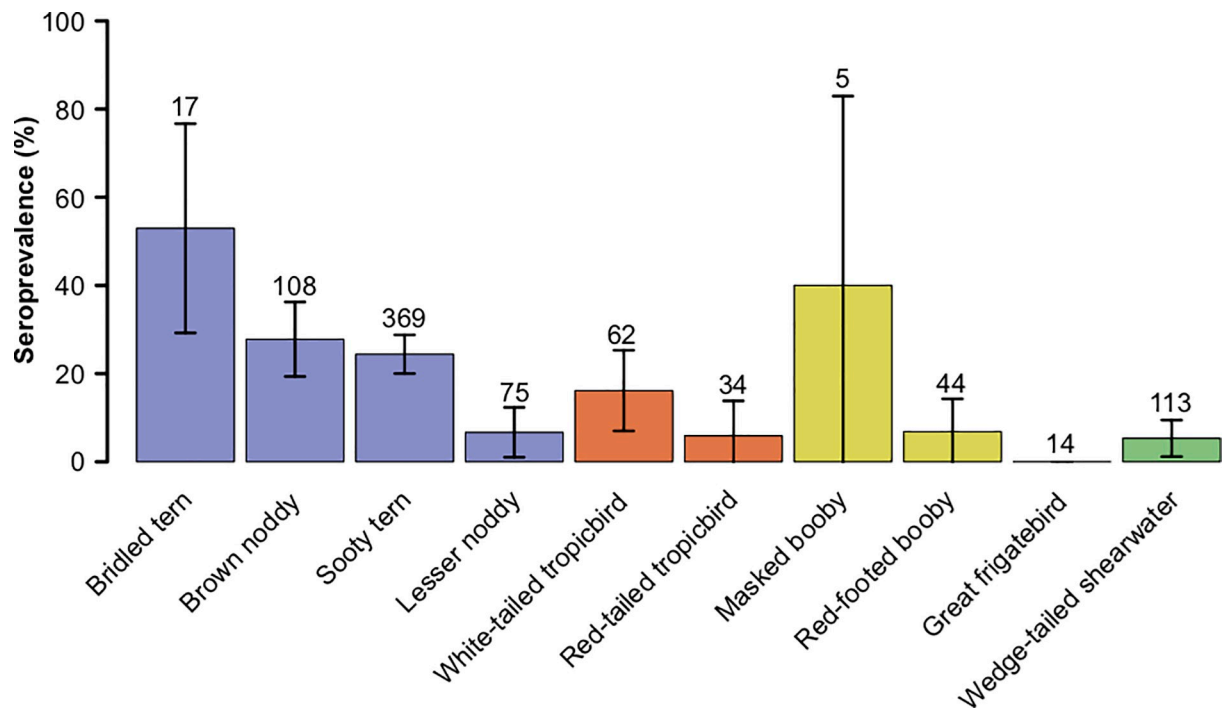


Fig 2. Seroprevalence of antibodies to *Toxoplasma gondii* per species in the adult seabirds sampled in the Western Indian Ocean (sample size and percentage with 95% confidence intervals). Colours indicate bird orders (blue: Charadriiformes, red: Phaethontiformes, yellow: Suliformes, green: Procellariiformes).

<https://doi.org/10.1371/journal.pone.0255664.g002>

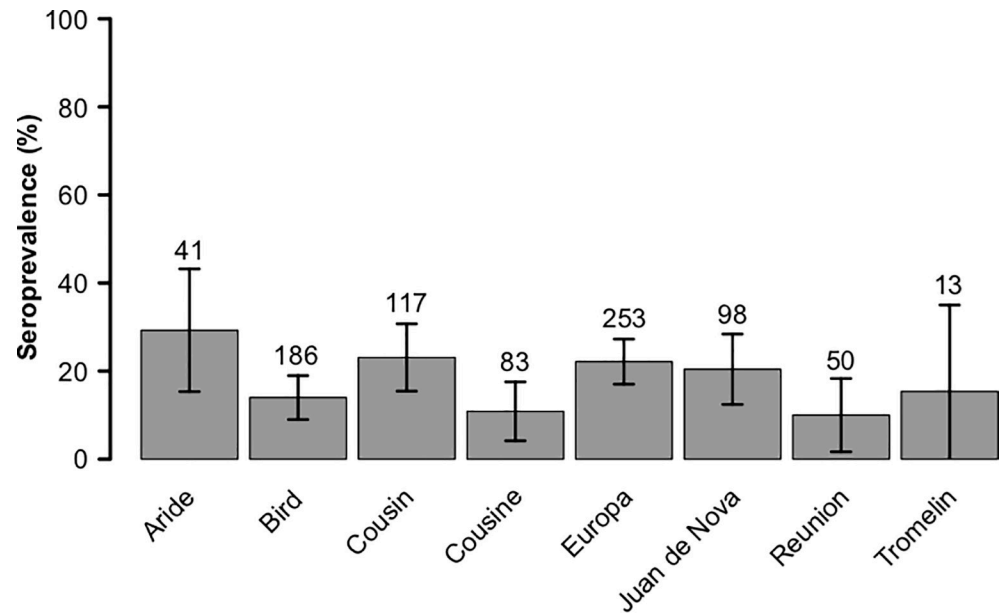


Fig 3. Seroprevalence of antibodies to *Toxoplasma gondii* per islands in the adult seabirds sampled in the Western Indian Ocean (sample size and percentage with 95% confidence intervals). Reunion and Juan de Nova are the only islands inhabited by cats.

<https://doi.org/10.1371/journal.pone.0255664.g003>

23.7% in bridled tern. Prevalence of *T. gondii* in adults was significantly lower in tree-nesting than ground-nesting species ($6\% \pm 4\%$ versus $21\% \pm 3\%$, $\chi^2 = 21$, $df = 839$, $p < 0.001$). The probability of detection of *T. gondii* antibodies in adults varied significantly between islands ($\chi^2 = 16$, $df = 833$, $p < 0.05$; Fig 3) but prevalence on islands inhabited by cats (Reunion and Juan de Nova) did not significantly differ from prevalence on cat-free islands ($\chi^2 = 0.38$, $df = 839$, $p < 0.53$).

Differences in the prevalence of *T. gondii* in adults were also detected between populations (*i.e.* islands) of the same species (Fig 4) in brown noddy ($\chi^2 = 6.7$, $df = 105$, $p < 0.05$), sooty tern ($\chi^2 = 16$, $df = 365$, $p < 0.001$), lesser noddy ($\chi^2 = 7.1$, $df = 72$, $p < 0.05$) and white-tailed tropicbird ($\chi^2 = 8.2$, $df = 59$, $p < 0.05$) but not in red-footed booby ($\chi^2 = 1.3$, $df = 42$, $p = 0.26$) and wedge-tailed shearwater ($\chi^2 = 5.6$, $df = 109$, $p = 0.14$).

