

Serological survey of *Toxoplasma gondii* infections in stray cats from Italy

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SUMMARY

Prevalence of *T. gondii* infection was determined in stray cats by a commercially available direct agglutination test. Overall, 40.7% of serum samples have given positive results. *T. gondii* infection seemed to be more prevalent in females than males (41.3% vs. 39%), in common European Shorthair cats than other breed (88.3% vs. 40%), in <5 year-old cats than older ones (42.3% vs. 38.3%), in cats from urban than suburban areas (45.8% vs. 35.7%), and during spring/summer than fall/winter months (45.8% vs. 35.4%). Despite these trends, differences between groups were not statistically significant by chi-square test. It is concluded that the seroprevalence in stray cats can be a good indicator of the spreading of *T. gondii* in the environment, highlighting the potential threat of infection to humans and other domestic or wild animals.

Keywords : Stray cat - *T. gondii* - Toxoplasmosis - Seroprevalence - Italy.

RÉSUMÉ

Étude sérologique de l'infestation par *Toxoplasma gondii* des chats errants en Italie. Par R. Papini, C. Sbrana, B. Rosa, A.M. Saturni, A.M. Sorrentino, M. Cerretani, G. Raffaelli et G. Guidi.

La prévalence de l'infestation par *Toxoplasma gondii* a été déterminée chez des chats errants par un test d'agglutination directe disponible dans le commerce. Au total, 40.7% des sérums ont donné des résultats positifs. L'infestation par *T. gondii* a semblé plus fréquente chez les femelles que chez les mâles (41.3% vs. 39%), chez les chats Communs Européens que chez les autres races (88.3% vs. 40%), chez les animaux de moins de 5 ans que chez les animaux plus âgés (42.3% vs. 38.3%), chez les chats vivant en milieu urbain que chez ceux vivant en milieu sub-urbain (45.8% vs. 35.7%), et pendant la période printemps/été que pendant la période automne/hiver (45.8% vs. 35.4%). Malgré ces tendances, les différences entre les groupes n'étaient pas significatives par le test du χ^2 . On peut en conclure que la séroprévalence des chats errants est un bon indicateur de l'étendue de l'infestation par *T. gondii* dans l'environnement, et reflète un risque potentiel d'infestation pour l'homme et les autres animaux domestiques ou sauvages.

Mots-clés : Chat errant - *T. gondii* - Toxoplasmose - Séroprévalence - Italie.

Introduction

T. gondii is definitely known as a cosmopolitan, zoonotic, protozoan parasite of cats with a very wide host range. Its life cycle, epidemiology, routes of transmission to animals and humans, as well as its pathogenicity in definitive, intermediate, and accidental hosts have been well established, as recently reviewed by TENTER *et al.* [22]. Numerous reports from various countries exist on the prevalence of *Toxoplasma* infections in stray cats, and infection rates using various serological tests have ranged from 10% to 70.2% [6, 20]. In Italy, serologic studies have shown widespread cases of infections in human beings [10, 17]. Also, serological evidence of toxoplasmosis in cattle, buffaloes, pigs, and dogs [22], as well as in wild animals [2], sheep and goats [12] has been reported in this country, demonstrating the endemicity of the infection. Despite this, the epidemiology of toxoplasmosis has not been extensively investigated in stray cats and, after searching in literature data bases, we found that only a few reports are available on the distribution and prevalence of the infection in this animal reservoir. The last of them remounts to almost ten years ago [4]. In addition, almost all serologic surveys on *T. gondii* have so far been made on limited numbers of stray cats. The prevalence of *T. gondii* infec-

tion reported in these definitive hosts has been ranging from 0.4% to 50.4% in various parts of Italy [11, 16]. However, population of stray cats is getting increased in this country and epidemiological aspects may be changing in last years. Therefore, little is actually known for toxoplasmosis of stray cats in Italy. The aim of the present study was to determine the seroprevalence of antibodies to *T. gondii* in stray cats residing in areas where a large stray cat population, among a relatively concentrated human population, results in almost ideal conditions for infection to have ecologic and public health impact.

Materials and methods

POPULATION STUDIED

Five-hundred and seventy-three stray cats from a geographic area of Tuscany (central Italy) were examined. They came from 48 managed stray cat colonies located in the Siena Municipality and in the surrounding suburban districts. Cats were systematically selected among cats captured for a population control program, from January 2003 to December 2004. This investigation was carried out on a monthly basis. Cats were considered as stray because no pre-

vious knowledge of ownership and no physical clues, such as collars, castration or ovariohysterectomy scars were available. All cats selected were free from overt disease. The information collected on each animal included sex, age, breed, area of capture, and date of blood collection. There were 409 females and 164 males. The median age of the cats was 1 year (range 6 months to 10 years). The relatively young animals (<5 year old) were more frequent (328/573, 57.2%) than older cats (>5 year old; 245/573, 42.8%). The greatest majority of the cats were common European Shorthair (n=538) and the remaining were purebred (9 Certosino and 8 Siamese) or crossbred (16 Persian crossbred and 2 Siamese crossbred).

