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Detection of *Toxoplasma gondii* in wild bivalves from the Kerguelen and Galapagos archipelagos: influence of proximity to cat populations, exposure to marine currents and kelp density

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ABSTRACT

Oocysts of the protozoan *Toxoplasma gondii* are found in felid feces and can be washed into coastal waters, where they persist for months, attaching to algae and accumulating in invertebrates. We used wild bivalves to assess contamination of coastal waters of the Kerguelen and Galapagos archipelagos by this zoonotic parasite. Additionally, we leveraged the contrasting situations of these archipelagos to identify some potential drivers of contamination. In the Galapagos, with a cat density reaching 142 per km^2, 15.38% of the sampled oysters (*Saccostrea palmula*) tested positive for *T. gondii* by quantitative real-time PCR (qPCR) (n = 260), and positive samples were found in all eight sampling sites. In Kerguelen, with 1-3 cats per km^2, 40.83% of 120 tested mussels (*Mytilus edulis platensis*) were positive, and positive samples were found in four out of the five sampling sites. These findings provide evidence of *T. gondii* contamination in the coastal waters of these archipelagos. Furthermore, *T. gondii*-positive bivalves were found on islands located 20 km away (Galapagos) and 5 km away (Kerguelen) from the nearest cat population, indicating that *T. gondii* oocysts can disperse through waterborne mechanisms over several kilometers from their initial deposition site. In the Galapagos, where runoff is infrequent and all sites are exposed to currents, the prevalence of qPCR-positive bivalves did not show significant variations between sites (p = 0.107). In Kerguelen where runoff is frequent and site exposure variable, the prevalence varied significantly (p < 0.001). The detection of *T. gondii* in Kerguelen mussels was significantly correlated with the site exposure to currents (odds ratio (OR) 60.2, p < 0.001) and the on-site density of giant kelp forests (OR 2.624, p < 0.001). This suggests that bivalves can be contaminated not only by oocysts transported by currents but also by consuming marine aggregates containing oocysts that tend to form in kelp forests.

1. Introduction

Reports of biological water contamination by land-derived pathogens affecting marine fauna and human health have increased sharply in recent decades (Stewart et al., 2008; Thorp and Covich, 2009; Bossart, 2011). These land-derived pathogens include a variety of microorganisms such as viruses (Smith et al., 1998; Van Bressem et al., 2009), bacteria (Norman et al., 2008; Van Bressem et al., 2009), fungi (Reif et al., 2006) and other parasites (Kreuder et al., 2003; Kuhn et al., 2011). Among these, *Toxoplasma gondii* is one of the most concerning for marine mammals and seabirds (Work et al., 2002; Shapiro et al., 2012, 2019b).

Toxoplasma gondii is the apicomplexan parasite responsible for toxoplasmosis, a disease that can affect all the homoeothermic species including humans (Dubey, 2021). Felids, and especially domestic cats (*Felis silvestris catus*), are the only source of environmental contamination with *T. gondii* oocysts since they serve as

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definitive hosts, shedding millions of oocysts in their feces (Hill et al., 2005; Gilot-Fromont et al., 2012). These oocysts can remain viable and infectious for months in moist soil (Lélu et al., 2012) and can be transported by runoff into river systems that reach freshwater and marine ecosystems (Simon et al., 2013; Shapiro et al., 2019a). They can also run off directly from coastal areas into coastal waters (VanWormer et al., 2013). Once in seawater, oocysts remain viable for up to 24 months (Lindsay and Dubey, 2009) and can be transported by fish (Massie et al., 2010). Experimental studies have also shown that many oocysts can attach to biofilms on the surface of kelp forest canopies (*Macrocystis pyrifera*) (Mazzillo et al., 2013; Shapiro et al., 2014). Lastly, the higher *T. gondii* prevalence in coastal birds than inland birds on cat-free islands suggests exposure of the former to oocysts carried by water at long distances from the felid populations (Poulle et al., 2021).

Bivalves are particularly useful sentinels of water contamination with pathogens such as *T. gondii* since they filter and accumulate particles, facilitating the detection of contaminants usually found in low concentrations in water (Toze, 1999; Thorp and Covich, 2009). In addition, their sessile way of life allows contamination to be localized. The molecular detection of *T. gondii* DNA in oysters (Marquis et al., 2019; Cong et al., 2021; Li et al., 2022) and mussels (Miller et al., 2008; Coupe et al., 2018; Santoro et al., 2020; Bigot-Clivot et al., 2022) has helped in the identification of various *T. gondii*-contaminated coastal sites and the factors involved. However, the contribution of each factor to this contamination may vary seasonally and from one environment to another (Yan et al., 2016). For example, in Weihai (China), Cong et al. (2021) found that the prevalence of *T. gondii* DNA in bivalves was significantly correlated with temperature and precipitation, while Li et al. (2022) found that it significantly correlated with temperature but not precipitation. Large domestic cat populations near coastal areas, high levels of rainfall associated with runoff, coastal geography and water movement dynamics have also been proposed to explain the local contamination of nearshore environments by *T. gondii* oocysts (Deem et al., 2010; Miller et al., 2002; Mosquera et al., 2023).