Discussion

Based on the analysis of 1014 seabirds belonging to ten species sampled in the Western Indian Ocean, we found an overall prevalence of 16.8% of seabirds carrying antibodies against *T. gondii*. This prevalence was higher than the one reported with the same threshold in the masked booby, the brown booby (*Sula leucogaster*) and the red-billed tropicbird (*Phaethon aethereus*) sampled in the Abrolhos archipelago, the south of Bahia State (Brazil) in the Atlantic Ocean (5.8% at MAT titre ≥ 10) [53]. If we had considered only MAT titres ≥ 25 , *T. gondii* prevalence would have been of 9.17% (93/1014), thus also higher than prevalence reported for other seabird species such as the Galapagos penguin (*Spheniscus mendiculus*) and the flightless cormorant (*Phalacrocorax harrisi*), both sampled in the Galapagos Archipelago (Ecuador) in the Pacific Ocean (2.3% at MAT titre ≥ 25) [54]. In birds, clinical signs associated with toxoplasmosis include anorexia, diarrhoea and respiratory distress, and may occasionally result in death [24, 25, 66]. Fatal toxoplasmosis has notably been reported in captive penguins [70, 71] and in a free-ranging red-footed booby died of disseminated toxoplasmosis on a Hawaiian

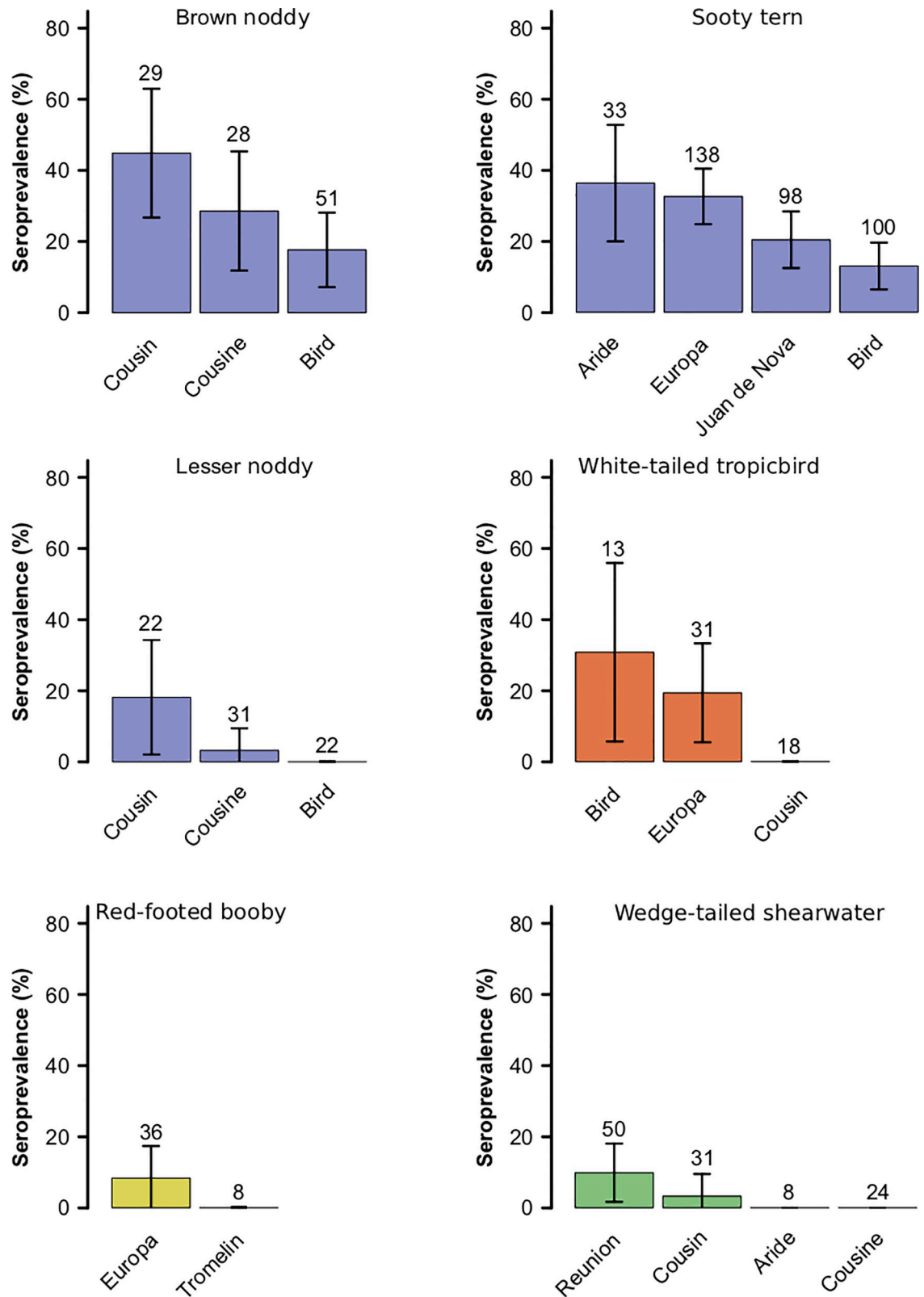


Fig 4. Seroprevalence of antibodies to *Toxoplasma gondii* per species and island in the adult seabirds sampled in the Western Indian Ocean (sample size and percentage with 95% confidence intervals). Sample sizes are indicated above bars. Colours indicate bird orders (blue: Charadriiformes, red: Phaethontiformes, yellow: Suliformes, green: Procellariiformes). Juan de Nova and Reunion

are inhabited by cats; Aride, Cousin and Cousine are less than 10 km away from the nearest island inhabited by cats; Bird, Europa and Tromelin are 80 km to 430 km away from the nearest feline population.

<https://doi.org/10.1371/journal.pone.0255664.g004>

island [72]. The relatively high exposure to *T. gondii* in the Western Indian Ocean therefore raises questions about the risk of induced mortality in seabird populations, although all birds sampled in this study were apparently healthy. Further investigations could be performed to detect clinical toxoplasmosis on these populations from necropsies and molecular analysis conducted on freshly dead birds, in particular in colonies with high *T. gondii* prevalence and/or in species susceptible to this infection, such as the red-footed booby.

