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Prevalence of *Toxoplasma gondii* infection in cats in southwestern Poland

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Abstract

Objective. An assessment of the prevalence of *Toxoplasma gondii* infection in cats from southwestern Poland using serology, coproscopy and PCR methods.

Materials and method. In total, 208 cats (139 females and 68 males), aged 0.5–12 years (mean=2.6) from 25 localities in southwestern Poland were examined by indirect immunofluorescence assay (IFAT) to estimate the *T. gondii* serological status. Faecal samples of 41 cats were examined for the presence of oocysts/DNA *T. gondii* by microscopy and Real-time/nested PCR. After flotation (with $NaNO_3$), pellets from faecal samples were disrupted by 10 cycles of freezing (liquid nitrogen) and warming. DNA was extracted using Qlamp DNA Stool Mini Kit (Qiagen), according to the manufacturer's instruction.

Results. The positive results in IFAT for anti-*T. gondii* IgG and IgM antibodies were found in 143 of 208 tested cats (68.8%). Among positive results, 14.5%, 34.1% and 51.4% were detected in titre ranges 128–512, 1,000–2,000 and ≥ 4,000, respectively. In 23.1% of cat sera anti- *T. gondii* IgM antibodies were found. The prevalence of anti-*Toxoplasma* antibodies was significantly greater in older cats (>1 year) (83.5%) than in younger cats (48.3%) ($P<0.05$), in females (74.1%) than in males (58.8%) ($P<0.05$), and in cats kept outdoors than indoors (69.7% vs. 16.7%) ($P<0.01$). Among the 41 faecal samples examined, the presence of structures resembling *T. gondii* oocysts was found in 2 samples (4.9%), and for one of these samples (2.4% of the total) the result was also confirmed by PCR.

Conclusions. *T. gondii* infection in domestic cats is highly prevalent in southwestern Poland. Information on the prevalence of infection in cats can be useful for assessing *T. gondii* environmental contamination and the risk for public health.

Key words

Poland, PCR, seroprevalence, *Toxoplasma gondii,* IFAT, cats, oocysts, coproscopy

INTRODUCTION

Toxoplasmosis is one of the more common parasitic zoonosis caused by the world-wide distributed protozoan *Toxoplasma gondii* that infects a variety of hosts including humans, domestic and wild animals [1]. *Toxoplasma gondii* infection, may pose a severe medical problem in congenital form, as cerebral and ocular damage in newborns, and as an acquired infection in immunocompromised humans and animals. The invasion may have an opportunistic character in relation to the host (i.e. in AIDS patients) [1, 2]. People usually become infected by *T. gondii* through consumption of undercooked meat containing parasite cysts, or by food and water contaminated with the parasite [2]. The ingestion of vegetables, fruit or water contaminated with sporulated oocysts has been considered the common means for acquisition of toxoplasmosis [3]. Human outbreaks of toxoplasmosis linked to drinking water contaminated with land runoff have been reported [4]. Soil contact and frequent gardening have also been identified as a risk factors for *T. gondii* infection [5, 6].

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Domestic cats and other Felidae, in which in epithelial cells of the small intestine the sexual reproduction of parasite occurs, there are the only definitive hosts of *T. gondii*, and they play a major role in the epidemiology of toxoplasmosis, shedding infective oocysts in faeces [2]. Infection of *T. gondii* in cats is usually asymptomatic; however, cats can also be an intermediate host for the parasite with the occurrence of clinical signs [7]. Clinical cases of *T. gondii* infection were mostly reported in kittens; the signs of infection included fever, anorexia, lethargy, ocular inflammation, abdominal discomfort and neurologic abnormalities [8]. Cats can become infected by eating the carcasses of small mammals (including mice and rats), birds infected with *T. gondii*, or by ingesting oocysts from the environment. The majority of cats infected primarily with tissue cysts, shed oocysts after a prepatent period of 3–10 days, with patency lasting up to 20 days. By contrast, about one-third of cats infected with oocysts shed the oocysts after a longer prepatent period of 18–49 days, for up to 10 days. Sporulated, infective oocysts (1–5 days after excretion by cat) are extremely resistant and under moderate conditions may survive for months, or even years in the environment [1]. Because infected cats excrete oocysts for a short period and mostly once in their lifetime, this causes difficulty in detecting *T. gondii* oocysts during single faecal examination [9]. Thus, the serological examination can be useful for determining the scale of *T. gondii* infection in the cat population. Previously, high *T. gondii* seroprevalence in

goats (70%) [10], pigs (10.4%) and cattle (19.3%) [11] were reported in the region of present study (Silesian province); human cases of toxoplasmosis were also reported from this area [12].