To identify contamination factors of wild bivalves with *T. gondii*, we compared the extent and distribution of this contamination in the Kerguelen (Southern Indian Ocean) and the Galapagos (Pacific Ocean) archipelagos. The presence of bivalves on islands with and without cats in these two remote environments offered an opportunity to study the influence of distance from the nearest cat population on bivalve contamination. The Kerguelen and Galapagos also differ in rainfall, coastline geography, marine currents and giant kelp forest presence that could influence the arrival, distribution and accumulation of *T. gondii* oocysts in bivalves. In the present study, we first explored the contamination of coastal waters by *T. gondii* in the Kerguelen and Galapagos archipelagos, using bivalves as indicators. Then we tested the associations between the occurrence of *T. gondii* DNA in bivalves and proximal environmental factors potentially related to the production, transport and retention of oocysts.

2. Materials and methods

2.1. Study areas

The Kerguelen archipelago (French Southern and Antarctic Territories, southern Indian Ocean) is more than 3800 km from the African continent (49° 21′ S, 70° 14′ E; Fig. 1). It includes one main island (Grande Terre) surrounded by more than 300 islands and islets. The cold sub-Antarctic climate of Kerguelen is characterized by high precipitation and windy conditions. Yearly rainfall can range from ∼ 8000 mm in the west to ∼ 800 mm in the east (Christophe et al., 2020) and snowfalls are frequent from June to September. The rugged coastline is dotted with gulfs and patches of the giant kelp *Macrocystis pyrifera*. Sea water temperature can vary between 1 °C to 9 °C (Féral, J.-P., Poulin, E., González-Wevar, C.A., Améziane, N., Guillaumot, C., Develay, E., Saucède, T., 2017. Long-term monitoring of coastal benthic habitats in the Kerguelen Islands: a legacy of decades of marine biology research, Symposium on Kerguelen plateau marine ecosystem and fisheries. Australian Antarctic Division, pp. 383–402. https://doi.org/10.5281/zenodo.3249143). Human activities are mainly localized in Grande Terre, around the scientific station of Port-aux-Français. The archipelago was free of felids until a few domestic cats were introduced on Grande Terre in the 1950s to combat rats and mice, which had also been introduced (Lesel and Derenne, 1975). Due to the harsh climatic conditions, cats are found at a low density (1–3 per km^2), mainly in the coastal areas (Martin et al., 2013). Over half have *T. gondii* antibodies (Afonso et al., 2007).

The Galapagos archipelago (Ecuador) comprised more than 100 volcanic islands and islets located in the Pacific Ocean, approximately 1,000 km from the South American continent (0° 46′ S, 91° 8′ W; Fig. 2). It has a subtropical climate with mostly deserted landscapes and sporadic rains from December to June (Larrea and Di Carlo, 2009). The mean annual rainfall is approximately 500 mm but the mean rainfall during the dry season can be as low as 130 mm (Paltán et al., 2021). The mean surface water temperature is ∼ 26 °C (Paltán et al., 2021). The human population is exclusively distributed on four islands: San Cristobal, Santa Cruz, Isabela and Floreana. The domestic cat was introduced on these islands during the 19th century (Phillips et al., 2012). Their populations locally reach 142 individuals per km^2 (Preston et al., 2022) and can be found from coastal areas to volcano summits (Konecny, 1987; MacLeod et al., 2020). Serological studies on San Cristobal and Isabela revealed that more than half of the individuals have *T. gondii* antibodies (Levy et al., 2008; Espín Alvarado, 2021). The uninhabited islands of the Galapagos archipelago are free of felids.

2.2. Bivalve sampling

Wild marine mussels (*Mytilus edulis platensis*) were collected on Kerguelen for an ecotoxicology study under order n°2017-115 of October 12, 2017 (Territoire des Terres Australes et Antarctiques Françaises, France). The mussels were sampled in January 2018 at three sites with cats (Anse de l'Echouage, Port Couvreux, Anse aux Ecueils) on the main island, and at two islands without cats (Ilot Channer, Ile Haute) (Fig. 1). The species' mean size of adults is reported as 6.5 cm (±0.4 cm) (Bultelle et al., 2021). Mussels were collected in the intertidal zone at low tide, at less than 5 m water depth, and transported to Port-aux-Français in a bucket filled with aerated seawater taken in the field. They were stored in zip-log bags at −20 °C until their transit to Stress Environnementaux et BIOsurveillance des milieux aquatiques laboratory (UMR-I 02 SEBIO) of the Reims Champagne Ardenne University (URCA, France) for the molecular detection of *T. gondii*.