As expected, the prevalence of *T. gondii* in seabirds sampled in the Western Indian Ocean varied significantly by age class, species and island, and was higher in ground nesting birds than tree-nesting birds confirming that ground-contact is a risk factor for seabirds to *T. gondii*. Of the species we sampled, the wedge-tailed shearwater has the most contact with the ground as it nests in a burrow [73]. On Reunion Island, wedge-tailed shearwaters burrow in cliffs frequented by cats. Those that tested positive for *T. gondii* antibodies had most likely ingested oocysts while preening their feathers stained with oocyst-contaminated soil. For sooty terns, the prevalence of anti-*T. gondii* antibodies was higher on Aride (where cats were eradicated several decades ago) than on Juan de Nova (where cats were present at the time of our sampling) raising the question of how long *T. gondii* oocysts can persist in the environment after cat eradication. Experimentally, the proportion of oocysts surviving in soil after 100 days is around 7% under dry conditions and 44% under damp conditions [17]. In Baja California, Mexico, the rate of recent human exposure to *T. gondii* (estimated via IgM detection) was 12–26% on five islands inhabited by cats and only 1.8% on the island where cats were eradicated seven years earlier [74]. The persistence of infectious oocysts for decades after eradication of the cats on Aride therefore seems unlikely. This implies that seabirds testing positive for *T. gondii* antibodies on Aride as well as Cousine and Bird (where cats were also eradicated several decades ago) were necessarily exposed to oocysts that were not produced locally and therefore dispersed from their shedding site. The medium to long-range dispersal of oocysts from land or islands inhabited by felids may also explain why we did not find a higher prevalence of *T. gondii* in seabirds on islands inhabited by cats than on cat-free islands.

Our data broadly suggest that birds visiting the shore are the most exposed to *T. gondii*. Indeed, the highest seroprevalence was observed in bridled terns, sooty terns and brown noddies (Charadriiformes) which nest close to the sea. In New Caledonia, bridled terns nest at less than five meters above the high tide level and at less than eight meters away from the water mark [75]. In the Seychelles, sooty terns nest on open sand or on sand with scattered low vegetation above the high tide level [76, 77]. Similarly, brown noddies nest both on the ground and in trees and often rest and collect nest material on the ground and on shores. Lesser noddies only nest in trees but spend time on the ground and shore, sunbathing during the day and collecting soil-borne materials for nesting (e.g. sticks and leaves) as well as material floating on the sea. In the Seychelles, the prevalence of *T. gondii* in sooty tern and noddy populations decreased with distance from the nearest cat population: for brown and lesser noddies, it was significantly higher on Cousin and Cousine islands (2 km and 5 km from Praslin) than on Bird island (80 km from Praslin); For sooty tern, it was higher on Aride island (9 km from Praslin) than on Bird. This pattern of *T. gondii* prevalence decrease with distance to cat populations was not observed for the white-tailed tropicbird, a more inland species than sooty tern and noddies. Taken together, these observations advise that *T. gondii* oocysts produced on cat-inhabited land could be transported by oceanic currents and deposited on distant shorelines, thereby contributing to the exposure of birds exploiting these habitats, such as terns and

noddies. Shapiro et al. [40] suggested that the attachment of *T. gondii* oocysts to marine aggregates may significantly influence the water transport of this terrestrial parasite. This association of oocysts with marine aggregates may also presumably facilitate their transport from islands colonised by cats. On arrival at distant shores, oocysts may be retained by high-water mark since they adhere to kelp [78]. The habit of noddies to collect seaweed for incorporation into their nests could prolong their exposure to *T. gondii*.

However, the detection of *T. gondii* antibodies in species that usually do not spend time in coastal habitats (tropicbirds, shearwater and boobies) suggests that a third source of contamination could also be involved in the transmission route of *T. gondii* to pelagic seabirds. Infectious *T. gondii* oocysts and/or *T. gondii* DNA have been detected in the intestines or tissues of Mullidae (goatfish), Carangidae (trevally, mackerel), Engraulidae (anchovies) and Clupeidae (herrings, shads, sardines) [43, 79]. Clupidae, Carangidae and Clupidae fishes are preyed by *Tursiops truncatus* and *Delphinus delphis* [80, 81] which are the two dolphin species most exposed to *T. gondii* in the Mediterranean Sea [46]. In the Seychelles, Carangidae and Engraulidae fishes are the secondary prey of white-tailed tropicbirds [82]. Similarly, on Europa, the red-tailed tropicbird and the red-footed booby occasionally take Carangidae and/or Mullidae fish [83, 84]. Therefore, the few white-tailed tropicbirds that tested positive on Bird and Europa, as well as the red-tailed tropicbirds and red-footed boobies that tested positive on Europa, may have been exposed to *T. gondii* by feeding on Carangidae or Mullidae fish carrying infectious oocysts. In the same way, the high *T. gondii* prevalence in sooty terns and brown noddies sampled in the Seychelles and the Mozambique Channel may not only result from their use of the shore but also to the significance of Mullidae and Carangidae in their diet, which also occasionally includes Clupeidae and Engraulidae [82, 83, 85]. Interestingly, comparable prevalence of *T. gondii* were detected in the Aride and Europa sooty tern populations (36.4% and 32.6%) which also have major similarities in diet composition [86]. Taken together, these observations suggest that Mullidae and Carangidae, and possibly Clupeidae and Engraulidae, may serve as biotic carriers for *T. gondii* in the Western Indian Ocean.

As expected, prevalence of *T. gondii* antibodies was lower in chicks than on adults in sooty tern (Juan de Nova: 0% versus 20.4%; Europa: 0% versus 32.6%) and in wedge-tailed shearwaters (Reunion: 4.3% versus 10%). However, prevalence of *T. gondii* was higher in chicks than in adults in red-footed boobies sampled on Europa (11.8% versus 8.3%) and Tromelin (1/1 positive versus 0/8). This unexpected result can be due to the persistence of maternal antibodies transferred via egg yolk [87, 88]. In long-lived birds such as wedge-tailed shearwater or red-footed boobies, specific maternal antibodies can have an estimated half-life of 25 days post-hatching [89, 90]. The low antibody levels detected in one shearwater and three red-footed booby chicks (MAT titres = 10 or 25) most likely resulted from maternal antibody transfer since antibody level might have been higher if chicks had produced antibodies in response to a recent environmental exposure to *T. gondii*. In contrast, the high antibody levels detected in nine juvenile masked boobies (MAT titres = 50, 100 or 200) from Tromelin, located 430 km away from the closest feline population, as well as in adult red-footed and masked boobies on Europa and Tromelin (300 km and 430 km away from the closest feline population) likely resulted of an environmental exposure to *T. gondii*. This result is intriguing because adult masked and red-footed boobies have a foraging range limited to the 150 km surrounding Europa and Tromelin [91–93]. The detection of antibodies to *T. gondii* in boobies from these islands could a result of the long-distance movements that juvenile boobies sometimes make before breeding [94–96] and/or the transport of oocysts across the ocean for hundreds of kilometres.

