Slov Vet Res 2016; 53 (2): 63-7
UDC 636.7.09:616.993.1:616-997.3:57.083.33(450)

Original Scientific Article

OCCURRENCE OF ANTI *Toxoplasma* ANTIBODIES IN OWNED DOGS FROM ITALY: A RETROSPECTIVE STUDY

Guido Rocchigiani, Simona Nardoni, Elisa Amato, Chiara Tempori, Francesca Mancianti

Dipartimento di Scienze Veterinarie, Università di Pisa, Viale delle Piagge 2, 56124 Pisa, Italy

*Corresponding author, E-mail: francesca.mancianti@unipi.it

Summary: Toxoplasma infection in human patients is still an important problem in Italy. Dogs seem to have a role in the epidemiology of human toxoplasmosis, being their presence associated with increased seroprevalence to *Toxoplasma gondii* in humans. Dogs can act as intermediate hosts of this parasite being able to harbor tissue cysts, but this way their reservoir importance for human infection is negligible. Their impact on human health could be due to their role in contaminating the household environment, so permitting the exposure to *T. gondii* the inhabitants. Serum samples of N. 1811 owned dogs randomly collected were examined by IFAT for antibodies against *T. gondii*. One hundred ninety two sera out of 1811 (10.6%) scored positive, with titers ranging from 1/20 to 1/640. Seroprevalence was significantly ($P< 0.01$) higher in adult than in juvenile dogs. On the contrary, it not significantly differed with regards to gender and feeding habits. This is the first report of occurrence of antibodies in an extensive specimen of dogs in Italy. The results of the present survey would indicate a relatively high occurrence of antibodies against *T. gondii* among owned dogs in the investigated area, confirming that attention should be paid in the management of this domestic species.

Key words: dog; *Toxoplasma gondii*; IFAT; seroprevalence

Introduction

Toxoplasma gondii is a zoonotic intracellular protozoan parasite with a worldwide distribution, infecting a large range of vertebrates. Humans become infected postnatally by ingesting tissue cysts from undercooked meat, consuming food or drink contaminated with oocysts, or by accidentally ingesting oocysts. Up to one-third of the world's population is chronically infected (1) and toxoplasmosis has been targeted by Center for Disease Control and Prevention as one of the five top priority parasitic diseases for public health action. However, only a small percentage of exposed adult humans or other animals develop clinical signs of disease (2). Toxoplasmosis is usually a self-limiting disease in immunocompetent individuals, but it is an important cause of morbidity and mortality in immunosuppressed individuals and can cause inflammation of the retinae in healthy adults (3). In human infants congenitally infected *T. gondii* can causes mental retardation, loss of vision and other health problems.

Dogs can act as intermediate hosts of this parasite being able to harbor tissue cysts, but

Received: 23 February 2015
Accepted for publication: 11 January 2016

this way their reservoir importance for human infection is negligible. Their impact on human health could be due to their role in contaminating the household environment so permitting the exposure to the inhabitants (4) by ingesting or rolling in cat feces that contain sporulated *T. gondii* oocysts. This result shows that canine feces could pose a risk of *T. gondii* infection to other species including humans because dogs may serve as mechanical vectors for parasite (5). There are few reports of primary toxoplasmosis in dogs, even if canine toxoplasmosis has been reported in several European and Asian countries as well as the United States (6) with common clinical signs including encephalitis, hepatitis and pneumonia (7). However seroprevalence rates are reported in different areas to vary from 85% in Turkey to 5% in China as reported by Tenter, (2000) (8). To the best of our knowledge there is a lack of data concerning the seroepidemiology of *Toxoplasma* infection in dogs from Italy, except for a report carried on 104 animals in a restricted area from South Italy (9).

The aim of the present paper was to investigate retrospectively about the occurrence of anti *Toxoplasma* antibodies in a sample of owned dogs from Central Italy, submitted to the lab of serology of Department of Veterinary Sciences of the University of Pisa for the annual control for prophylaxis of leishmaniosis. This information could be useful to evaluate the possible circulation of the parasite among dog population living in strict contact with humans.