On the Galapagos, wild mangrove oysters (*Saccostrea palmula*) were collected under the research permits MAAE-DBI-CM-2021–0195 and N°074-2022-EXP-CM-FAU-DBI/MAATE (Ministerio del Ambiente, Agua y Transición Ecológica, Ecuador). The sampling occurred between October and November 2021 on three islands with cats (San Cristobal, Santa Cruz and Isabela) and on Santiago island without cats (Fig. 2). Oysters were collected from volcanic rocks at less than 5 m depth in the intertidal zone of mangrove forests at low tide. Adults were prioritized for collection. Their size is approximately 6–7 cm (Cabrera Peña et al., 2001). The research permit authorized the collection of a maximum of 100 oysters per island but only 33 to 88 oysters were collected per island because few individuals were present at most of the sampled sites.

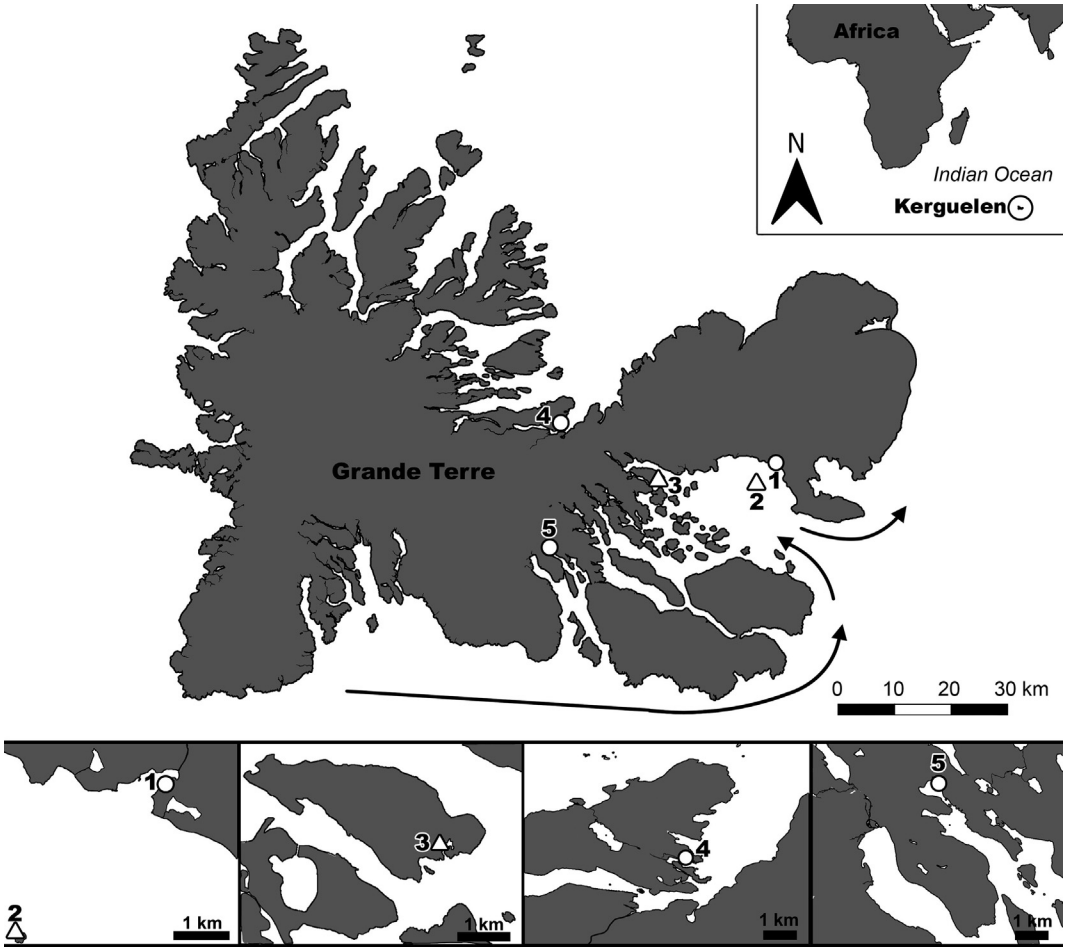

Fig. 1. Location of the mussel collection sites in the Kerguelen archipelago, Indian Ocean, and focus on their coastlines (white circles: islands with cats; white triangles: islands without cats). 1, Anse de l'Echouage; 2, Ilot Channer; 3, Ile Haute; 4, Port Couvreux; 5, Anse aux Ecueils. Contamination on islands without cats can occur via oocysts carried by currents coming from islands with cats. Black arrows represent the flow of currents that enter the Morbihan Gulf where sites 1 and 2 are.