To conclude, this study clearly demonstrates that *T. gondii* has efficiently colonized the marine realm of the tropical Indian Ocean. Three non-exclusive routes of contamination

could be involved: (i) by the ingestion of oocysts locally deposited on islands colonised by cats; (ii) by the ingestion of oocysts transported by currents and deposited on the shore of distant islands; (iii) by the ingestion of oocysts carried by Mullidae, Carangidae, Clupeidae or Engraulidae fish. It is interesting to note that the only species for which no seropositive bird was found—i.e. great frigatebird breeding on Europa—was also the least exposed to these routes of contamination. Indeed, on the cat-free island of Europa, great frigatebirds nest and roost in trees and bushes and have a diet essentially composed of flying-fish and Ommastrephid squids [55, 97]. Further investigations are needed to confirm that *T. gondii* oocysts could be transported over tens or hundreds of kilometres across the ocean and to better identify the ecological processes allowing the pathway of this protozoa in the tropical seabird community.

Supporting information

S1 Table. Information on seabirds sampled in the western Indian Ocean for the detection of antibodies against *Toxoplasma gondii* in their sera.

(PDF)

Acknowledgments

We are very grateful to the Savy family for their warm hospitality and support for the fieldwork on Bird Island. We are also grateful to Mr F. Keeley for the opportunity and support to work on Cousine Island. We thank the Island Conservation Society and Nature Seychelles for organizing transportation and providing fieldwork support from their teams (both staff and volunteers) on Aride and Cousin Islands, respectively. We also thank Eric Buffard, Sophie Bureau, Licia Calabrese, Jacques Fayon, Tom Hiney, Laurence Hoareau, Sébastien Lefort, Aurélien Prudor, David Ringler, Bernard Rota and Julie Tourmetz for their assistance in the field. Léon Biscornet, Graham Govinden and Brigitte Pool are also thanked for providing support for sample conservation at the Victoria Hospital, Mahé, Seychelles.

Author Contributions

Conceptualization: Marie-Lazarine Poulle, Dominique Aubert, Isabelle Villena, Camille Lebarbenchon.

Data curation: Marie-Lazarine Poulle.

Formal analysis: Marie-Lazarine Poulle, Camille Lebarbenchon.

Funding acquisition: Isabelle Villena, Camille Lebarbenchon.

Investigation: Marie-Lazarine Poulle, Matthieu Le Corre, Matthieu Bastien, Elsa Gedda, Chris Feare, Audrey Jaeger, Christine Larose, Nirmal Shah, Nina Voogt, Byron Göpper, Erwan Lagadec, Gérard Rocamora, Camille Lebarbenchon.

Resources: Régine Geers.

Supervision: Marie-Lazarine Poulle, Matthieu Le Corre, Matthieu Bastien, Chris Feare, Audrey Jaeger, Christine Larose, Nirmal Shah, Nina Voogt, Byron Göpper, Erwan Lagadec, Gérard Rocamora, Camille Lebarbenchon.

Validation: Marie-Lazarine Poulle, Camille Lebarbenchon.

Visualization: Audrey Jaeger, Camille Lebarbenchon.

Writing – original draft: Marie-Lazarine Poulle.

Writing – review & editing: Marie-Lazarine Poulle, Matthieu Le Corre, Matthieu Bastien, Chris Feare, Audrey Jaeger, Erwan Lagadec, Gérard Rocamora, Dominique Aubert, Isabelle Villena, Camille Lebarbenchon.