OBJECTIVE

The aim of present study was to evaluate the occurrence of *T. gondii* infection in cats from southwestern Poland (Silesia) using serology, coproscopy and PCR methods, to assess the potential environmental contamination by the parasite, and the threat for human and animal health.

MATERIALS AND METHOD

Region of study. The Silesian province is located in southwestern Poland (Fig. 1) and constitutes nearly 3.9% of the country's area. The region is inhabited by almost 5,000,000 people, living in a high density of 393 people/km^2. The region covers an area of varied landscape, from mountains through the woodlands to the urbanized areas. The region has numerous water reservoirs which supply water to the cities and towns.

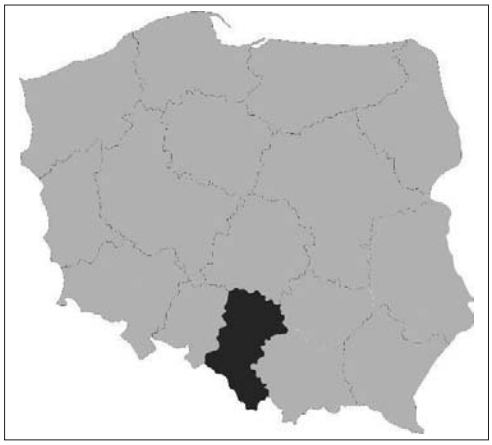

Figure 1. Location of the study area in Poland

Data collection. Questionnaires were collected with data provided by animals owners about age, gender, breed, outdoor access, type of location (urban/rural), and diet.

Serological examination. In total, 208 European breed and cross-bred cats – 139 females and 68 males (data of gender for one cat was unavailable), aged 0.5–12 years (mean=2.6) from 25 localities in southwestern Poland were examined. Sera were obtained by venipuncture of the brachial or jugular vein, left to clot, and after centrifugation (2,100 × g for 10 min) kept at the temperature of –20 °C until analysis.

For the detection of *T. gondii* IgM and IgG antibodies, sera of cats were examined using immunofluorescence antibody test (IFAT). Twenty microliters of each serum diluted in PBS was applied to slides coated with whole tachyzoites of *T. gondii* RH strain as antigen (BioMerieux), and incubated in a humid chamber for 30 min at 37 °C. Slides were washed with PBS (2 × 5 min) and incubated in a humid chamber for 30 min at 37 °C with 20 µl of FELINE IgM and IgG FITC anti-immunoglobulin conjugates (VMRD, Inc., USA). After washing (as described above) and drying, the slides were viewed under a UV microscope (Olympus BX51). The cut-off of the fluorescence test was established at the dilution of sera – 1:128 and 1:16 for *T. gondii* IgG and IgM positive feline origin controls, respectively (VMRD, Inc., USA). The bright green colour of the tachyzoites surface under ultraviolet light was considered a positive result.

Faecal examination. Stool samples of 41 cats were collected and examined. Two grams of each sample were placed in a test-tube (15 ml), mixed with 0.7 ml redistilled H_2O and homogenized. Subsequently, a seven-fold volume of flotation fluid $NaNO_3$ was added to the tube, after which the tube was left for 20 minutes. In the next step, the surface liquid of the suspension (150–200 µl) was taken using a Pasteur pipette and placed into an Eppendorf tube. Each pellet was divided into 2 parts, of which one part was examined by microscopy, while the second was used for DNA extraction.

DNA was extracted using the commercial set QIamp DNA Stool Mini Kit (Qiagen) according to the producer's instruction, followed by 10 cycles of freezing (in liquid nitrogen) and warming (in a water bath at 60 °C) in order to disrupt the oocysts. DNA samples were examined by Real time PCR according to the method described by Lin et al. [13], and by nested PCR using the method described by Grigg and Boothroyd [14].

Statistical analysis. The results were analysed with $\chi 2$ test, using STATISTICA v. 5.1 package (Statsoft, Tulsa, OK, USA).

