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Short communication

# Seroepidemiology and risk assessment of *Toxoplasma gondii* infection in captive wild birds and mammals in two zoos in the North of Portugal



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### ABSTRACT

Toxoplasmosis, caused by the protozoan *Toxoplasma gondii*, is one of the most widespread zoonoses in the world. It can affect most warm-blooded animals but only felids are its definitive hosts. We determined seroprevalence and associated risk factors in birds and mammals kept in two zoological parks in northern Portugal. Sera from 77 birds and 42 mammals were assayed for the presence of *T. gondii* antibodies by the modified agglutination test (MAT, cut-off titre 20); 34.5% (41/119) were seropositive. All seropositive animals were apparently healthy except one seropostive mandarin (*Aix galericulata*) which had chorioretinitis. This is the first report on *T. gondii* seroprevalence in wild animals in captivity in Portugal. The present findings indicate a widespread exposure of zoo animals in Portugal to *T. gondii*.

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# 1. Introduction

*Toxoplasma gondii* is a global zoonotic protozoan that infects most warm-blooded animals, including human beings. Domestic and wild felids are the only recognized definitive hosts of the parasite and play an essential role in the dissemination of infection (García-Bocanegra et al., 2010). Toxoplasmosis is an important risk for public health, causing life-threatening illness in developing foetuses and in immunocompromised people (Dubey, 2010). Infection may occur by the ingestion of sporulated oocysts from the environment, by consumption of infected tissues or by transplancental transmission (Dubey, 2010). Herbivore intermediate hosts acquire infection by ingesting food or water contaminated with sporulated *T. gondii* oocysts. Carnivores and omnivores may additionally become infected mainly by ingesting meat containing cysts (Lopes et al., 2011).

*T. gondii* infections in animals kept in zoological parks are of clinical and public health importance. Many species of mammals (e.g.,

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Pallas cats and Australasian marsupials) and birds (canaries and finches) die of clinical toxoplasmosis. Excretion of *T. gondii* oocysts by felids housed in zoos or feral cats roaming in zoos, can expose the staff, and visitors (including children) to *T. gondii* infection (Dubey and Jones, 2008; Jones and Dubey, 2010).

Here we report prevalence of antibodies to *T. gondii* and associated risk factors in birds and mammals from two zoological parks in the North of Portugal, first such study from Portugal.

## 2. Material and methods

## 2.1. Animals and samples

Blood samples were collected from animals in two zoological parks located in the municipalities of Maia (Zoo A;  $41^{\circ}13'58''N$ ,  $08^{\circ}37'18''W$ ) and of Vila Nova de Gaia (Zoo B;  $41^{\circ}09'27''N$ ,  $08^{\circ}53'75''W$ ), district of Oporto, North region of Portugal (Fig. 1), between November 2014 and March 2015.

Information on gender, age group (juvenile or adult), country of origin (Portugal or abroad), type of food received and clinical status (apparently healthy or clinically sick) was collected. Age was estimated on the basis of size, weight, coat features and tooth wear and eruption pattern (Saénz-de-Buruaga et al., 1991). Estimated ages of birds ranged from 2 to 20 years and those of mammals from



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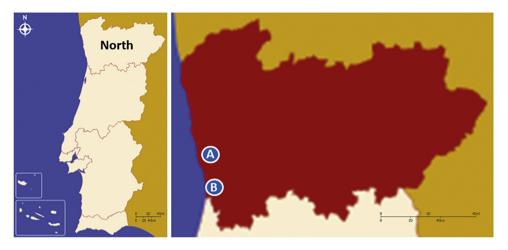


Fig. 1. North region of Portugal and location of the two zoological parks studied. The distance between Zoo A and Zoo B is 20 km (adapted from Wikipedia).

3 months to 15 years. Animals were classified as juvenile or adult depending on their species and specific sexual maturity.

Blood (0.5 ml) samples were collected by venipuncture of manually restrained or sedated animals. Following centrifugation of clotted blood, serum aliquots were stored at -20 °C until analysis. A total of 119 animals from 30 genera (18 avian and 12 mammalian) and 38 defined species (23 avian and 15 mammalian), including two unspeciated mammals were tested (Tables 1 and 2).