References

1. Appelbee AJ, Thompson RCA, Olson ME. *Giardia* and *Cryptosporidium* in mammalian wildlife—current status and future needs. *Trends Parasitol.* 2005; 21: 370–376. <https://doi.org/10.1016/j.pt.2005.06.004> PMID: 15982929
2. Fayer R., Dubey JP, Lindsay DS. Zoonotic protozoa: from land to sea. *Trends Parasitol.* 2004; 20, 531–536. <https://doi.org/10.1016/j.pt.2004.08.008> PMID: 15471705
3. Oates SC, Miller MA, Hardin D, Conrad PA, Melli A, Jessup DA, et al. Prevalence, environmental loading, and molecular characterization of *Cryptosporidium* and *Giardia* isolates from domestic and wild animals along the central California coast. *Appl Environ Microbiol.* 2012; 78, 8762–8772. <https://doi.org/10.1128/AEM.02422-12> PMID: 23042185
4. Srisuphanunt M, Karanis P, Charoenca N, Boonkhao N, Ongertth JE. *Cryptosporidium* and *Giardia* detection in environmental waters of southwest coastal areas of Thailand. *Parasitol Res.* 2010; 106, 1299–1306. <https://doi.org/10.1007/s00436-010-1795-0> PMID: 20232084
5. Miller M, Miller W, Conrad P, James E, Melli A, Leutenegger C, et al. Type X *Toxoplasma gondii* in a wild mussel and terrestrial carnivores from coastal California: New linkages between terrestrial mammals, runoff and toxoplasmosis of sea otters. *Int J Parasitol.* 2008; 38, 1319–1328. <https://doi.org/10.1016/j.ijpara.2008.02.005> PMID: 18452923
6. Dubey JP. Toxoplasmosis—a waterborne zoonosis. *Vet Parasitol.* 2004; 126, 57–72. <https://doi.org/10.1016/j.vetpar.2004.09.005> PMID: 15567579
7. Shapiro K, Bahia-Oliveira L, Dixon B, Dumètre A, de Wit LA, VanWormer E, et al. Environmental transmission of *Toxoplasma gondii*: Oocysts in water, soil and food. *Food Waterborne Parasitol.* 2019; 15: e00049. <https://doi.org/10.1016/j.fawpar.2019.e00049> PMID: 32095620
8. VanWormer E, Carpenter TE, Singh P, Shapiro K, Wallender WW, Conrad PA, et al. Coastal development and precipitation drive pathogen flow from land to sea: evidence from a *Toxoplasma gondii* and felid host system. *Sci Rep.* 2016; 6: 29252. <https://doi.org/10.1038/srep29252> PMID: 27456911
9. Tenter AM, Heckeroth AR, Weiss LM. *Toxoplasma gondii*: from animals to humans. *Int J Parasitol.* 2000; 30: 1217–1258. [https://doi.org/10.1016/s0020-7519\(00\)00124-7](https://doi.org/10.1016/s0020-7519(00)00124-7) PMID: 11113252
10. Dubey JP, Murata FHA, Cerqueira-Cézar CK, Kwok OCH, Grigg ME. Recent epidemiologic and clinical importance of *Toxoplasma gondii* infections in marine mammals: 2009–2020. *Vet Parasitol.* 2020; 288: 109296. <https://doi.org/10.1016/j.vetpar.2020.109296> PMID: 33271425
11. Simon A, Chambellant M, Ward BJ, Simard M, Proulx JF, Levesque B, et al. Spatio-temporal variations and age effect on *Toxoplasma gondii* seroprevalence in seals from the Canadian Arctic. *Parasitology.* 2011; 138, 1362–1368. <https://doi.org/10.1017/S0031182011001260> PMID: 21813043
12. Forman D, West N, Francis J, Guy E. The sero-prevalence of *Toxoplasma gondii* in British marine mammals. *Mem Inst Oswaldo Cruz.* 2009; 104: 296–298. <https://doi.org/10.1590/s0074-02762009000200024> PMID: 19430656
13. Obusan MC, Villanueva RME, Siringan MA, Rivera WL, Aragonés LV. *Leptospira* spp. and *Toxoplasma gondii* in stranded representatives of wild cetaceans in the Philippines. *BMC Vet Res.* 2019; 15:372. <https://doi.org/10.1186/s12917-019-2112-5> PMID: 31655601
14. Di Guardo G, Proietto U, Di Francesco CE, Marsilio F, Zaccaroni A, Scaravelli D, et al. Cerebral toxoplasmosis in striped dolphins (*Stenella coeruleoalba*) stranded along the Ligurian sea coast of Italy. *Vet Pathol.* 2010; 47: 245–253. <https://doi.org/10.1177/0300985809358036> PMID: 20118319
15. Schares G, Vrhovec MG, Pantchev N, Herrmann DC, Conraths FJ. Occurrence of *Toxoplasma gondii* and *Hammondia hammondi* oocysts in the faeces of cats from Germany and other European countries. *Vet Parasitol.* 2008; 152: 34–45. <https://doi.org/10.1016/j.vetpar.2007.12.004> PMID: 18226453
16. Frenkel JK, Ruiz A, Chinchilla M. Soil survival of *Toxoplasma* oocysts in Kansas and Costa Rica. *Am J Trop Med Hyg.* 1975; 24: 439–443. <https://doi.org/10.4269/ajtmh.1975.24.439> PMID: 1098494
17. Lélou M, Villena I, Dardé M-L, Aubert D, Geers R, Dupuis E, et al. Quantitative Estimation of the Viability of *Toxoplasma gondii* Oocysts in Soil. *Appl Environ Microbiol.* 2012; 78, 5127–5132. <https://doi.org/10.1128/AEM.00246-12> PMID: 22582074
18. Yilmaz SM, Hopkins SH. Effects of different conditions on duration of infectivity of *Toxoplasma gondii* oocysts. *J Parasitol.* 1972; 58: 938–939 PMID: 5078600

