Journal of Coastal Life Medicine

journal homepage: www.jclmm.com

Original article doi: 10.12980/jclm.4.2016j5-251

Detection of antibodies against *Toxoplasma gondii* in *Mirounga leonina* Linnaeus, 1758 (Pinnipedia, Phocidae) from Elephant Island

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ARTICLE INFO

Article history:
Received 19 Dec 2015
Accepted 5 Feb 2016
Available online 1 Mar 2016

Keywords:
Antarctic
Hemagglutination
Marine mammals
Parasite
Protozoan
Zoonosis

ABSTRACT

Objective: To investigate the presence of antibodies against *Toxoplasma gondii* (*T. gondii*) in *Mirounga leonina* (*M. leonina*) Linnaeus from Elephant Island, Antarctica.
Methods: The animals were anesthetized, restrained, measured, weighed and had their blood collected by venipuncture of intervertebral lumbar epidural vein. Blood samples were collected from 102 individuals. Indirect hemagglutination and serum dilution at a proportion of 1:25 was used for specific immunoglobulin G anti-*T. gondii* detection.
Results: Only one (0.98%) specimen, a newly weaned calf, was seropositive.
Conclusions: This study is the highest serological survey for antibody detection against *T. gondii* in *M. leonina*. The results suggest a low level of exposure to *T. gondii*, probably due to the absence of felids in the study area. The seropositivity presented by the elephant seal may be related to the presence of oocysts in water or cysts in their preys. Despite being reported the presence of the parasite in fish and molluscs, there are no records of tissue cysts or oocysts in squid or fish of the diet of *M. leonina*. Thus, further parasitological studies focused on preys of elephant seals are needed for a better understanding of infection of *M. leonina* by *T. gondii*.

1. Introduction

The protozoan *Toxoplasma gondii* (*T. gondii*) has a worldwide distribution and is parasitic on a wide variety of domestic and wild animals, including humans[1,2]. As a parasite of wild species, *T. gondii* is incriminated in causing encephalitis in marine mammals in different regions of the planet[3]. The presence of antibodies against *T. gondii* in marine top predators indicates infections of their preys with tissue cysts, or contaminations of the marine environment with oocysts[4]. Marine mammals, including the southern elephant seals [*Mirounga leonina* (*M. leonina)*], are considered good bioindicators of environmental changes[5]. *M. leonina* has circumpolar distribution with breeding colonies in sub-Antarctic islands. The three main populations are concentrated in South Georgia in the South Atlantic with 350 000 individuals; Heard and Kerguelen Islands in the Southern Indian Ocean with 237 000 individuals; and Macquarie Island in the South Pacific with 136 000 individuals[6]. Even at a distance, the negative influences of human activities can affect the health status of marine mammal populations. Elephant seals are also affected, even living in inhospitable environments for humans. For this reason, there is a growing concern in monitoring the health of the antarctic region fauna. Therefore, the aim of the present study was to investigate the presence of antibodies against *T. gondii* in southern elephant seals from Elephant Island, Antarctic Peninsula.

2. Materials and methods

2.1. Animals and sampling

Blood samples were collected from 102 specimens of *M. leonina* on the coast of Elephant Island, Antarctic Peninsula. The animals were anesthetized, restrained, identified, measured, weighed and had their blood collected by venipuncture of intervertebral lumbar epidural vein. An anesthetic protocol was employed by using a

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All experimental procedures involving animals were conducted in accordance to the Scientific Committee on the Antarctic Research (SCAR) guidelines and approved by this Committee (permit #49.0039/98-7).

Foundation Project: Supported by Brazilian National Council of Technological and Scientific Development (CNPq) (Institutional process number 67.0018/98-8).

The journal implements double-blind peer review practiced by specially invited international editorial board members.

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compound of the phencyclidine family (tiletamine) associated with a compound of the benzodiazepine family (zolazepam), on a proportion of 1:1 (Zoletil 50®, Virbac, Brazil). A dose of 1 mg/kg was used intramuscularly[7,8]. During the collection, the palpebral reflexes and awakening from anesthesia were monitored. Blood samples were centrifuged (1500 r/min for 10 min) to obtain serum, and stored at –20 °C until analyzed.

2.2. Serological research

For detection of anti-*T. gondii* antibodies, the technique used was the indirect hemagglutination by using the commercial kit Imuno-HAI (WAMA Diagnóstica®, Brazil). The technique relies on the detection of specific immunoglobulin G in serum by agglutinating with sensitized antigens of *T. gondii*. All samples were tested in triplicate to confirm the results.

3. Results

Antibodies against *T. gondii* were detected in one of 102 samples of *M. leonina* from Elephant Island, representing 0.98% of the total samples. The seropositive sample came from a male individual with 191 kg of weight, 202 cm of total length and 133 cm of chest girth. These data indicated that it was a newly weaned calf.

4. Discussion

Antibodies against *T. gondii* have been detected in several species of marine mammals[5,9-12]. Not only the occurrence of antibodies to *T. gondii* is known, but the clinical toxoplasmosis has been diagnosed in different species such as *Mirounga angustirostris, Callorhinus ursinus, Phoca vitulina richardii, Zalophus californianus, Tursiops truncatus, Stenella coeruleoalba, Tursiops truncatus aduncus, Stenella longirostris, Grampus griseus, Sotalia fluviaatilis guinensis*, *Trichechus manatus, Delphinapterus leucas*[9,10]. Studies with several species of mammals around the world bring relevant information about the presence of the parasite *T. gondii* in the marine environment. The present study is the largest serological survey realized in *M. leonina* for the detection of antibodies against *T. gondii*, and the first one to be performed in the Elephant Island. The hemagglutination is a widely used tool to detect antibodies against *T. gondii* in a variety of marine mammals. Among the different available serological tests, the hemagglutination has the advantage of being used in several species dispensing species specific conjugated antibody, with a high sensitivity and specificity[4,9,13,14].