RESULTS

Serology. In IFAT, positive results for IgG and IgM antibodies were found in 143 of 208 tested cats (68.8%). Among IgG positive results (66.3%), 14.5%, 34.1% and 51.4% were detected in titre ranges 128–512, 1,000–2,000 and ≥ 4,000, respectively. *T. gondii* specific IgM antibodies occurred in 23.1% of cats (Tab. 1). Five cats showed only IgM, but no IgG antibodies, 43 cats showed both, IgM and IgG antibodies, whereas in 95 cats, IgG antibodies only were detected. (Fig. 2). Among cats with IgM positive results, the mean age of cats with low titre range (128 -512) of IgG was significantly lower (mean 1.69, SD=0.8) than the mean age of cats with higher IgG titre range (≥1,000) (mean 3.69, SD=3.1) (P<0.001).

Statistical analysis. It demonstrated the significantly greater prevalence of overall (IgG and IgM) anti-*Toxoplasma* antibodies in older cats (>1 year) (83.5%) than in younger cats (48.3%) (P<0.05), in females (74.1%) than in males (58.8%) (P<0.05), and in cats kept outdoors than indoors (69.7% *vs.* 16.7%) (P<0.01) (Fig. 2). Prevalence of anti-*T. gondii* antibodies was greater in cats from rural (72.9%) than urban (60.9%) areas, although the difference was not statistically significant (p=0.2687).

Result of stool examination. Among the examined 41 fecal samples of cats, the presence of structures resembling *T. gondii* oocysts was found in 2 samples (4.9%), and for one of these samples (2.4% of the total) the result was also confirmed by Real Time PCR.

Table 1. Percentage of cats sera positive for *T. gondii* in IFAT

No. of examined cats	Positive results								
	IgG		IgG titre ranges (%)			IgM		Total IgG and IgM	
208	N	%	128–512	1,000–2,000	≥4,000	N	%	N	%
	138	66.3	14.5	34.1	51.4	48	23.1	143	68.8

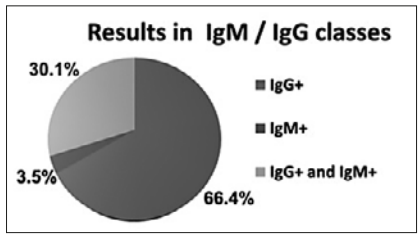

Figure 2. Percentages of positive results of cats in particular class of antibodies

DISCUSSION

The presented study showed 68.8% of seropositive results (IgG and IgM) among cats living in southwestern Poland. This result is similar to those obtained previously in other regions of Poland. A high percentage (70.6%) of seropositive results was reported for cats living in the city of Poznań [15]. Similar results (70.6% and 65.2%) were recorded for cats in the Olsztyn urban area [16, 17] and a little higher percentage (75%) for cats in the Lublin region [18]. A smaller percentage (52.5%) was reported for cats from the southwestern region of Poland [19].

In Europe, results similar to those presented in the current study were obtained in Belgium (70.2%) [20] and in the Czech Republic (61.3%) [21]. A lower seroprevalence was noted in Hungary (47.6%) [22] and in Italy 33%–50.4% [23, 24, 25]. A few studies have been performed in Spain where the seroprevalence ranged from 32.3%–63.4% [26, 27].

A study performed in Italy, showed results similar to the current study, in that the seropositive results were more frequent among females compared to males, but the difference did not attain a significant level [23]. The difference in seroprevalence between cats living in rural and urban area (72.9% and 60.9%) determined in the present study, may result from the difference in access to reservoirs of the parasite, i.e. prey animals or oocysts in the environment. Contrary to the current results, Papini et al. [23] reported a reverse dependence.

Worldwide, numerous studies have been carried out in cat populations, and depending on the geographical region, the method used, outdoor access of cats, the seroprevalence of *T. gondii* in cats ranges from 2.7%, up to 85% [26, 27, 28, 29, 30]. Higher prevalence was mostly reported in stray than in household cats, and in older than younger cats [19, 23, 27, 28, 31, 32, 33]. The correlation between seropositivity and increasing age of cats recorded in the presented study correspond with the results obtained by other authors [34, 35, 36]; however, a reverse dependence was also found [37, 38, 39].

The high seroprevalence stated in this study among the examined cats (68.8%) can be explained by a large proportion of animals having outdoor access and their possibility to catch prey. Many of cats may be given raw meat in their diet, which also provides an opportunity for infection with *T. gondii*. Results of the study by Knaus and Fehler [40] showed significant differences between strays, with a seroprevalence of 72.5%, and household cats, with 40%. A smaller difference in seroprevalence was reported by Śmielewska-Loś and Pacoń [19] between cats kept in shelters (55%) and indoors (50%). The presented study showed a large difference in seropositivity between outdor (69.7%) and indoor cats (16.7%).