Each oyster was opened on site and collected without its shell since it was attached to the rock. They were transported in zip-log bags inside a cooler with ice packs. Oysters were stored at −20 °C at the Galapagos Science Center (San Cristobal Island, Ecuador) from the University San Francisco of Quito (USFQ) until their transit to the Epidémio-Surveillance et Circulation des Parasites dans les Environnements laboratory (UR 7510 ESCAPE, URCA, Reims, France) for the molecular detection of *T. gondii*.

2.3. Assessment of T. gondii detection method in oysters

This assessment was conducted on commercially available Pacific oysters (*Crassostrea gigas*), bought fresh and stored at −20 °C before use. We evaluated the detection method by spiking tissues of the Pacific oysters with serially diluted solutions containing 1, 5, 10, 10^2, 10^3 and 10^4 oocysts. The spiked samples were kept at 4 °C for 24 h before the pre-treatment step with trypsin. The 95% limit of detection (LOD_{95}) was calculated as described by Berrouch et al. (2023). LOD_{95} was obtained using five replicates for 1, 5, 10 and 10^2 dilutions and three replicates for the 10^3 and 10^4 dilutions. It is important to note that the *T. gondii* detection protocol in bivalve tissues used in our laboratory was initially validated on marine mussels (Cazeaux et al., 2022). However, due to the greater mass of oyster tissues compared with mussel tissues, we adapted the protocol using a DNA extraction kit which differed from that described by Cazeaux et al. (2022) (see section 2.4).

2.4. Pre-treatment, DNA extraction and quantitative real-time PCR (qPCR) detection

The pre-treatment of all wild bivalves with trypsin was performed following the protocol of Cazeaux et al. (2022). Each wild bivalve was shredded with a scalpel and digested in 20 mL of 0.9% NaCl and 2 mL of 10X trypsin (Thermo Fisher Scientific, France) for 1.5 h at 37 °C on a shaker (90 rpm) in stomacher bags with side filters (lateral strainer bag, Bagpage R400, Interscience, France). The filtrates were collected by hand pressure and rinsing the bags with a 0.9% NaCl solution. The filtrates were centrifuged at 2,500 *g* for 10 min (10 °C), and the supernatants were discarded. The pellets were then washed with 25 mL of 0.9% NaCl, vortexed and centrifuged again at 2,500 *g* for 10 min at 10 °C. The supernatants were discarded, and pellets were kept for total genomic DNA extraction.

For mussels, DNA was extracted using a FastDNA® SPIN Kit for Soil according to the manufacturer's instructions (MP Biomedicals, France) and stored at −20 °C until use (Cazeaux et al., 2022). For