Materials and methods

Serum samples of N. 1811 owned dogs were randomly collected from those submitted to the lab of serology of Department of Veterinary Sciences of the University of Pisa, for the annual control for prophylaxis of leishmaniosis. The animals were 1052 males and 759 females, 269 of them were young (age ≤ 1 year), the other 1542 adult with ages ranging to 13 months to 15 years. All the animals had an indoor/outdoor lifestyle, spending part of their life outdoor every day and belonged to many different breeds, except toy dogs. Six hundred forty nine (35.8%) dogs were fed on commercial pet food, 531 (29.4%) on homemade dog food and 631 (34.8%) on both.

Anti *Leishmania* specific antibodies have been detected by immunofluorescent antibody test (IFAT) performed as described elsewhere (10) and positive and negative results for each dog have been recorded.

To evaluate the presence of anti *Toxoplasma* antibodies an IFAT was performed on sera, using Toxospot® (BioMérieux, Marcy l'Etoile, France) as antigen and an anti dog-IgG FITC antibody produced in rabbit (Sigma-Aldrich; PBS dilution 1:32). All serum samples were screened with a threshold dilution 1:20, and positive ones were end-titrated using 2-fold dilutions. Cut off dilution was chosen following Macrì et al (2009) (11).

The differences between the seroprevalence values obtained from animals of different gender and age, and fed on different food were evaluated by means of x^2 test.

Table 1: Distribution of antibody titers and coinfection with *Leishmania* of *Toxoplasma* seropositive dogs.

anti *Toxoplasma* antibody titres	number of animals	(%)	animals coinfected with *Leishmania*	(%)
20	26	13.5	1	3.8
40	132	68.8	9	6.8
80	27	14.1	5	18.5
160	4	2.1	2	50
320	2	1	0	0
640	1	0.5	0	0
total number	192		17	

Results

One hundred ninety two sera out of 1811 (10.6%) revealed anti *Toxoplasma* antibodies, with titers ranging from 1/20 to 1/640. Among the whole sample 92 (5.1%) sera scored positive for *Leishmania* antibodies and 17 of them (8.8%) resulted coinfected by *Toxoplasma*. More detailed data are reported in Table 1.

One hundred four out of 1052 (9.9%) males and 88 out of 759 (11.6%) females scored positive for antibodies against *Toxoplasma*, respectively. Nine young animals out of 269 (3.3%) and 183 out of 1542 adult (11.8%) were seropositive, also.

Among seropositive animals 72 (37.5%) were fed on both commercial and homemade food, 53 (27.6%) on homemade food and 67 (34.9%) on commercial pet food.

Seroprevalence was significantly ($P<0.01$) higher in adult than in juvenile dogs. On the contrary, the comparison of antibody presence was not significantly different with regards to gender and feeding habits.

Discussion

This retrospective study showed that 10.6% of examined dogs had antibodies against *T. gondii*. This remark agrees with data from literature even if there is a wide range of prevalences, due both to different serological techniques employed and to canine population selected. Recent surveys on domestic dogs have been performed in Portugal (12) with a global prevalence of 38%, in China (13) with 21.5% of positives and in Korea with 5.1% of domestic dogs positive versus 18.5% of stray dogs (14). Our data fully agree with Yang et al (2013) (15) who reported 10% of infected pet dogs from the Northeast of China. On the basis of this value the Authors stated that this relatively high prevalence of *T. gondii* infection in pet dogs, may pose a risk for human health.

There was not observed any correlation between gender and seropositivity, and this finding is in agreement with other Authors (14-18), while a positive correlation between age and seroprevalence, was reported by others (13,16). Feeding habits seem do not impact on presence of antibodies as observed by Ali et al. (2003) (16).