The results obtained with the hemagglutination technique suggested a low level of exposure to *T. gondii* in this population of elephant seals. Different results were found by Rengifo-Herrera *et al.*[5], which reported 76.9% seropositivity in the same species by employing the same techniques and diagnostic dilution of serum. However, these authors had analyzed only 13 samples collected in the Shetland Islands and Antarctic Peninsula. In another work conducted in southern elephant seals at Macquarie Island (south of New Zealand), 11 of 48 animals (22.9%) showed agglutination to antibodies against *T. gondii* at 1:40 dilution, which indicated that these animals were exposed to the protozoan. The Macquarie Island is a nature reserve inhabited by feral cats (*Felis catus*) which facilitates the contact with the protozoan and may explain the elephant seals infected with *T. gondii*[15]. Differently from Macquarie Island, the presence of the definitive hosts of *T. gondii* is not reported for the Elephant Island (Antarctic Peninsula). This fact could explain the low occurrence of seropositive animals in our study. For this reason, the cycle of the parasite does not have its full development in the target area of this study. Even in Antarctica, in serological survey of penguins (*Pygoscelis adeliae, Pygoscelis papua* and *Pygoscelis antarctica*), no antibodies against *T. gondii* were detected, which confirmed that there is a low prevalence of the parasite in the region[16].

Genetic and migration studies reveal that there is no significant gene flow among populations of *M. leonina*, because the species shows strong philopatry behavior. However, southern elephant seals have been found in remote regions of his native colony reaching the mainland, such as Uruguay and Brazil[17]. Therefore, these individuals may be contaminated from pollution sources originated from the continent. The manner in which interaction occurs with *T. gondii* and parathenic host is not fully understood at marine environment. At times of flooding in coastal regions, the contamination of the seas by oocysts and the flow of contaminated water is acknowledged[9]. Lindsay *et al.*[18] demonstrated the ability of *T. gondii* sporulated oocysts remained infective for six months at a temperature of 4 °C in seawater.

The presence of singly sporulated oocyst in the sea does not guarantee the permanence of the parasite in this environment, since marine mammal species rarely ingest seawater. When they do it accidentally, it is in small amounts. Marine mammals get the water they need from their preys[19]. *Engraulis mordax* and *Sardinops sagax* are fish species which are able to ensure the continuity of *T. gondii* in marine environment, as they act as concentrators and disseminators of oocysts, and they are part of the diet of many marine mammals[20]. Invertebrate organisms such as molluscs also act as phoretic agents[18]. The molluscs cephalopods are the main components of the diet of *M. leonina*. The main species consumed by southern elephant seals have restricted antarctic circumpolar distribution. *Psychroteuthis glacialis* is the most abundant species of molluscs at the coast of the antarctic continent[21-23]. A small portion of the diet of southern elephant seals is composed by fish species[21,24]. According to the dietary habits of elephant seals, it is possible that they ingest preys infected by *T. gondii*.

The seropositivity presented by young newly weaned southern elephant seal analyzed in the present study may be related to the presence of oocysts in water or cysts in their food items. Transplacental and transmammary transmission are not ruled out in this case, since it was not possible to capture the mother of this animal for the detection of antibodies against *T. gondii*. This possibility should be considered, since sea otters have been proven congenital transmission[3]. Despite being reported the presence of the parasite in fishes and molluscs, there are no records of tissue cysts or oocysts adhered to the gills or any other part of the body of squids, and cysts in the muscles of fishes which constitutes the diet of *M. leonina*. Therefore, parasitological studies targeting species eaten by elephant seals should receive greater attention in the future.

Young specimens such as the seropositive southern elephant seal analyzed in the present study perform, on average, more trips to the marine environment for food as compared to adults, but the trips are not so far from the shore and occupy a much smaller area[25]. For this reason, the exposure to *T. gondii* probably has been occurred in the vicinity of Elephant Island, which revealed a local water contamination or infection of coastal ichthyofauna and/or malacofauna populations.

The wildlife of Antarctica has no felines in its composition. This fact indicates that southern elephant seals in this region should be being infected from the consumption of squids, fishes and small crustaceans containing cysts or oocysts of *T. gondii* served by the water. In 1991, the Madrid Protocol on Environmental Protection to the Antarctic Treaty, prohibited the introduction of any species to preserve the native wildlife from the possible introduction of diseases[5]. The fulfillment of the Madrid Protocol indicates that elephant seals may reflect the status of the spread of *T. gondii* through the marine environment. The distance of definitive host ensures for these animals the condition of biomarkers for the presence of the parasite in their regions of occurrence. A significant increase in cruise ship traffic and human activity can potentially act as a source of introduction of *T. gondii* in new areas, such as in Antarctica[15]. The effect of these human activities can be evaluated with studies focused on the detection of antibodies against *T. gondii* in marine mammals at Antarctica. These studies should be conducted more frequently to obtain more information about the distribution of the parasite in order to avoid the presence and permanence of *T. gondii* in the Antarctic fauna.

Even only one animal showing detectable levels of immunoglobulin G against *T. gondii*, this result is very important, because it reveals that even remote regions in the ocean, such as the Elephant Island, are achieved to the protozoan life cycle. This result also serves as a warning to the increase and advancement of oceanic pollution with waste arising from urban centers, which is able to spread *T. gondii* oocysts in the most diverse ecosystems of the planet.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments

This work was supported by Brazilian National Council of Technological and Scientific Development (CNPq) and is registred by the institutional process # 67.0018/98-8.

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