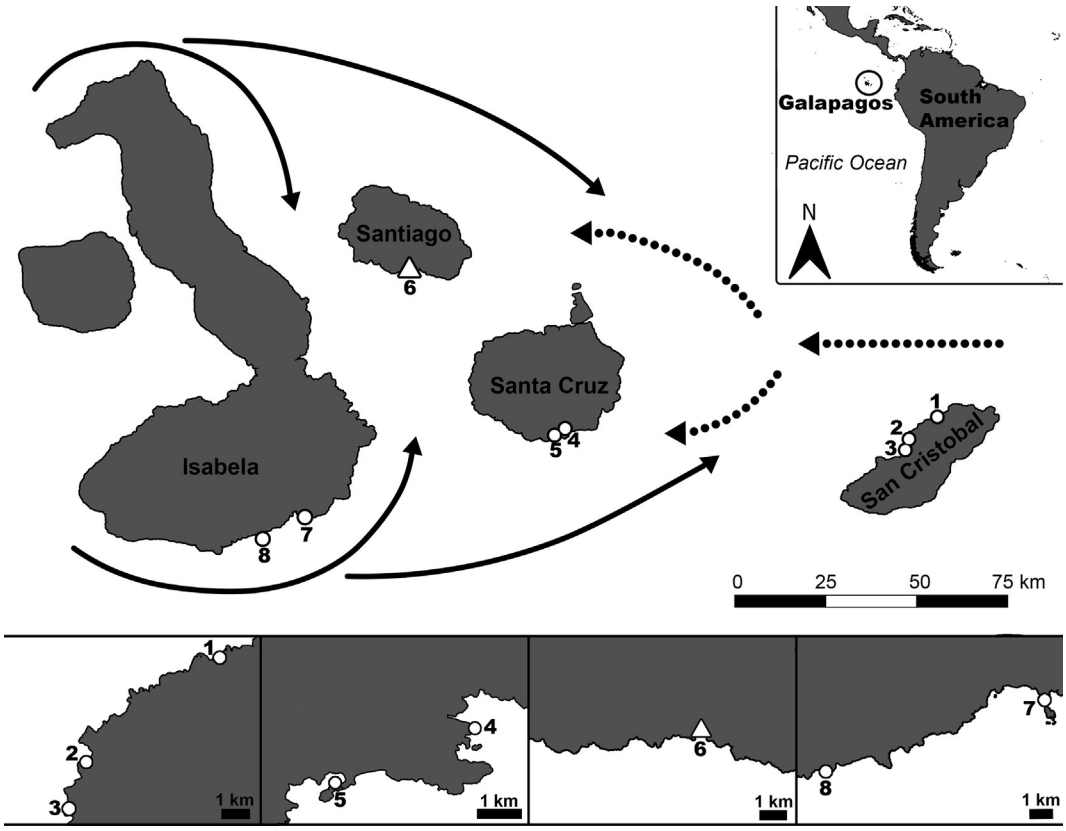

Fig. 2. Location of the oyster collection sites in the Galapagos archipelago, Pacific Ocean, and focus on their coastlines (white circles: islands with cats; white triangle: island without cats). 1, Puerto Grande; 2, Cerro Brujo; 3, La Tortuga; 4, Punta Estrada; 5, Tortuga Bay; 6, Poza de las Azules; 7, Bahia Concha de Perla; 8, La Union. Contamination on the island without cats can occur via oocysts carried by currents coming from islands with cats. Black solid arrows and black dotted arrows represent the directions of the Cromwell Current and the South Equatorial Current, respectively.

oysters, DNA was extracted using an E.Z.N.A.® Soil DNA Kit according to the manufacturer's instructions (omega BIO-TEK, USA) and stored at −20 °C until use. As the extraction kits used for the mussel and oyster samples differed, only the detection of *T. gondii* DNA oocysts was measured and not the quantity of oocysts, to avoid an experimental bias.

Detection of *T. gondii* DNA was performed by TaqMan real-time qPCR targeting the 529 bp repeat region AF487550.1 (Lélu et al., 2011). For mussels, the detection was performed in 2021 using a CFX96 TOUCH™ thermocycler (Bio-Rad) at the UMR-I 02 SEBIO laboratory (URCA, France). For the oysters, detection was performed in 2023 using a QuantStudio 5 (Thermo Fisher) at the UR 7510 ESCAPE laboratory (URCA, France). All amplification experiments were performed in duplicate and a no-template control was added. A sample was considered positive when the threshold cycle (Ct) was less than 40 as previously described by Géba et al. (2020) and Cazeaux et al. (2022).

To confirm contamination with *T. gondii* in the Kerguelen, two amplicons from Anse aux Ecueils and Port Couvreux (sites with cats) were sequenced, as well as three amplicons from Ilot Channer and Ile Haute (sites without cats). Regarding the Galapagos, an amplicon from Puerto Grande, Cerro Brujo, La Tortuga, Punta Estrada, Tortuga Bay, Bahia Concha de Perla, and La Union (sites with cats) were sequenced. Four amplicons from Poza de las Azules (site without cats) were also sequenced. Thus, the amplicons were purified and submitted for Sanger sequencing by Microsynth France SAS. The sequences obtained were compared with GenBank reference sequences using the Basic Local Alignment Search Tool (BLAST; https://blast.ncbi.nlm.nih.gov/Blast.cgi).

2.5. Statistical analysis

The homogeneity of the distribution of the prevalence of qPCR-positive bivalves across sites in each archipelago was tested using the Fisher exact test in R (R Core Team, R., 2013. R: A language and environment for statistical computing). For analysis of the occurrence of *T. gondii* DNA in bivalves, the explanatory variables considered were: i) the presence/absence of cats on the sampling island, ii) the site's exposure to currents as potential vectors of waterborne oocysts and, iii) the giant kelp forest density at the site.

Each site's exposure to currents was scored from 1 (the least exposed) to 3 (the most exposed) based on whether the sites are minimally, partially, or completely exposed to the influence of marine and tidal currents coming from islands with cats, according to their geographic features described in Table 1.

The density of giant kelp forests was considered to influence the presence of *T. gondii* in bivalves given the ability of this giant kelp to retain oocysts (Shapiro et al., 2012; Mazzillo et al., 2013). This density was obtained using the high-resolution global map of giant kelp established by Mora-Soto et al. (2020). The Kelp Difference (KD) Index was obtained by calculating the mean values of the nine closest pixels around each collection site.

Logistic regression models built with the package "stats" v3.6.2 and "oddsratio" v2.0.1 (R Core Team, R., 2013. R: A language and environment for statistical computing; Schratz, P., 2017. R package 'oddsratio': Odds ratio calculation for GAM (M) s & GLM (M) s, version: 1.0. 2. https://doi.org/10.5281/zenodo.1095472) were used to estimate the odds ratio (OR) between the detection of *T. gondii* DNA in bivalves and presence/absence of cats on islands, exposure to

Table 1
Prevalence of *Toxoplasma gondii* DNA in wild bivalves from sampling sites in the Kerguelen and Galapagos archipelagos as estimated by quantitative real-time PCR (qPCR), and variables tested as explanatory factors for this prevalence.