Occurrence of anti *Toxoplasma* antibodies was reported by IFAT in 17% of 104 dogs from the province of Benevento, Italy (9). To the best of our knowledge this is the only report by this country. *Toxoplasma* infection in human patients is still an important problem in Italy. Despite a substantial decrease in *T. gondii* seroprevalence in humans (from 40 to 20-30% in the adult population in the last 20 years) (19) in fact, 1-2 congenital *Toxoplasma* cases per 10,000 births are currently estimated (20) and 1-4% of them are at risk of death or serious neurological sequelae (21). Dogs seem to have a role in the epidemiology of human toxoplasmosis. Previous studies indicate that the presence of dogs is associated with increased seroprevalence to *T. gondii* in humans (22, 23). Free ranging stray dogs can act as sentinel, furthermore being the most common pets in the world, dogs also reflect the extent of *T. gondii* infection in the domestic environment (24) and can act as reservoir, living in strict contact with people and cats.

The results of the present survey would indicate a moderate occurrence of antibodies against *T. gondii* among owned dogs in the investigated area, confirming that attention should be paid in the management of this domestic species.

References

1. Dubey JP. Toxoplasmosis of animals and humans. 2nd ed. Boca Raton, Florida : CRC Press, 2010: 313 pp.

2. Dubey JP, Rajendran C, Ferreira LR, et al. High prevalence and genotypes of *Toxoplasma gondii* isolated from goats, from a retail meat store, destined for human consumption in the USA Int J Parasitol 2011; 41: 827–33.

3. Montoya JG, Liesenfeld O. Toxoplasma. Lancet 2004; 363:1965–76.

4. Lindsay DS, Dubey JP, Butler JM, Blagburn BL. Mechanical transmission of *Toxoplasma gondii* oocysts by dogs. Vet Parasitol 1997; 73: 27–33.

5. Schares G, Pantchev N, Barutzki D, Heydorn AO, Bauer C, Conraths FJ. Oocysts of *Neospora caninum, Hammondia heydorni, Toxoplasma gondii* and *Hammondia hammondi* in faeces collected from dogs in Germany. Int J Parasitol 2005; 35:1525–37.

6. Dubey JP, Beattie CP. Toxoplasmosis of animals and man. Boca Raton, FL : CRC Press, 1988: 220 pp.

7. Dubey JP, Jones JL. *Toxoplasma gondii*

infection in humans and animals in the United States. Int J Parasitol 2008; 38: 1257–78.

8. Tenter AM, Heckeroth AR, Weiss LM. *Toxoplasma gondii*: from animals to humans. Int J Parasitol 2000; 30:1217–58.

9. Bartoli M, Nacca A, Licciardi V, Veneziano V, Cringoli G. Anticorpi verso *Toxoplasma gondii* in cani e gatti della provincia di Benevento. Acta Med Vet 1996; 42:191–6.

10. Mancianti F, Meciani N. Specific serodiagnosis of canine leishmaniasis by indirect immunofluorescence, indirect hemagglutination, and counterimmunoelectrophoresis. Am J Vet Res 1988; 9: 1409–11.

11. Macrì G, Sala M, Linder AM, Pettirossi N, Scarpulla M. Comparison of indirect fluorescent antibody test and modified agglutination test for detecting *Toxoplasma gondii* immunoglobulin G antibodies in dog and cat. Parasitol Res 2009; 105: 35–40.

12. Lopes AP, Santos H, Neto F, et al. Prevalence of antibodies to *Toxoplasma gondii* in dogs from northeastern Portugal. J Parasitol 2011; 97: 418–20.

13. Li Y, Liu Q, Li S, Wei F, Jin H, Yang M. Seroprevalence of *Toxoplasma gondii* infection in dogs in Sichuan Province, southwestern China. J Parasitol 2012; 98: 209–10.

14. Nguyen TD, Choe SE, Byun JW, Koh HB, Lee HS, Kang SW. Seroprevalence of *Toxoplasma gondii* and *Neospora caninum* in dogs from Korea. Acta Parasitol 2012; 57: 7–12.

15. Yang N, Mu M, Li H et al. Seroprevalence of *Toxoplasma gondii i*nfection in pet dogs in Shenyang, northeastern China. J Parasitol 2013; 99:176–7.