BLOOD COLLECTION

Blood was withdrawn by venipuncture of the cephalic or Jugular vein into sterile 2.5 ml syringes equipped with a 22-gauge needle after intramuscular injection of animals with a Xylazine (Rompum®, 1 mg/kg,) Ketamine (Ketalar®, 10 mg/kg) combination by veterinary personnel. Serum specimens were collected into coded eppendorf microtubes following centrifugation of clotted blood at 1000 g for 10 min, and then were stored at 20°C until needed. If serum specimens could not be tested within 24-48 hours, they were frozen at -20°C. Frozen serum samples were thawed at room temperature (20-25°C) before use.

SEROLOGICAL TESTS

T. gondii-specific IgG antibodies were detected by a commercially available direct agglutination test using a sensitised antigen (Toxo-Screen® DA, Bio Mériex Italia, Roma, Italy), according to manufacturer's instructions. The test, with specificity of 98.80% and sensitivity of 96.22%, was performed on sera dilutions of 1:40 and 1:4000 in U-bottomed microtitre plates, as previously described by other authors [4, 6, 20]. IgG titres were expressed in international units per millilitre. A serologic analysis was considered to be positive for IgG titres >4 IU/ml, as determined by a positive agglutination test result.

ANALYSIS OF RESULTS

Seroprevalence was defined as the percentage (number of positive results (P) /total cat population (N)) and corresponding 95% confidence interval ($P \pm 1.96 \sqrt{P(1-P)/N}$) of samples testing positive for IgG antibodies to *T. gondii*. Chi-square test was used to determine significant association for sex (female versus male), age (<5 versus ≥5 years old), breed (common European Shorthair versus other breed), area of capture (urban versus suburban area), and date of blood collection (spring/summer versus fall/winter) and *T. gondii* seroprevalence. Differences were considered significant at $p < 0.05$.

Results

Results are shown in Table I. The present seroepidemiological survey of stray cats for *Toxoplasma* antibodies revealed a high infection rate. A total of 233 (40.7%) samples were found to be seropositive for *T. gondii* IgG at titres of 1:40 or 1:4000, and the more frequent titre was 1:4000 (188 cases, i.e. 80.7% of positive results). There were 41.3% (169/409) positive female cats and 39% (64/164) males. Higher prevalence was detected in relatively young animals (age <5 years) than in older cats (age ≥5 years; 139/328, 42.3% versus 94/245, 38.3%). Two-hundred and nine-positive animals out of 248 (88.3%) were common European Shorthair cats and 40% (14/35) were purebred or crossbred. With respect to the areas of capture, IgG were found in 45.8% (128/279) cat from the urban area and 35.7% (105/294) from the suburban area. With respect to the period of sampling, *T. gondii* infections resulted to be more prevalent in spring/summer (132/288, 45.8%) than in fall/winter (101/285, 35.4%) months. Despite the trends reported, when the sampled population was analysed by data collected from each cat, no significant association was found between *T. gondii* positivity and sex, breed, age, area of capture, or the period of sampling. Therefore, our results show that stray cats of any sex, age and breed are susceptible to *T. gondii* infection.

Variables		No. of positive samples	Total samples	Prevalence % (95% CI)
Sex	Females	169	409	41.3 (36.2-45.8)
	Males	64	164	39 (31.6-46.4)
Age	<5 years	139	328	42.3 (37-47.6)
	≥5 years	94	245	38.3 (35.2-41.4)
Breed	European Shorthair	219	248	88.3 (84.3-92.3)
	Others	14	35	40 (17.3-62.7)
Area of capture	Urban	128	279	45.8 (40.1-51.4)
	Suburban	105	294	35.7 (30.5-40.9)
Period of sampling	Spring/summer	132	288	45.8 (40.4-51.2)
	Fall/winter	101	285	35.4 (30.1-40.7)
Total		233	573	40.7 (36.6-44.6)

TABLE I. — Prevalence rates (%) and corresponding 95% confidence intervals (95% CI) of IgG antibodies to *T. gondii* in stray cats testing positive by Toxo-Screen® DA test in Italy.

Discussion

The total prevalence of serum antibodies against *T. gondii* in stray cats from Tuscany was 40.7%. No statistically significant influence of sex, age, breed and season was evidenced in the present study, suggesting that the exposure of stray cats to this parasite may be accidental. The present results are partially different from those of other authors, which found statistically significant correlation of male sex, increasing age and/or summer season with overall seropositivity in owned and stray cats [14, 19]. In agreement with those authors [14, 19], it is possible that the higher prevalence rate found in relatively older cats may be due to repeated infections with the parasite occurred during their life. Otherwise, the increased infection rate occurring in the spring/summer period may be related to increased availability of infected preys for cats in these seasons. On the other hand, male sex may be associated with a higher prevalence rate because of territorial habits: indeed, male cats generally live in a wider territory than females and, thus, have increased possibility to find a great number of sources of the infection.