19. Dubey JP, Frenkel JK. Feline toxoplasmosis from acutely infected mice and the development of *Toxoplasma* cysts. *J Protozool.* 1976; 23: 537–546. <https://doi.org/10.1111/j.1550-7408.1976.tb03836.x> PMID: 1003342
20. Dubey JP, Cerqueira-Cezara CK, Murataa FHA, Kwoka OCH, Yangb YR, Su C. All about toxoplasmosis in cats: the last decade. *Vet Parasitol.* 2020b; 283: 109145. <https://doi.org/10.1016/j.vetpar.2020.109145> PMID: 32645556
21. Cabezón O, García-Bocanegra I, Molina-López R, Marco I, Blanco JM, Höfle U, et al. 2011. Seropositivity and risk factors associated with *Toxoplasma gondii* infection in wild birds from Spain. *PLoS One.* 2011; 6: e29549. <https://doi.org/10.1371/journal.pone.0029549> PMID: 22216311
22. Lopes AP, Sargo R, Rodrigues M, Cardoso L. High seroprevalence of antibodies to *Toxoplasma gondii* in wild animals from Portugal. *Parasitol Res.* 2011; 108: 1163–1169. <https://doi.org/10.1007/s00436-010-2158-6> PMID: 21104273
23. Olsen A, Berg R, Tagel M, Must K, Deksne G, Enemark HL, et al. Seroprevalence of *Toxoplasma gondii* in domestic pigs, sheep, cattle, wild boars, and moose in the Nordic-Baltic region: A systematic review and meta-analysis. *Parasite Epidemiol Control.* 2019; 5, e00100. <https://doi.org/10.1016/j.parepi.2019.e00100> PMID: 30906889
24. Campbell K. An investigation of an infection with a protozoan parasite causing mortalities in Little Penguins (*Eudyptula minor*) on Penguin Island, Western Australia. Master of science thesis, Murdoch University 2015. Available: <https://researchrepository.murdoch.edu.au/id/eprint/28175/>
25. Last RD, Shivaprasad HL. An outbreak of toxoplasmosis in an aviary collection of Nicobar pigeons (*Caloenas nicobarica*). *Jl S.Afr.vet.Ass.* 2008; 79(3): 149–152
26. Hollings T, Jones M, Mooney N, McCallum H. Wildlife disease ecology in changing landscapes: mesopredator release and toxoplasmosis. *Int J Parasitol Parasites Wildl.* 2013; 2: 110–118. <https://doi.org/10.1016/j.ijppaw.2013.02.002> PMID: 24533323
27. Hartley WJ, Dubey JP. Fatal Toxoplasmosis in Some Native Australian Birds. *J Vet Diagn Invest.* 1991; 3: 167–169. <https://doi.org/10.1177/104063879100300213> PMID: 1892936
28. Work TM, Massey JG, Rideout BA, Gardiner CH, Ledig DB, Kwok OCH, et al. Fatal toxoplasmosis in free-ranging endangered 'Alala from Hawaii. *J Wildl Dis.* 2000; 36: 205–212. <https://doi.org/10.7589/0090-3558-36.2.205> PMID: 10813600
29. Miller MA, Grigg ME, Kreuder C, James ER, Melli AC, Crosbie PR, et al. An unusual genotype of *Toxoplasma gondii* is common in California sea otters (*Enhydra lutris nereis*) and is a cause of mortality. *Int J Parasitol.* 2004; 34, 275–284. <https://doi.org/10.1016/j.ijpara.2003.12.008> PMID: 15003489
30. Carlson-Bremer D, Colegrove KM, Gulland FMD, Conrad PA, Mazet JAK, Johnson CK. Epidemiology and pathology of *Toxoplasma gondii* in free-ranging California sea lions (*Zalophus californianus*). *J Wildl Dis.* 2015; 51: 362–373. <https://doi.org/10.7589/2014-08-205> PMID: 25588007
31. Bressemer MFV, Raga JA, Guardo GD, Jepson PD, Duignan PJ, Siebert U, et al. Emerging infectious diseases in cetaceans worldwide and the possible role of environmental stressors. *Dis Aquat Org.* 2009; 86: 143–157. <https://doi.org/10.3354/dao02101> PMID: 19902843
32. Gibson AK, Raverty S, Lambourn DM, Huggins J, Magargal SL, Grigg ME. Polyparasitism is associated with increased disease severity in *Toxoplasma gondii*-infected marine sentinel species. *PLoS Negl Trop Dis.* 2011; 5: e1142. <https://doi.org/10.1371/journal.pntd.0001142> PMID: 21629726
33. Lauriano G, Di Guardo G, Marsili L, Maltese S, Fossi MC. Biological threats and environmental pollutants, a lethal mixture for Mediterranean cetaceans? *J Mar Biol Assoc U K.* 2014; 94: 1221–1225.
34. Conrad PA, Miller MA, Kreuder C, James ER, Mazet J, Dabritz H, et al. Transmission of *Toxoplasma*: Clues from the study of sea otters as sentinels of *Toxoplasma gondii* flow into the marine environment. *Int J Parasitol.* 2005; 35: 1155–1168 <https://doi.org/10.1016/j.ijpara.2005.07.002> PMID: 16157341
35. Shapiro K, VanWormer E, Aguilar B, Conrad PA. Surveillance for *Toxoplasma gondii* in California mussels (*Mytilus californianus*) reveals transmission of atypical genotypes from land to sea. *Environ Microbiol.* 2015; 17: 4177–4188. <https://doi.org/10.1111/1462-2920.12685> PMID: 25367256
36. VanWormer E, Miller MA, Conrad PA, Grigg ME, Rejmanek D, Carpenter TE, et al. Using molecular epidemiology to track *Toxoplasma gondii* from terrestrial carnivores to marine hosts: implications for public health and conservation. *PLoS Negl Trop Dis.* 2014; 8: e2852. <https://doi.org/10.1371/journal.pntd.0002852> PMID: 24874796
37. Lindsay DS, Dubey JP. Long-term survival of *Toxoplasma gondii* sporulated oocysts in seawater. *J Parasitol.* 2009; 95: 1019–1020. <https://doi.org/10.1645/GE-1919.1> PMID: 20050010
38. Arkush KD, Miller MA, Leutenegger CM, Gardner IA, Packham AE, Heckerroth AR, et al. Molecular and bioassay-based detection of *Toxoplasma gondii* oocyst uptake by mussels (*Mytilus galloprovincialis*). *Int J Parasitol.* 2003; 33: 1087–1097. [https://doi.org/10.1016/s0020-7519\(03\)00181-4](https://doi.org/10.1016/s0020-7519(03)00181-4) PMID: 13129531