Archipelago / Island / Site	Inhabited with cats	Exposure to currents (justification)	Kelp density (KD)	Number of bivalves	Number of positive bivalves	Prevalence (%)	p-values Fisher exact test
Kerguelen / Grande Terre / Anse de l'Echouage	Yes	Low (semi-enclosed shallow bay)	0	24	0	0.00	<0.001
Kerguelen / Ilot Channer	No	High (open sea)	19	24	21	87.50	
Kerguelen / Ile Haute	No	Low (semi-enclosed shallow bay)	0	24	5	20.83	
Kerguelen / Grande Terre / Port Couvreux	Yes	Medium (enclosed inlet)	8	24	10	41.67	
Kerguelen / Grande Terre / Anse aux Ecueils	Yes	Medium (enclosed inlet)	4	24	13	54.17	
Galapagos / San Cristobal / Puerto Grande	Yes	High (open sea)	0	38	7	18.42	0.107
Galapagos / San Cristobal / Cerro Brujo	Yes	High (open sea)	0	23	3	13.04	
Galapagos / San Cristobal / La Tortuga	Yes	High (open sea)	0	27	10	37.04	
Galapagos / Santa Cruz / Punta Estrada	Yes	High (open sea)	0	18	2	11.11	
Galapagos / Santa Cruz / Tortuga Bay	Yes	Low (semi-enclosed shallow bay)	0	60	7	11.66	
Galapagos / Santiago / Poza de las Azules	No	High (open sea)	0	61	7	11.48	
Galapagos / Isabela / Bahia Concha de Perla	Yes	High (open sea)	0	25	2	8.00	
Galapagos / Isabela / La Union	Yes	High (open sea)	0	8	2	25.00	

marine currents and density of giant kelp forests (Table 1). Statistical difference was considered if $p < 0.001$.

3. Results

3.1. Detection method developed for Toxoplasma gondii in oysters

Our detection method led to the same LOD_{95} at 50 oocysts (Table 2) as the method initially used for mussels by Cazeaux et al. (2022), with a regression coefficient (R^2) of 0.9955.

Table 2
Detection of *Toxoplasma gondii* DNA by quantitative real-time PCR (qPCR) in Pacific oysters (*Crassostrea gigas*) spiked with serially diluted solutions containing *Toxoplasma gondii* oocysts.

Oocysts seeded	Cq mean ± S.D.	Positive oysters/total samples
10,000	25.87 ± 0.30	3/3
1,000	29.46 ± 0.30	3/3
100	32.43 ± 0.50	5/5
50	34.01 ± 0.28	5/5
10	36.72 ± 0.17	4/5
5	Not amplified	0/5
1	Not amplified	0/5

3.2. Prevalence of T. gondii DNA in wild bivalves

Toxoplasma gondii DNA was detected in 40.83% (49/120) of the mussels collected in Kerguelen and 15.38% (40/260) of the oysters collected in Galapagos. Except for Anse de l'Echouage on Kerguelen, *T. gondii* DNA was detected in bivalves at all sites of both archipelagos. The prevalence of *T. gondii* DNA between sites in Kerguelen varied significantly from 0% to 87.5% ($p < 0.001$, Table 1). In comparison, it ranged from 8% to 25% with no significant differences between sites in Galapagos ($p = 0.107$, Table 1).

The sequencing confirmed that 8/10 DNA samples from the Kerguelen and 1/10 DNA samples from the Galapagos correspond to *T. gondii* (between 96.77 and 100% identity). For the Kerguelen archipelago, only one out of three amplicons from Ilot Channer and one out of two amplicons from Anse aux Ecueils were not successfully sequenced. Thus, the contamination with *T. gondii* is confirmed for all sites with cats (Anse aux Ecueils and Port Couvreux) and without cats (Ilot Channer and Ile Haute). The only sample from the Galapagos successfully sequenced is from Puerto Grande (San Cristobal Island).

3.3. Factors associated with T. gondii prevalence in wild bivalves

Logistic regression analyses showed that the presence of cats was associated with a significant reduction in the odds ratio (OR)

for Kerguelen (OR 0.397; 95% Confidence Interval (95% CI): 0.185–0.838; $p = 0.0153$). Interestingly, the exposure to marine currents had a significant association with the presence of *T. gondii* DNA in mussels on Kerguelen. At the site with the highest exposure to currents (Ilot Channer, located in an open sea shoreline), the probability of finding a qPCR-positive mussel increased 60 times compared with the site least exposed to currents (Anse de l'Echouage, a semi-enclosed shallow bay; OR 60.2; 95% CI: 14.987–334.098; $p < 0.001$). We also found that the density of giant kelp forests at this archipelago showed a significant association with the presence of *T. gondii* DNA, with an increment of 2.624 (95% CI: 1.860–3.979; $p < 0.001$) in the odds ratio for every five unit increment in the kelp density (Table 3).