Although cat is the animal reservoir of *T. gondii* infection, only a few reports are available on the prevalence of *T. gondii* oocysts in faeces and on sera antibodies against *T. gondii* in stray cats from Italy, and broad surveys of the infection are limited in this country. In Emilia-Romagna, PAMPIGLIONE *et al.* [16] reported a prevalence of 0.4% in stray cats (one case on 250) for *T. gondii* infection revealed by microscopic examination of faeces. In Piedmont, faecal samples from 60 stray cats were screened for *T. gondii* oocysts and the positivity rate was 6.6% [8]. Using the direct agglutination test, 58 of 115 (50.4%) stray cats were seropositive for *T. gondii* in the town of Rome [11]. In another larger study carried out in Venetia, antibodies to *T. gondii* were detected in sera from 163 of 490 (33%) stray cats using Toxo-Screen® DA [4]. In particular, a serological survey among pet and stray cats carried out in the same geographic area, indicated that overall 29 of 50 (58%) cats were positive for *T. gondii* antibodies, as determined by Sabin-Feldman dye test (SFDT) [1]. In our study, 40.7% of stray cats showed IgG to the parasite. Therefore, this result falls within the reference range previously reported in Italy, and in addition, it suggests a less spreading of *Toxoplasma* in the same examined area at least for the last years comparing with the situation in the past (58%), although the transmission cycle among stray cats is still active. Numerous studies have been carried out in stray cats worldwide. Although it is inaccurate to compare prevalence data of studies which used different serological tests with variable sensitivity and specificity, the prevalence of 40.7% for toxoplasmosis found in the present study is very close to 39.6% and 41% recorded in stray cats from Egypt [18] and United States [9] by SFDT, respectively, and to 40% from Brazil by enzyme linked immunosorbent assay (ELISA) [13]. However, different prevalence rates were found throughout the world, ranging from 10% to 70.2% [6, 13-15, 19, 20, 22]. These variations are probably due to differences in the number of animals and type of population surveyed, in the geographical areas studied or in different diagnostic procedures used. The SFDT is still considered as

the “gold standard” for serological diagnosis of toxoplasmosis in animals and humans [22]. The ELISA test is often used because of its high sensitivity and specificity when compared with other test methods [13, 19]. In the present study, the Toxo-Screen® DA was chosen due to the ease of its preparation and application, as well as due to the good correlation in detection of *T. gondii* specific IgG when compared with SFDT [5] and ELISA [19]. In addition, this test has successfully been used for previous serologic investigations in cats [4, 6, 20], and has been reported as the most sensitive and specific for determining *T. gondii* antibodies in cats [20].

The number of stray cats in Italy is difficult to estimate accurately, but the overall population is widely concerned to be growing. The major problem about stray cats (especially those which reside in colonies near human habitations) is that they might serve as reservoir for infectious agents that can be transmitted to humans. Indeed, several zoonotic agents, including *T. gondii*, have been associated with stray cat populations [15]. However, it is difficult to put the potential health risk that stray cats pose into perspective without knowing their health status. Though stray cats in the present study were from a limited geographic area, the prevalence of 40.7% for toxoplasmosis provides information that the parasite is widely distributed in the environment. The seropositive cats are likely to have already shed a great number of *T. gondii* oocysts with wide-spread contamination of the environment. It is known that *Toxoplasma* transmission takes place by ingestion of oocysts excreted in cat faeces, which can contaminate water and raw vegetables, or by ingestion of raw or undercooked meat that contains parasite cysts. The prevalence of *T. gondii* infections in urban areas can thus be related to environmental contamination with oocysts. Moreover, it has recently been established that infectious environmentally resistant oocysts shed in the faeces of felids can be transported via freshwater runoff into the marine ecosystem, where represent a major source of infection and mortality in some marine mammals [3]. A direct measure of the environmental contamination by oocysts counting is unfeasible for technical reasons. An interesting alternative for measuring *T. gondii* environmental spreading is the seroprevalence in free-living animal reservoirs such as stray cats that can be used as good indicators in densely built urban areas. In addition, stray cats may be one of the best indicators for such studies in urban areas because they are usually captured by Veterinary Public Health Service for population control programs. To support this rationale, some authors compared prevalence rate values in humans and cats. They found that the seroprevalence of toxoplasmosis among humans highly correlates to individual contact with both owned and stray cats and with *T. gondii* seroprevalence in this animal species [7, 21].

To conclude, the determination of prevalence of *T. gondii* infection in stray cats is of epidemiological importance because stray cats can serve as potential source of oocysts for the environment and thus for a very wide range of hosts. Therefore, they can be used to estimate the spreading of the parasite and to allow timely intervention for the control of toxoplasmosis. Our serological study provides adequate information on the prevalence of *T. gondii* infection in a

population of stray cats in Italy, which indicate the persistence of the necessary epidemiological conditions for the parasitic life cycle and for the source of this agent to humans and other domestic or wild animals. It is also believed that our results may contribute to an updated international bank of data on toxoplasmosis of cats throughout the world.

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