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Short communication

Evaluation of antibodies against *Toxoplasma gondii* and *Leptospira* spp. in Magellanic penguins (*Spheniscus magellanicus*) on Magdalena Island, Chile

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ABSTRACT

Toxoplasmosis has been reported in many avian species, but little information is available from wild penguin populations. *Leptospira* can infects domestic and wild animals. *Spheniscus magellanicus* belong to the order Sphenisciformes, family Spheniscidae, and are colonial birds. These seabirds live in temperate waters along the Atlantic shores of South America, and their total population has been estimated to be 1,300,000 breeding pairs. Magdalena Island (Chile) hosts an important breeding colony but, over recent decades, a marked decline in the number of birds has been seen. The objective of this study was to determine occurrences of antibodies against *T. gondii* and *Leptospira* spp. in penguins (*Spheniscus magellanicus)* on Magdalena Island, from where no previous data on these agents were available. Serum samples were collected from 132 penguins on Magdalena Island. Antibodies against *Toxoplasma gondii* were detected using the modified agglutination test (Titer ≥20), and anti-*Leptospira* spp. antibodies were detected using the microscopic agglutination test (Titer ≥100). *T. gondii* antibodies were detected in 57 (43.18%) of the 132 serum samples, with titers that ranged from 20 to 320. None of the penguins in this study was reactive to anti-*Leptospira* spp. antibodies. This is the first report of *T. gondii* seropositivity in free-living Magellanic penguins in Chile.

1. Introduction

Toxoplasma gondii is a coccidian parasite found worldwide. It infects virtually all warm-blooded animals, including humans, but only cats (domestic and wild) are its definitive hosts. Toxoplasmosis has been reported in many avian species, but little information is available from wild penguin populations (Dubey, 2010). Leptospirosis is a bacterial infection determined by pathogenic spirochetes of the genus *Leptospira* that infects domestic and wild animals, as well as humans. Leptospirosis infection in wild fauna has been identified in varius biomes of Latin America, such as in the Atlantic Forest, Amazon, Equatorial Forest, Pantanal, Ocean and Savanna (Vieira et al., 2018).

Magellanic penguins belong to the order Sphenisciformes, family Spheniscidae, and are colonial birds. These seabirds live in temperate waters along the Atlantic shores of South America, and their total population has been estimated to be 1,300,000 breeding pairs. They reproduce on the coasts of Argentina and Patagonian Chile and on the Falkland Islands during the months of September to March. Before winter, they migrate northwards from these locations towards the southern and southeastern coasts of Brazil, searching for places with greater availability of food (Boersma et al., 2013).

Magdalena Island, in Chile, hosts an important breeding colony of Magellanic penguins. However, over recent decades, a marked decline in the number of birds has been seen due to a series of factors relating especially to commercial fishing, climate change and oil contamination (Bingham and Herrmann, 2008; Calvert et al., 2013).

A study conducted in the Galapagos Islands, Ecuador, reported that the endemic Galapagos penguin (*Spheniscus mendiculus*) was seropositive to *T. gondii*. Antibodies were detected at a seroprevalence rate of 2.3% among 298 specimens (Deem et al., 2010). To date, this study remains the sole evidence of *T. gondii* exposure in wild populations of penguins.

On the other hand, *T. gondii* was reported in a captive blue penguin (*Eudyptula minor*) in Tasmania, Australia, by means of

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immunohistochemical analyses (Mason et al., 1991). In South Africa, Graczyk et al. (1996) also detected antibodies against *T. gondii* in African penguins (*Spheniscus demersus)* at a rehabilitation center. A fatal case of toxoplasmosis was also described in three captive African penguins in the Netherlands (Ploeg et al., 2011). Ratcliffe and Worth (1951) found *T. gondii* lesions in one Magellanic penguin and in six African penguins after they were imported into a zoo in the United States, but Fleischmann et al. (1968) considered that the lesions described by those authors might have been related to *Plasmodium* and might have been due to avian malaria.

In Brazil, using a modified agglutination test (MAT $\geq$20), antibodies to *T. gondii* were detected in captive Magellanic penguins, with a prevalence of 28%. However, the majority of the birds (72%) had been kept in captivity for many years and it was impossible to conclude whether infection had occurred before or after they arrived in captivity (Gennari et al., 2016a).

