Veterinary Parasitology 200 (2014) 8–12

Contents lists available at ScienceDirect

Veterinary Parasitology

journal homepage: www.elsevier.com/locate/vetpar

# *Toxoplasma gondii* prevalence in cats from Lisbon and in pigs from centre and south of Portugal

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ARTICLE INFO

*Article history:*
Received 18 June 2013
Received in revised form 1 November 2013
Accepted 19 December 2013

*Keywords:*
Toxoplasmosis
*Toxoplasma gondii*
Cats
Pigs
Seroprevalence
Molecular analysis

ABSTRACT

Toxoplasmosis is an important zoonosis worldwide. Here we determined the presence of *Toxoplasma gondii* antibodies in sera and *T. gondii* DNA in faeces of 215 domestic cats from veterinary clinics in the Lisbon area; 44 (20.5%) had anti-*T. gondii* IgG antibodies by the modified agglutination test (cut-off 1:40) and DNA was detected in 16 (35.6%) of 45 cat faeces tested. Risk factor analysis indicated increase of seroprevalence with age of the cats. Sera and tissues of 381 pigs from a slaughterhouse were also tested for *T. gondii* infection; 27 (7.1%) of the 381 pigs were seropositive. *T. gondii* DNA was demonstrated in diaphragms and/or brains of seven (35.0%) of 20 anti-*T. gondii* seropositive pigs tested by the B1 nested-PCR. Results indicate very high prevalence of *T. gondii* DNA in the faeces (oocysts) of definitive hosts and relatively low, but still worrying, seroprevalence of *T. gondii* antibodies in pigs destined for human consumption.



## 1. Introduction

Toxoplasmosis is a worldwide zoonosis. The importance of *Toxoplasma gondii* infection in domestic cats is due to their role as definitive hosts in the parasite's life cycle and to their proximity to humans, as pets. Several studies strongly suggest that the consumption of raw or undercooked pork contaminated with *T. gondii* cysts is a major source of *T. gondii* transfer to humans (Hill and Dubey, 2002; García-Bocanegra et al., 2010; Torrey and Yolken, 2013).

The aims of the present study were to survey the seroprevalence of anti-*T. gondii* antibodies as well as the presence of *T. gondii* DNA in biological samples obtained from domestic cats in the Lisbon area, and from pigs raised in the centre and south of Portugal for human consumption.

## 2. Materials and methods

### 2.1. Sampling

Between October 2007 and March 2008 samples from 215 domestic cats (*Felis catus*) were collected during routine veterinary practice, in Lisbon, Portugal. Data on gender, age and inhabited floor (ground floor, in which there is an increased accessibility and potential contact with the outside; upper floors between 1 and 15) were collected. A total of 215 serum samples (2 ml each), 45 faecal samples (3 g each) (collected from litter box) and 15 brain tissue samples (10 g each) (obtained through necropsy of cats died of various causes, without suspicion of *T. gondii* infection) were

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0304-4017/$ – see front matter © 2014 Published by Elsevier B.V.
http://dx.doi.org/10.1016/j.vetpar.2013.12.017

collected from the cats included in the study. The samples collected had origin in domestic cats of people living in the urban area of Lisbon. These cats usually do not have access to outdoors; however most of them were adopted from litters of stray cats. Domestic cats are usually fed with dry commercial food, although sometimes owners offer them canned food, as well as, in some cases, raw meet.

Samples from 381 pigs (*Sus scrofa*), collected between November 2007 and June 2008 during slaughter in the Regional Slaughterhouse of Alto Alentejo, Sousel, Portugal (38 57 N, 7 40 W), were studied for *T. gondii* infection. These animals were raised in 16 properties, from central and southern regions of Portugal. Data on gender, age group (juveniles, 6–12 months; young adults, 12–24 months), breed and farm management practices (intensive or extensive pig farming) was collected. A total of 381 serum samples (10 ml each), 218 diaphragm samples (50 g each) and 122 brain tissue samples (50 g each) were obtained from the pigs for *T. gondii* infection study.

Blood samples were centrifuged and serum was recovered and stored at −20 °C. The other samples (faeces and tissues) were collected and transported on ice to the laboratory, processed and stored at −20 °C. Faecal samples were processed by modified Ritchie's method (Casemore et al., 1985). Faecal samples (3 g) were solved in ether (2 ml) and water (10 ml), then filtered with two layered gauze and centrifuged (735 × *g*, 5 min). The supernatant was centrifuged again (2205 × *g*, 10 min) and the sediment was recovered and stored at 4 °C for further analysis. Tissue samples (brains and diaphragms) were macerated, homogenised, digested with trypsin (0.2% (w/v) in PBS, 3 h) and then centrifuged and washed three times with PBS (3000 × *g*, 10 min).