39. Esmerini PO, Gennari SM, Pena HFJ. Analysis of marine bivalve shellfish from the fish market in Santos city, São Paulo state, Brazil, for *Toxoplasma gondii*. *Vet Parasitol*. 2010; 170: 8–13. <https://doi.org/10.1016/j.vetpar.2010.01.036> PMID: 20197214
40. Shapiro K, Silver MW, Largier JL, Conrad PA, Mazet JAK. Association of *Toxoplasma gondii* oocysts with fresh, estuarine, and marine macroaggregates. *Limnol Oceanogr*. 2012; 57: 449–456.
41. Krusor C, Smith WA, Tinker MT, Silver M, Conrad PA, Shapiro K. Concentration and retention of *Toxoplasma gondii* oocysts by marine snails demonstrate a novel mechanism for transmission of terrestrial zoonotic pathogens in coastal ecosystems. *Environ Microbiol*. 2015; 17: 4527–4537. <https://doi.org/10.1111/1462-2920.12927> PMID: 26033089
42. Mazzillo FFM, Shapiro K, Silver MW. A new pathogen transmission mechanism in the ocean: the case of sea otter exposure to the land-parasite *Toxoplasma gondii*. *PLoS One*. 2013; 8, e82477. <https://doi.org/10.1371/journal.pone.0082477> PMID: 24386100
43. Massie GN, Ware MW, Villegas EN, Black MW., 2010. Uptake and transmission of *Toxoplasma gondii* oocysts by migratory, filter-feeding fish. *Vet Parasitol*. 2010; 169: 296–303. <https://doi.org/10.1016/j.vetpar.2010.01.002> PMID: 20097009
44. Lafferty KD. Sea otter health: Challenging a pet hypothesis. *Int J Parasitol Parasites Wildl*. 2015; 4: 291–294. <https://doi.org/10.1016/j.ijppaw.2015.05.005> PMID: 26155464
45. Miller MA, Gardner IA, Kreuder C, Paradies DM, Worcester KR, Jessup DA, et al. Coastal freshwater runoff is a risk factor for *Toxoplasma gondii* infection of Southern sea otters (*Enhydra lutris nereis*). *Int J Parasitol*. 2002; 32: 997–1006. [https://doi.org/10.1016/s0020-7519\(02\)00069-3](https://doi.org/10.1016/s0020-7519(02)00069-3) PMID: 12076629
46. Cabezón O, Resendes AR, Domingo M, Raga JA, Agustí C, Alegre F, et al. Seroprevalence of *Toxoplasma gondii* antibodies in wild dolphins from the Spanish Mediterranean coast. *J Parasitol*. 2004; 90: 643–644. <https://doi.org/10.1645/GE-257R> PMID: 15270114
47. Gonzales-Viera O, Marigo J, Ruoppolo V, Rosas FCW, Kanamura CT, Takakura C, et al. Toxoplasmosis in a Guiana dolphin (*Sotalia guianensis*) from Paraná, Brazil. *Vet Parasitol*. 2013; 191: 358–362. <https://doi.org/10.1016/j.vetpar.2012.09.012> PMID: 23063774
48. Mikaelian I, Boisclair J, Dubey JP, Kennedy S, Martineau D. Toxoplasmosis in beluga whales (*Delphinapterus leucas*) from the St Lawrence estuary: two case reports and a serological survey. *J Comp Pathol*. 2000; 122: 73–76. <https://doi.org/10.1053/jcpa.1999.0341> PMID: 10627393
49. Measures LN, Dubey JP, Labelle P, Martineau D. Seroprevalence of *Toxoplasma gondii* in Canadian pinnipeds. *J Wildl Dis*. 2004; 40: 294–300. <https://doi.org/10.7589/0090-3558-40.2.294> PMID: 15362830
50. Jensen S-K, Nymo IH, Forcada J, Godfroid J, Hall A. Prevalence of *Toxoplasma gondii* antibodies in pinnipeds from Antarctica. *Vet Rec*. 2012; 171: 249.2–249. <https://doi.org/10.1136/vr.100848> PMID: 22798344
51. Rengifo-Herrera C, Ortega-Mora LM, Álvarez-García G, Gómez-Bautista M, García-Párraga D, García-Peña FJ, et al. Detection of *Toxoplasma gondii* antibodies in Antarctic pinnipeds. *Vet Parasitol*. 2012; 190: 259–262. <https://doi.org/10.1016/j.vetpar.2012.05.020> PMID: 22726387
52. Acosta ICL, Souza-Filho AF, Muñoz-Leal S, Soares HS, Heinemann MB, Moreno L, et al. Evaluation of antibodies against *Toxoplasma gondii* and *Leptospira* spp. in Magellanic penguins (*Spheniscus magellanicus*) on Magdalena Island, Chile. *Vet Parasitol Reg Stud Reports*. 2019; 16: 100282. <https://doi.org/10.1016/j.vprsr.2019.100282> PMID: 31027597
53. Gennari SM, Niemeyer C, Soares HS, Musso CM, Siqueira GCC, Catão-Dias JL, et al. Seroprevalence of *Toxoplasma gondii* in seabirds from Abrolhos Archipelago, Brazil. *Vet Parasitol*. 2016; 226: 50–52. <https://doi.org/10.1016/j.vetpar.2016.06.016> PMID: 27514883
54. Deem SL, Merkel J, Ballweber L, Vargas FH, Cruz MB, Parker PG. Exposure to *Toxoplasma gondii* in Galapagos Penguins (*Spheniscus mendiculus*) and Flightless Cormorants (*Phalacrocorax harrisi*) in the Galapagos Islands, Ecuador. *J Wildl Dis*. 2010; 46: 1005–1011. <https://doi.org/10.7589/0090-3558-46.3.1005> PMID: 20688714
55. Schreiber EA, Burger J. *Biology of marine birds*, CRC marine biology series. Boca Raton, Fla, CRC Press; 2002.
56. Schott FA, Xie S-P, McCreary JP Jr. Indian Ocean circulation and climate variability. *Rev Geophys*. 2009, 47, RG1002. <https://doi.org/10.1029/2007RG000245>.
57. Faulquier L, Fontaine R, Vidal E, Salamolard M, Le Corre M. Feral Cats *Felis catus* threaten the endangered endemic Barau's petrel *Pterodroma baraui* at Reunion Island (Western Indian Ocean). *Waterbirds*. 2009; 32(2):330–336.
58. Ringler D, Russell JC, Le Corre M. Trophic roles of black rats and seabird impacts on tropical islands: Mesopredator release or hyperpredation? *Biol Conserv*. 2015; 185: 75–84.
59. Feare CJ. Ecology of Bird Island, Seychelles. *Atoll Res Bull*. 1979; 226: 1–29.