16. Ali CN, Harris JA, Watkins JD, Adesiyun AA. Seroepidemiology of *Toxoplasma gondii* in dogs in Trinidad and Tobago. Vet Parasitol 2003; 113: 179–87.

17. Alvarado-Esquivel C, Romero-Salas D, Cruz-Romero A, et al. High prevalence of *Toxoplasma gondii* antibodies in dogs in Veracruz, Mexico. BMC Vet Res 2014; 10: e191 (4 p.). http://bmcvetres.biomedcentral.com/articles/10.1186/s12917-014-0191-x

18. Duan G, Tian YM, Li BF, et al. Seroprevalence of Toxoplasma gondii infection in pet dogs in Kunming, Southwest China. Parasit Vectors 2012; 5: e118 (4 p.). http://parasitesandvectors.biomedcentral.com/articles/10.1186/1756-3305-5-118

19. De Paschale M, Agrappi C, Manco MT, Cerulli T, Clerici P. Implementation of screening for Toxoplasma gondii infection in pregnancy. J Clin Med Res 2010; 2: 112–6.

20. Stagni L, Romano MA, Romano A, et al. Prenatal screening for congenital toxoplasmosis in Campania: preliminary report on activities and results. Mem Inst Oswaldo Cruz 2009; 104: 374–7.

21. Gilbert RE, Peckham CS. Congenital toxoplasmosis in the United Kingdom: to screen or not to screen? J Med Screen 2002; 9:135–41.

22. Frenkel JK, Lindsay DS, Parker BB, Dobesh M. Dogs as possible mechanical carriers of Toxoplasma, and their fur as a source of infection of young children. Int J Infect Dis 2003; 7:292–3.

23. Etheredge GD, Michael G, Muehlenbein MP, Frenkel JK. The roles of cats and dogs in the transmission of *Toxoplasma* infection in Kuna and Embera children in eastern Panama. Rev Panam Salud Publ 2004; 16:176–86.

24. Esch KJ, Petersen CA. Transmission and epidemiology of zoonotic protozoal diseases of companion animals. Clin Microbiol Rev 2013; 26:58–85.

PRISOTNOST PROTITELES PROTI TOKSOPLAZMI PRI DOMAČIH PSIH V ITALIJI: RETROSPEKTIVNA ŠTUDIJA

G. Rocchigiani, S. Nardoni, E. Amato, C. Tempori, F. Mancianti

Povzetek: Okužba s toksoplazmo je v Italiji še vedno pomemben zdravstveni problem. Predvideva se, da imajo psi pomembno vlogo pri epidemiologiji človeške toksoplazmoze, saj je njihova prisotnost v okolju ljudi povezana s povečano koncentracijo protiteles proti *Toxoplasmi gondii* pri ljudeh. Psi so lahko vmesni gostitelji tega parazita, ki lahko preživi v tkivnih cistah, vendar je pomen tega rezervoarja za prenos okužbe na ljudi zanemarljiv. Bolj pomembna je vloga psov pri onesnaževanju okolja s *T. gondii* in s tem na povečano stopnjo izpostavljenosti ljudi. V raziskavi smo pri 1811 naključno izbranih psih analizirali prisotnost protiteles proti *T. gondii* v serumu z analizo IFAT. Ugotovili smo, da je bilo 192 psov (10,6 %) pozitivnih, s titri od 1 : 20 do 1 : 640. Prisotnost protiteles je bila statistično značilno ($p < 0,01$) višja pri odraslih psih v primerjavi z mladimi, medtem ko ni bilo razlik med spoloma in tudi ne glede na različne prehranjevalne navade. To je prvo poročilo o pojavljanju protiteles proti *T. gondii* v obsežnem vzorcu psov v Italiji, ki nakazuje, da je seroprevalenca protiteles proti *T. gondii* pri psih relativno visoka. Zato je potrebno pozornost nameniti tudi kontroli okužb pri psih.

Ključne besede: pes; *Toxoplasma gondii*; IFAT; seroprevalenca