The objective of this study was to determine occurrences of antibodies against *T. gondii* and *Leptospira* spp. in *Spheniscus magellanicus* on Magdalena Island, from where no previous data on these agents were available.

2. Material and methods

In January 2011 and 2015, serum samples were collected from 132 penguins (*Spheniscus magellanicus*) on Magdalena Island. This island forms a nature reserve called “Monumento Natural Los Pinguinos” in the Strait of Magellan, near Punta Arenas, southern Chile (52°55′10.0″ S; 70°34′37.7″ W). Blood was collected through puncturing the jugular or cephalic vein and the serum samples were kept at −20 °C until analyses.

The fieldwork on Magdalena Island was conducted under license no. 645/2009, issued by the National Forests Corporation (Corporación Nacional Forestal; CONAF), and under permit no. 3283 issued by the National Fisheries Service (Servicio Nacional de Pesca; SERNAPESCA), Chile.

It was not possible to determine the penguins' sex, since no genetic tests were used. The birds in the present study were all adults, according to their plumage color and size (Boersma et al., 2013).

Antibodies against *T. gondii* were detected using the modified agglutination test (MAT) (Dubey and Desmonts, 1987). The serum samples were initially screened at 1:20 dilution and the positive samples were then end-titrated. Positive and negative serum samples from chickens were used as controls. The MAT is considered to be the most sensitive test for detecting *T. gondii* antibodies in animal serum samples and, based on studies on bird species, an antibody titer of 20 was considered indicative of *T. gondii* exposure among penguins (Dubey et al., 2016; Gennari et al., 2016a,b).

Detection of anti-*Leptospira* spp. antibodies was performed using the microscopic agglutination test (MAT-Leptospira). This was done in accordance with the standard procedure recommended by the World Organization for Animal Health (OIE) (OIE, 2012), using the 23 serovars listed in Table 1. The serum samples were first tested with the whole antigen collection, at a dilution of 1:100, and then titrated with the reacting antigens in a double dilution series. The titer for positive reactions was the reciprocal of the highest serum dilution in serum-antigen mixtures that showed 50% agglutinated leptospires per microscopic field.

3. Results

Overall, antibodies against *T. gondii* were found in 57 (43.18%) of the 132 serum samples, with titers that ranged from 20 to 320 (Table 2). None of the penguins in this study was reactive to anti-*Leptospira* spp. antibodies.

4. Discussion

The MAT used in the present study for detection of *T. gondii* antibodies is the most-used test for wild animals. It presents good sensitivity and specificity and does not require species-specific conjugates. This last attribute is very useful for studies on wild species (Dubey, 2010). Although not yet validated for detection of *T. gondii* in penguins, it has already been widely used for other aquatic or non-aquatic species of birds (Dubey, 2010; Deem et al., 2010; Gennari et al., 2016a,b). Although Dubey et al. (2016) isolated *T. gondii* from chickens using a 1:5 titer in a MAT in a validation study, the cutoff of 1:20 that was used in the present study was suggested by Gennari et al. (2016a). The latter authors considered that 1:20 was a more conservative dilution.

In the present study, based on a MAT with titer $\geq$20, the seroprevalence among the birds reached 43.18% (57/132). This proportion is higher than what was found by Deem et al. (2010), based on a MAT with a cutoff of 50 that was conducted on samples from free-living Galapagos penguins (*Spheniscus mendiculus)* in Ecuador. If the cutoff of the present study had been 1:80, i.e. higher than what was used by Deem et al. (2010), the occurrence rate would have continued to be higher (21%) than what was found among the penguins in Galapagos Island (2.3%).

Although Magdalena island is considered to be cat-free, there is very frequently a human presence, consisting of researchers. The chances of bringing sporulated *T. gondii* oocysts into this environment therefore exists.

Our results from adult penguins were also 32% higher than the occurrence rate of 28% found by Gennari et al. (2016a) among captive *S. magellanicus*. However, 55% of the birds from their study were juveniles, and all of those birds were caught on the Brazilian coast, having originated from Argentina (primarily) and Falklands (secondarily) than from Chile.

Magellanic penguins feed primarily on fish and squid, while crustaceans are of minor importance. All of these three components of Magellanic penguins' diet vary in their proportions over consecutive breeding seasons (Pütz et al., 2001).