#### 2.2. Serological testing for T. gondii antibodies

Serum samples were tested for IgG antibodies to T. gondii at serial dilutions of 1:20 to 1:1280, by using a commercial kit (Toxo-Screen DA<sup>®</sup>, bioMérieux, Lyon, France) as described by Dubey and Desmonts (1987). Positive and negative controls originated from goats were supplied with the kit and included in each testing plate. The results obtained with the MAT were expressed as an antibody titre, i.e. the reciprocal of the highest dilution at which agglutination (at least one half of the well's diameter) was still visible after 5-18 h incubation at room temperature. The cut-off titre of 20 (2 IU/ml in relation to a WHO international reference serum) was chosen to maximize specificity of the test (Zhang et al., 2000; Lopes et al., 2011). The MAT has been used widely for determining antibodies to T. gondii in all hosts, including humans and avian species (Dubey, 2010) because it does not require species specific reagents. The MAT is the only serological test that has been validated in naturally infected pigs and chickens using isolation of viable parasites as the gold standard (Dubey et al., 1995, 2016), and the isolation rate increased with the MAT titre in chickens (Dubey et al., 2016). Because there is no easy way to validate serology results in zoo animals we present all titres in Tables 1 and 2.

#### 2.3. Data analysis

The exact binomial test was used to calculate confidence intervals (CI) for the proportions, with a 95% confidence level. The Chi-square or Fisher's exact tests were used to compare positivity values by independent variables, i.e. class (birds and mammals), gender, age group, country of origin, diet type and clinical status. A probability (p) value <0.05 was considered as statistically significant. Independent variables with a significant difference between categories were subject to multiple logistic regression analysis in order to identify risk factors for *T. gondii* infection (Petrie and Watson, 2013).

#### 3. Results

The seroprevalence of *T. gondii* infection was 22.1% in the 77 birds (Tables 1 and 3) and 57.1% in the 42 mammals (Tables 2 and 4). Seropositive individuals were found in 10 (43.5%) of the avian and 10 (66.7%) of the mammalian species. The overall prevalence of seropositive animals was 34.5% (CI: 26.0-43.7%). Twenty (52.6%) of all the captive wild animal species had seropositive individuals.

The independent variables zoological park and diet type (Table 3) were not confirmed as risk factors for *T. gondii* infection in birds after multiple logistic regression analysis ( $p \ge 0.999$ ).

One adult female mandarin (*Aix galericulata*) had lesions of chorioretinitis compatible with ocular toxoplasmosis. This bird had an antibody titre of 80, it was born in Portugal and was only fed on a commercial mixture of corn and seeds.

#### 4. Discussion

The present work revealed antibodies to *T. gondii* in 34.5% of the captive wild warm-blooded animals from two zoological parks in the North of Portugal. Although some species were only represented by one individual, this is the most comprehensive assessment of *T. gondii* infection in the captive wild fauna in this country. In the present study, more than half (52.6%) of all captive wild species studied had at least one seropositive animal confirming that this parasite can infect a wide variety of warm-blooded animals.

Currently, there are no commercial serological tests to distinguish between infections acquired through the consumption of cysts in meat or through the ingestion of sporulated oocyst. Most of the evidence is based on epidemiological investigations and prevalence studies in animals (Dubey, 2010). A comparison of results between different studies is difficult due to factors related to the host, and the characteristics of the test used (Dubey et al., 1995). Information on *T. gondii* prevalence in zoos worldwide was reviewed previously and is not repeated here (Dubey, 2010).

The current work showed that almost a quarter (22.1%) of the captive birds were seropositive. A higher seroprevalence of 50% was reported in wild birds by Lopes et al. (2011). Most seropositive birds had titres of 40 (41.2%) and only one bird (5.9%) had a titre of  $\geq$ 1280. These results are different from those observed in the study by Lopes et al. (2011) in which the titre 20 was the most frequent.

Out of 23 bird species (Table 1), 12 (52.2%) were found seropositive, a fact which suggests a considerable susceptibility to *T. gondii* infection of the avian specimens included in the study. However, Download English Version:

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