For Galapagos, we found no significant association between the presence of *T. gondii* DNA in oysters, the presence/absence of cats on the sampling island, or the exposure of the sampling site to currents (Table 3). Kelp density at the sampling site could not be included as a variable for the Galapagos since all sampling sites on this archipelago had a KD value of zero (Table 1).

4. Discussion

The detection of *T. gondii* DNA in 41% of mussels collected in Kerguelen and 15% of the oysters collected in Galapagos highlights, for the first known time, the contamination of coastal waters of these two archipelagos by this land-derived parasite. Moreover, in Kerguelen the sequencing of positive samples confirms the contamination of islands (with or without cats) with *T. gondii*. At the Galapagos, only one out of the 10 samples could be successfully sequenced but this still allowed the identification of *T. gondii*. The sequencing failure of the nine Galapagos samples is likely due to inadequate quality and quantity of DNA for successful sequencing.

By taking advantage of the ability of bivalves to concentrate oocysts that are probably in very low density in the ocean, we detected *T. gondii* DNA in most sampling sites, including those on islands without cats. This finding suggests widespread contamination of the coastal environments of these two archipelagos during the one to two centuries following the introduction of cats onto some of their islands. In Hawaii (USA), where cats were introduced during the 18th Century (Hess et al., 2007) and where prevalence of *T. gondii* in their population is greater than 30% (Danner et al., 2007), cases of acute toxoplasmosis leading to mortality have already been documented in native species such as monk seals (*Monachus monachus*) and seabirds (*Corvus hawaiiensis* and *Sula sula*) (Work et al., 2000, 2002; Honnold et al., 2005). These reports raise concerns about the potential occurrence of such cases in marine mammals and seabirds that are endemic to the Kerguelen and Galapagos archipelagos, long isolated from felines and whose immune systems may not have had time to adapt to *T. gondii*.

As cat density is much lower on Kerguelen than on Galapagos (Martin et al., 2013; Preston et al., 2022) a lower overall prevalence of *T. gondii* DNA in bivalves would have been expected for Kerguelen. However, the opposite was observed (41% on Kerguelen versus 15% on Galapagos). This unexpected result cannot be explained by a difference in the size of the bivalves leading to a difference in the reduction in the filtration capacity (Jones et al., 1992; Chinnadurai et al., 2022) since Kerguelen mussels and Galapagos oysters are approximately the same size. However, the difference in bivalve collection period, imposed by field constraints, may be partly responsible for the higher prevalence in Kerguelen than Galapagos since Kerguelen mussels were collected in January, i.e. after the period of heavy rain and snowfall leading to a constant runoff, while oysters from Galapagos were collected in November, i.e. during the dry season when runoff is very limited. Lastly, the cats' way of life may also explain the unexpected result for prevalence. On Kerguelen, harsh climatic conditions inland and distribution of main prey for cats (i.e. rabbits and breeding birds) restrict the feral cat population to the coast and, notably, on the beaches where oocysts may be accumulated by the constant runoff product of high precipitation and snow melting. By contrast, on the Galapagos, cats are more scattered in the four inhabited islands, rains are sporadic and happen mainly from December to June, and the perennial flows of surface water are limited to the southeastern central side of San Cristobal Island (Violette et al., 2014). Differences in water temperatures between the two archipelagos also must be considered as those may affect the survival and infectivity of *T. gondii* oocysts. Experimentally, sporulated oocysts retain their infectivity for 54 months in fresh water at 4 °C, and for 15 months at 20–25 °C (Dubey et al., 1998). In seawater (15 ppt NaCl), oocysts kept at 4 °C for 24 months were orally infective for mice, while those kept at room temperature were not (Lindsay and Dubey, 2009). Thus, the difference in water temperature between the two archipelagos could contribute to explaining the higher detection level of *T. gondii* in the bivalves from Kerguelen, which has colder waters (between 1 °C and 9 °C) (Féral et al., 2016) compared with Galapagos (between 22.2 °C and 26.9 °C) (Palacios, 2004).

Until this study, the distinction between exposure of seabirds nesting on islands without cats to *T. gondii* oocysts carried by currents and oocysts carried by fish was impossible because all the seabird species studied feed on fish (Deem et al., 2010; Mosquera et al., 2023; Poulle et al., 2021). By contrast, the present study is based on bivalves for which the oocysts retained during water filtration is the only possible route of contamination. Thus, bivalves yielding positive qPCR results collected around the cat-free islands of Ile Haute and Ilot Channer (Kerguelen) and those collected around Santiago (Galapagos), respectively, less than 1 km, ~4 km

Table 3
Parameters of the variable tested as explanatory factors for the detection of *Toxoplasma gondii* DNA in wild bivalves collected in the Kerguelen and Galapagos archipelagos.