### 2.2. Serological screening

A modified direct agglutination method (MAT) (Toxo-Screen DA, Biomérieux® SA) was used for detection of anti-*T. gondii* IgG. All samples were tested in duplicate at the dilutions of 1:40, 1:60 and 1:180. Serum with a titre of 1:40 or higher was considered positive. Results were expressed by the antibody titre (reciprocal of the highest dilution agglutination). Anti-*T. gondii* IgM detection was performed using an immune sorbent agglutination assay (Toxo-ISAGA, Biomérieux® SA). All samples were tested in duplicate at the dilutions of 1:100, 1:150 and 1:200. Serum samples from animals with positive anti-*T. gondii* IgM were tested for IgG to the 1:4860 dilution.

### 2.3. Molecular analysis

Total DNA was extracted by the Mini-BeadBeater/guanidinium thiocyanate-silica method (Alves et al., 2001). In cats, DNA was extracted from the 45 faecal samples and 15 brain samples. In pigs, DNA was obtained from 20 diaphragms and brain tissues of the *T. gondii* seropositive animals and from 42 diaphragms and brain tissues randomly selected from 42 *T. gondii* seronegative animals. Briefly, 1 ml of processed faecal or tissue sample was centrifuged (20,000 × *g*, 15 min) and 300 μl of the sediment was added to 900 μl of lysis buffer (guanidinium thiocyanate 7 M, Tris–HCl 50 mM pH 6.4, EDTA 25 mM pH 8.0, Triton X-100 1.5% (v/v)), 60 μl of isoamyl alcohol and 0.3 g of 0.5 mm diameter zirconia beads. The samples were shaken in a Mini-BeadBeater (Stratech Scientific) at maximum speed (2 min). The supernatant was recovered and mixed with 25 μl of a coarse activated silica suspension (1%, w/v, pH 2.0) and incubated at room temperature under gentle shaking (1 h). The mix was centrifuged (20,000 × *g*, 1 min) and the supernatant was removed. The silica pellet was washed twice with 200 μl of wash buffer (guanidinium thiocyanate 7 M, Tris–HCl 50 mM pH 6.4), once with 200 μl of ethanol (80%, v/v) and 200 μl of acetone. The pellet was dried (55 °C, 10 min) and DNA was eluted after suspension and incubation of the silica pellet in 60 μl of sterile water (70 °C, 10 min). Finally, the supernatant with DNA was recovered and stored at −20 °C after centrifugation (20,000 × *g*, 2 min). DNA detection was performed by nested-PCR directed to the *T. gondii* B1 gene, followed by direct sequencing (Burg et al., 1989; Mason et al., 2010).

### 2.4. Data analysis

A descriptive statistical analysis was performed for qualitative data. Pearson Chi-Square and Fisher's exact tests were applied to investigate associations between qualitative categorical variables at a significance level of 0.05 using SPSS v.20.0 software (SPSS Inc., Chicago, IL, USA).

## 3. Results

### 3.1. Cats

Of the 215 cats studied, 44 (20.5%) were positive for anti-*T. gondii* IgG. Among the 44 *T. gondii* seropositive cats, 4 (1.9%) were positive at dilution 1:40, 6 (2.8%) at dilution 1:180 and 34 (15.8%) at dilution ≥1/180. The distribution of *T. gondii* seropositive and seronegative cats according to their gender, age and inhabited floor are depicted in Table 1. A statistical association between *T. gondii* seroprevalence and age groups was observed. Seroprevalence was undoubtedly higher in older animals ($P < 0.01$). No statistical associations were observed between the results of anti-*T. gondii* IgG test and cats' gender or inhabited floor. *T. gondii* IgM was detected in 2 (0.9%) of the 215 cats (both with ISAGA index of nine according to the Toxo-ISAGA kit instructions); these cats had IgG titre of 1:4860 and *T. gondii* DNA in faeces. *T. gondii* DNA was detected in 16 (35.5%) of the 45 faeces, distributed by 10 (22.2%) anti-*T. gondii* IgG seronegative cats and 6 (13.3%) anti-*T. gondii* IgG seropositive cats. Of the 15 brains analysed by PCR, the one (6.7%) with *T. gondii* DNA belonged to an anti-*T. gondii* IgG seronegative cat that was also positive for B1 gene in faeces. This two years old male cat was adopted from outdoors, just before he died, probably in immunodepression, suggested by severe signs of infectious rhinotracheitis syndrome. Statistical analysis pointed out for absence of correlation between B1 nested-PCR and serologic results.