60. Stoddart DR, Fosberg FR. Bird and Denis Islands, Seychelles. Atoll Res Bull. 1981; 252: 1–50.
61. Warman S, Dodd D. A biological survey of Aride Island nature reserve, Seychelles. Biol Conserv. 1984; 28: 51–71.
62. Rocamora G, Henriette E. Invasive Alien Species in Seychelles. Why and how to eliminate them? Identification and management of priority species. Island Biodiversity & Conservation centre, University of Seychelles. Biotope Editions, Mèze, MNHM Paris [Inventaires & Biodiversité series]; 2015.
63. Russell JC, Le Corre M. Introduced mammal impacts on seabirds in the Iles Eparses, Western Indian Ocean. Mar. Ornithol. 2009; 37: 121–128.
64. Lebarbenchon C, Jaeger A, Feare C, Bastien M, Dietrich M, Larose C, et al. Influenza A virus on oceanic islands: host and viral diversity in seabirds in the Western Indian Ocean. PLoS Pathog. 2015; 11: e1004925. <https://doi.org/10.1371/journal.ppat.1004925> PMID: 25996394
65. Dubey JP, Desmonts G. Serological responses of equids fed *Toxoplasma gondii* oocysts. Equine Vet J. 1987; 19: 337–339. <https://doi.org/10.1111/j.2042-3306.1987.tb01426.x> PMID: 3622463
66. Dubey JP. A review of toxoplasmosis in wild birds. Vet Parasitol. 2002; 106: 121–153. [https://doi.org/10.1016/s0304-4017\(02\)00034-1](https://doi.org/10.1016/s0304-4017(02)00034-1) PMID: 12031816
67. Dubey JP, Laurin E, Kwok OCH. Validation of the modified agglutination test for the detection of *Toxoplasma gondii* in free-range chickens by using cat and mouse bioassay. Parasitology. 2016; 143: 314–319. <https://doi.org/10.1017/S0031182015001316> PMID: 26625933
68. Dubey JP, Felix TA, Kwok OCH. Serological and parasitological prevalence of *Toxoplasma gondii* in wild birds from Colorado. J Parasitol. 2010; 96: 937–939. <https://doi.org/10.1645/GE-2501.1> PMID: 20950101
69. R Development Core Team. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna A, editor. Vienna; 2015.
70. Mason RW, Hartley WJ, Dubey JP. Lethal toxoplasmosis in a little penguin (*Eudyptula minor*) from Tasmania. J Parasitol. 1991; 77(2): 328. PMID: 2010870
71. Ploeg M, Ultee T, Kik M. Disseminated toxoplasmosis in Black-footed penguins (*Spheniscus demersus*). Avian Dis. 2011; 55(4): 701–703. <https://doi.org/10.1637/9700-030411-Case.1> PMID: 22312996
72. Work TM, Massey JG, Lindsay DS, Dubey JP. Toxoplasmosis in three species of native and introduced Hawaiian birds. J Parasitol. 2002; 88: 1040–1042. [https://doi.org/10.1645/0022-3395\(2002\)088\[1040:TITSON\]2.0.CO;2](https://doi.org/10.1645/0022-3395(2002)088[1040:TITSON]2.0.CO;2) PMID: 12435157
73. Bancroft WJ, Hill D, Roberts JD, 2004. A new method for calculating volume of excavated burrows: the geomorphic impact of Wedge-Tailed Shearwater burrows on Rottneest Island. Funct Ecol. 2004; 18: 752–759.
74. de Wit LA, Croll DA, Tershy B, Correa D, Luna-Pasten H, Quadri P, et al. Potential public health benefits from cat eradications on islands. PLoS Negl Trop Dis. 2019. 13: e0007040. <https://doi.org/10.1371/journal.pntd.0007040> PMID: 30763304
75. Villard P, Bretagnolle V. Breeding biology of the bridled tern (*Sterna anaethetus*) in New Caledonia. Waterbirds 2010; 33: 246–250.
76. Feare CJ, Gill EL, Carty P, Carty HE, Ayrton VJ. Habitat use by Seychelles sooty terns *Sterna fuscata* and implications for colony management. Biol. Conserv. 1997; 81: 69–76.
77. Vesey-Fitzgerald D. XXIX. Further contributions to the ornithology of the Seychelles islands. Ibis 1941; 83: 518–531.
78. Shapiro K, Krusor C, Mazzillo FFM, Conrad PA, Largier JL, Mazet JAK, et al. Aquatic polymers can drive pathogen transmission in coastal ecosystems. Proc R Soc B. 2014; 281: 20141287. <https://doi.org/10.1098/rspb.2014.1287> PMID: 25297861
79. Marino AMF, Giunta RP, Salvaggio A, Castello A, Alfonzetti T, Barbagallo A, et al. *Toxoplasma gondii* in edible fishes captured in the Mediterranean basin. Zoonoses Public Health 2019; 66: 826–834. <https://doi.org/10.1111/zph.12630> PMID: 31278858
80. Blanco C, Salomón O, Raga JA. Diet of the bottlenose dolphin (*Tursiops truncatus*) in the western Mediterranean Sea. J Mar Biol Assoc UK. 2001; 81, 1053–1058.
81. Silva MA. Diet of common dolphins, *Delphinus delphis*, off the Portuguese continental coast. J Mar Biol Assoc U K. 1999; 79: 531–540.
82. Catry T, Ramos J, Jaquemet S, Faulquier L, Berlincourt M, Hauselmann A, et al. Comparative foraging ecology of a tropical seabird community of the Seychelles, western Indian Ocean. Mar Ecol Prog Ser. 2009; 374: 259–272.
83. Chereil Y, Le Corre M, Jaquemet S, Ménard F, Richard P, Weimerskirch H. Resource partitioning within a tropical seabird community: new information from stable isotopes. Mar Ecol Prog Ser. 2008; 366: 281–291.

84. Le Corre M, Cherel Y, Lagarde F, Lormée H, Jouventin P. Seasonal and inter-annual variation in the feeding ecology of a tropical oceanic seabird, the red-tailed tropicbird *Phaethon rubricauda*. *Mar Ecol Prog Ser*. 2003; 255: 289–301.
85. Feare C.J. The breeding of the sooty tern *Sterna fuscata* L. in the Seychelles, and the effect of experimental removal of its eggs. *J. Zool. Lond.* 1976; 179: 317–360.
86. Jaquemet S, Potier M, Cherel Y, Kojadinovic J, Bustamante P, Richard P, et al. Comparative foraging ecology and ecological niche of a superabundant tropical seabird: the sooty tern *Sterna fuscata* in the southwest Indian Ocean. *Mar Biol*. 2008; 155: 505–520
87. Boulinier T, Staszewski V. Maternal transfer of antibodies: raising immuno-ecology issues. *Trends Ecol Evol*. 2008; 23: 282–288. <https://doi.org/10.1016/j.tree.2007.12.006> PMID: 18375011
88. Hasselquist D, Nilsson J-Å. Maternal transfer of antibodies in vertebrates: trans-generational effects on offspring immunity. *Philos Trans R Soc B Biol Sci*. 2009; 364: 51–60. <https://doi.org/10.1098/rstb.2008.0137> PMID: 18926976
89. Garnier R, Ramos R, Staszewski V, Militão T, Lobato E, González-Solís J, et al. Maternal antibody persistence: a neglected life-history trait with implications from albatross conservation to comparative immunology. *Proc R Soc B Biol Sci*. 2012; 279: 2033–2041. <https://doi.org/10.1098/rspb.2011.2277> PMID: 22189405
90. Ramos R, Garnier R, González-Solís J, Boulinier T. Long antibody persistence and transgenerational transfer of immunity in a long-lived vertebrate. *Am Nat*. 2014; 184: 764–776. <https://doi.org/10.1086/678400> PMID: 25438176
91. Kappes M, Weimerskirch H, Pinaud D, Le Corre M. Variability of resource partitioning in sympatric tropical boobies. *Mar Ecol Prog Ser*. 2011; 441: 281–294.
92. Mendez L, Cotté C, Prudor A, Weimerskirch H. Variability in foraging behaviour of red-footed boobies nesting on Europa Island. *Acta Oecologica*. 2016; 72: 87–97.
93. Weimerskirch H, Le Corre M, Jaquemet S, Marsac F. Foraging strategy of a tropical seabird, the red-footed booby, in a dynamic marine environment. *Mar Ecol Prog Ser*. 2005; 288: 251–261.
94. Kohno H, 2000. Visits of Immature Blue-faced and Red-footed Boobies to Nakanokamishima, South Ryukyus, Japan. *Bull Inst Oceanic Res & Develop Tokai Univ*. 2000; 21: 111–117.
95. O'Brien RM, Davies J. A new subspecies of masked booby *Sula dactylatra* from Lord Howe, Norfolk and Kermadec Islands. *Mar Ornithol*. 1990; 18: 1–7.
96. O'Neill P, Heatwole H, Preker M, Jones M. Populations, movements, and site fidelity of brown and masked boobies on the Swain Reefs, Great Barrier Reef, as shown by banding recoveries. CRC Reef Research Centre Technical Report No. 11 Townsville; 1996.
97. Weimerskirch H, Le Corre M, Jaquemet S, Potier M, Marsac F. Foraging strategy of a top predator in tropical waters: great frigatebirds in the Mozambique Channel. *Mar Ecol Prog Ser*. 2004, 275: 297–308.