Serological examination is useful for indirect diagnosis of *T. gondii* infection and in epidemiological studies. The differences in titres of positive sera using different assays depends on the antigen profiles presented in each test for antibody detection. IFAT (Indirect Fluorescent Antibody Test) used in the presented study, is considered as a reference method. Comparison of MAT (modified agglutination test) and IFAT results performed by Macri et al. [41] showed a large degree of agreement for tested cat sera.

High anti-*T. gondii* IgM antibody levels suggest an active infection. In the course of *T. gondii* infection, IgM antibodies appear early, within 1–3 weeks, and decline rapidly within 3–16 weeks, while the specific IgG can be detected within 2–4 weeks, and remains detectable for many years. IgM can be present in kittens sera as the result of transplacental or lactational transfer of *T. gondii* infection. IgG alone indicate the chronic stage of infection and their presence suggest that the cat probably no longer excretes the oocysts [1, 42, 43].

In the current study, 23.1% of cats were IgM positive, which may indicate a recent infection, especially in the cases of cats with IgM alone or cats with an accompanying low titre range of IgG antibodies. The mean age of cats suspected of a recent infection was significantly lower in comparison with the mean age of cats with probable distant infection (IgM positive and high titre of IgG). Because IgM are occasionally also detected in the serum of cats with chronic infection, or as a false-positive IgM caused by autoimmune antibodies (rheumatoid factor or antinuclear antibodies) [43], it is likely that not all tested cats with anti-*T. gondii* IgM, had been recently infected. It is noteworthy that more than half of the cats reacting positively in the presented study showed a very high IFAT titre (≥4,000), which seems to indicate an active disease process.

Although cats are the animal reservoir of *T. gondii* infection, only a few reports are available on the *T. gondii* oocysts prevalence in cat faeces. The high *T. gondii* seroprevalence stated in presented study (68.8%) suggests a potentially wide distribution of oocysts in the environment. The seropositive cats are likely to have already shed a great number of *T. gondii* oocysts into environment. Oocysts become infective in the environment within 1–5 days (after sporulation) and can survive for months in soil and water [42]. Humans can become infected by consumption of water, vegetables and fruits contaminated with oocysts, or by ingestion of oocysts after contact with contaminated soil [3, 18, 44, 45, 46, 47]. However, microscopy has a low sensitivity and it is not fully reliable to discriminate between *T. gondii* oocysts and other coccidians [48]. *T. gondii* oocysts are morphologically and morphometrically similar to e.g. *Hammondia hammondi* [49, 50], which can give false positive results in faeces examination. Detection of *T. gondii* oocysts during routine examination of a cat population is rare (with up to several percent). In Italy, Pampiglione et al. [51] reported a 0.4% level of oocysts

prevalence in stray cats revealed by microscopy. In another study in Italy, a 6.6% positivity rate of *T. gondii* oocysts in faecal samples of 60 stray cats was reported [52]. Other studies also showed a low level of *T. gondii* oocysts detection: 1.3% in the Czech Republic, 0.11–1.1% in Germany, 0.3% in The Netherlands, 0.23% in France, 0.4% in Switzerland, 0.9–1.8% in USA and 1.3% in Brazil [53]. In the study performed by Schares et al. [54], microscopical identification was confirmed by PCR, and finally 0.11% of cats were found to shed *T. gondii* oocysts. The results of the presented study, in which only 2 samples of cat stool were identified as positive by microscopy (4.9%), and one sample was found to be positive by PCR (2.4%), seem to correspond with the results obtained by other authors.

CONCLUSIONS

The results of this study show a high seroprevalence of anti-*T. gondii* antibodies, mostly in very high titres of IgG, and a relatively low incidence of *T. gondii* oocysts excretion among the tested cat population from southwestern Poland. Nevertheless, if the fact is considered that infective oocysts are shed by cats only for a short time in their life, sometimes only once, the very high seropositivity stated in the presented study should be regarded as a more reliable index of the potential risk of human toxoplasmosis due to exposure to cats in a given area, as seropositive cats have most probably been either past or future oocyst shedders. In other words, the common presence of *T. gondii* antibodies in cats also suggests a large contamination of the environment by oocysts, which constitute a source of infection for humans and animals. Thus, information on the seroprevalence of infection in cats can be useful for assessing *T. gondii* environmental contamination and the risk for public health.

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