Oocysts of *T. gondii* can sporulate and survive in seawater for several months (Lindsay et al., 2003; Fayer et al., 2004). Marine mammals in distinct groups (cetaceans, pinnipeds and sirenians) and waterfowl can become infected through consumption of water containing the oocysts. Cole et al. (2000) suggested that *T. gondii* oocysts from cat feces could enter the marine environment and contaminate both the water and many species of invertebrates, and that these could function as transportation hosts to mammals and waterfowl. Lindsay et al. (2001) were able to infect mice that were experimentally fed with *T. gondii*-contaminated oysters (*Crassostrea virginica*), and they proved that *T. gondii* can survive for several months in these oysters (Lindsay et al., 2004).

Bigot-Clivot et al. (2016) proved that freshwater crustaceans can bioaccumulate *T. gondii* oocysts, and crustaceans are part of penguins' food chain. Massie et al. (2010) experimentally infected anchovies and Pacific sardines with *T. gondii* oocysts and observed that migratory filter feeders serve as biotic vectors for this parasite.

A study conducted solely on Magdalena Island during the breeding season 1996/97 examined the stomach contents of Magellanic penguins and found that these consisted exclusively of sprats (*Sprattus fuegensis*) (Radl and Culik, 1999). Recently, in a study conducted in Golfo San Jorge, Argentina, a total of 112 adult Magellanic penguins were flushed and at least 15 prey taxa were found, including fish, cephalopods, crustaceans and polychaetes (Yorio et al., 2017)

This was the first study to investigate anti-leptospiral antibodies in penguins and, as in other studies that have analyzed the presence of anti-*Leptospira* antibodies in birds in Latin America, all the penguins were seronegative (Cordeiro et al., 1981; Everard et al., 1983 apud Vieira et al., 2018). However, birds have already been identified as renal carriers of *Leptospira* (Jobbins and Alexander, 2015), and marine animals have also been identified through serological tests (Colegrove

Table 1
Antigens of the genus *Leptospira* sp. used in the microscopic agglutination test, listed according to species, serogroup, serovar and strain.

Leptospira species	Serogroup	Serovar	Strain
L. interrogans	Australis	Australis	Ballico
	Autumnalis	Autumnalis	Akyiami
	Bataviae	Bataviae	Van Tienem
	Australis	Bratislava	Jez Bratislava
	Canicola	Canicola	Hond Utrecht IV
	Icterohaemorrhagiae	Copenhageni	M-20
	Icterohaemorrhagiae	Icterohaemorrhagiae	RGA
	Sejroe	Hardjoprajitno	Hardjoprajitno
	Hebdomadis	Hebdomadis	Hebdomadis
	Pomona	Pomona	Pomona
	Pyrogenes	Pyrogenes	Salinem
	Djasiman	Sentot	Sentot
L. borgpetersenii	Ballum	Castellonis	Castellon −3
	Sejroe	Hardjobovis	Sponselee
	Javanica	Javanica	VeldradtBataviae - 46
	Tarassovi	Tarassovi	Perepelitsin
	Celledoni	Whitcombi	Whitcombi
L. kirschneri	Cynopteri	Cynopteri	3522C
	Grippotyphosa	Grippotyphosa	Moskva-V
	Autumnalis	Butembo	Butembo
L. santarosai	Sejroe	Guaricura	BOV- 6
	Shermani	Shermani	1342-K
L. noguchi	Panama	Panama	CZ −214

Table 2
Frequency of occurrence of seropositive penguins ($n = 57$) presenting antibodies against *Toxoplasma gondii* in the modified agglutination test (MAT), according to titer.

Antibody titer	N° positive	%
20	32	56.1
40	13	22.8
80	8	14.1
160	2	3.5
320	2	3.5

et al., 2005) and molecular methods (Cameron et al., 2008).

The area where the penguins of the present study were caught is a cold region, with a mean temperature of 4.7 °C (summer 10.5 °C and winter 1.1 °C), which could interfere with the prevalence of *Leptospira* spp. (Jensen and Magnussen, 2016).

This was the first report of *T. gondii* seropositivity in free-living Magellanic penguins (*S. magellanicus*) in Chile.

Conflict of interest

The authors do not have any conflict of interest.

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