Archipelago	Variable	Positive	Total	Odds ratio (OR CI_{95})	*p*-value
Kerguelen	**Cats**				
	No	26	48	Reference	
	Yes	23	72	0.397 (0.185–0.838)	0.0153
	Exposure to currents				
	Low	5	48	Reference	
	Medium	23	48	7.912 (2.855–25.960)	<0.001
	High	21	24	60.2 (14.987–334.098)	<0.001
	Kelp Density (KD)			Increment of 5 KD	
	Range (0–19)	49	120	2.624 (1.860–3.979)	<0.001
Galapagos	**Cats**				
	No	7	61	Reference	
	Yes	33	199	1.534 (0.675–3.955)	0.3201
	Exposure to currents				
	Low	7	60	Reference	
	High	33	200	1.496 (0.658–3.861)	0.3502

and ~ 20 km from the two closest cat-populated islands, demonstrate the waterborne dispersal of *T. gondii* oocysts at least 20 km from their place of deposition.

The prevalence of qPCR-positive bivalves per site on Kerguelen varied significantly from 0% (Anse de l'Echouage) to 87.5% (Ilot Channer), while it did not vary between sites on Galapagos. This suggests a relatively homogeneous *T. gondii* contamination of coastal waters on the Galapagos versus a heterogeneous distribution of this contamination on Kerguelen. The westward South Equatorial Current and the eastward Equatorial Undercurrent that drive water all around the Galapagos archipelago (Liu et al., 2014) might be implicated in the dispersal of *T. gondii* contamination of coastal waters between the sampling sites. These currents could potentially carry oocysts from eastern (Isabela and Floreana) and western (San Cristobal and Santa Cruz) islands with cats towards islands without cats such as Santiago. On Kerguelen, the very irregular contours of the coasts and the west-east direction of marine currents lead to differences in the exposure of sites to currents potentially carrying oocysts, leading to a significant association between this exposure and the occurrence of qPCR-positive mussels. The two lowest *T. gondii* prevalence values in mussels were found in the sampling sites of Anse de l'Echouage and Ile Haute, which are highly sheltered due to their location in an enclosed shallow bay protected from the influx of water brought by marine currents. No positive mussel was found at Anse de l'Echouage, located opposite to the closest river, a potential source of contamination with oocysts carried by runoff from land. The second and third highest prevalence values on Kerguelen were found in mussels from sampling sites located on inlets at Port Couvreux and Anse aux Ecueils, where the influence of marine currents seems limited. In contrast, a higher prevalence was found at Ilot Channer, a site at the main flow of the current entering Baie du Morbihan (Féral et al., 2017, symposium paper cited earlier) and exposed to south-western swell and north-western surface currents that could carry oocysts from Grande Terre Island, where cats are present.

This study also demonstrated a significant association between the occurrence of qPCR-positive mussels and the density of giant kelp forests in Kerguelen. This finding can be explained by the large quantities of oocysts that can attach to the biofilms of this giant kelp (Shapiro et al., 2012; Mazzillo et al., 2013), whose detritus constitutes the second main source of food for Kerguelen mussels (Le Bourg et al., 2022). The dense kelp forest surrounding Ilot Channer and the medium density of this forest in Port Couvreux and Anse aux Ecueils may contribute to the high and medium prevalence of *T. gondii* in bivalves at these sites.

To conclude, our study sheds light on the widespread contamination of coastal waters by *T. gondii* in the Kerguelen and Galapagos archipelagos, even without a long-standing feline presence. Using bivalves as sentinels has proven to be an invaluable tool for assessing the extent of this contamination. It is noteworthy that the use of hemolymphatic liquid biopsies collected from sentinel bivalves can be a valuable method for monitoring infectious diseases. Recently, we employed this effective and simple-to-implement approach to detect pathogens in bivalves in remote areas like Kerguelen (Ferchiou et al., 2022a, 2022b). Moreover, a recent study conducted by Kim et al. (2023) demonstrated the high sensitivity and affordability of PCR-based detection of *T. gondii* in hemolymph samples, making it an effective method for screening the presence of protozoan DNA. This study also provides compelling evidence of the role of marine currents in the dispersal of oocysts. In addition, giant kelp forests seem to favor the accumulation of oocysts in bivalves on Kerguelen, highlighting the need for further research to understand the full extent of their ecological impacts and to develop strategies for mitigating the potential threats to coastal biodiversity. Toxoplasmosis could, for example, affect the Kerguelen shag, *Phalacrocorax verrucosus*, because this endemic seabird lives mainly in bays, fjords and heavily indented coasts where it feeds on kelp beds during summer (Cook et al., 2008). In Galapagos, the presence of stray cats on beaches of semi-enclosed and shallow bays, as reported in Tortuga Bay (Laurie, 1983), might lead to the local *T. gondii* contamination of coastal waters, as suggested under similar conditions along Californian (USA) coasts (Miller et al., 2002).

CRediT authorship contribution statement

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