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ORIGINAL ARTICLE

Seroepidemiological study of canine *Leishmania infantum* and *Toxoplasma gondii* infections in Shanghai, China, and analysis of risk factors

Wei Jiang[1], Yan Wang[2], Yingchun Liu[1], Tao Li[1], Yongjun Chen[1], Shaohui Wang[1], Xiangan Han[1], Quan Wang[1]

[1] *Chinese Academy of Agricultural Sciences, Shanghai Veterinary Research Institute, Shanghai, PR China*
[2] *Animals, Plants and Food Inspection and Quarantine Technical Center of Shanghai Entry-Exit Inspection and Quarantine Bureau, Shanghai, PR China*

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Abstract

The aim of this study was to determine the seroprevalence of *Leishmania infantum* and *Toxoplasma gondii* among household dogs in Shanghai (the most important industrial and commercial city in China), and to assess the possible risk factors associated with the infection. During 2014–2015, a total of 408 sera were collected from healthy household dogs and tested for *L. infantum* and *T. gondii* infection using commercial ELISA kits. The endemic characteristics according to gender, age group and breed were revealed by statistical descriptions and inference. The positive rates of *L. infantum* infection (24/408, 5.9%) were lower than those of *T. gondii* infection (37/408, 9.1%), and co-infection with both parasites was detected in seven dogs (7/408, 1.7%). Seropositivity for either parasite was more likely associated with age: the seroprevalence of *T. gondii* infection ranged from 1.3% (dogs≤1 year) – 18.7% (dogs>6 years), whereas that of *L. infantum* ranged from 1.3% (dogs≤1 year) – 9.9 % (dogs>6 years). Interestingly, the rates of exposure to both *L. infantum* and *T. gondii* were higher in males than in females. Relatively higher exposure rates for *L. infantum* and *T. gondii* were also observed in crossbred dogs compared with purebred dogs. However, neither gender nor breed is likely a determining factor for infection with these two parasites ($P > 0.05$). Identification of the risk factors that underlie these differences may help in the prevention of *L. infantum* and *T. gondii* infection in household dogs. To the best of our knowledge, this is the first report of *L. infantum* and *T. gondii* infection in household dogs in Shanghai, which shows that these two important parasites are still prevalent in this region. Therefore, it is necessary to take integrated strategies for prevention and control of infection in animals, which could help to reduce human infection in the region.

Key words

seroprevalence, *Leishmania infantum, Toxoplasma gondii*, household dogs, risk factors

INTRODUCTION

Dogs may be naturally infected by a variety of widely-distributed parasites and are considered potential vectors for the transmission of zoonotic and veterinary diseases [1, 2]. Unlike wild, feral or stray dogs, household dogs are closely associated with humans and thus play particularly important roles in public health. The protozoan parasites *Leishmania infantum* and *Toxoplasma gondii* are agents of zoonotic diseases and are of considerable public health importance worldwide [2, 3, 4].

Visceral leishmaniasis is a potentially fatal parasitic disease caused by the parasite *L. infantum*; this disease is transmitted to human and animal hosts through the bite of phlebotomine sand flies [5, 6]. Infected individuals can develop severe clinical syndromes that are characterised by prolonged fever, splenomegaly, weight loss, hepatomegaly and pancytopenia, and exhibit a greater than 90% case-fatality rate in the absence of treatment [7]. Dogs are the main urban reservoirs of *L. infantum* and represent a major source of contagion due to their high prevalence of infection and intense cutaneous parasitism [1]. In terms of public health, human leishmaniasis has become a serious concern in many countries, including 12 countries in the Americas and 65 countries in Europe, Asia and Africa; approximately 500,000 new cases occur worldwide each year [8, 9]. An estimated 12 million people are infected with this parasite, leading to approximately 20,000–40,000 deaths per year [9, 10]. This disease remains one of the most serious parasitic diseases endangering human health in China and is highly prevalent in the vast rural areas north of the Yangtze River, particularly in the plain regions of Shandong, Jiangsu, Anhui, Henan, and Hebei Provinces [11]. Despite the worldwide importance of *L.infantum*, little information is available regarding the infection of dogs by this parasite in Shanghai, which is situated on the banks of the Yangtze River Delta in southern China.

Toxoplasmosis is a worldwide endemic disease caused by *T. gondii*, which infects a wide spectrum of vertebrate hosts, including humans. *T. gondii* infection can cause toxoplasmic encephalitis in immunocompromised patients, blindness,

Address for correspondence: Quan Wang, Chinese Academy of Agricultural Sciences, Shanghai Veterinary Research Institute, CAAS, 518 Ziyue Road, Minhang, Shanghai 200241, PR China
E-mail: wangquan@shvri.ac.cn

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abortion, foetal abnormalities and even prenatal death in congenital cases [4, 12]. Dogs are an important intermediate host of *T. gondii*, the consumption of improperly cooked infected meat is a significant health risk for consumers in areas where dog meat is served as food [13]. Serosurveys of *T. gondii* in dogs have been performed extensively worldwide, and the seropositivity rates range from 12.9% – 91.0%, depending on the country and region [1, 14, 15]. The prevalence of *T. gondii* infection is also relatively high in dogs in various provinces of China [16, 17, 18].

Shanghai is the most important industrial and commercial city in China, ranked first in terms of population and population density. The household dog population has increased significantly with the recent improvement in human living standards and the increased awareness of animal welfare. Understanding the prevalence of these pathogens in household dogs may aid the development of appropriate disease management strategies and could therefore benefit both animal and human health. The purpose of this study was to analyse the seroprevalence of *L. infantum* and *T. gondii* in dogs living in Shanghai from the aspect of improving the control of these pathogens.

MATERIALS AND METHODS

Study design and blood sampling. From May 2014 – January 2015, 408 dogs (212 male, 196 female) were selected at random from dogs presented for annual vaccination at the animal health authorities in Shanghai, China. Biometric data for these dogs, including information on age, gender and breed, were obtained from the owners. All the dogs were between the ages of 2 months and 12 years. More purebred (322/408; 78.9%) than mixed-breed (86/408; 21.1%) dogs were included. The dogs were classified as juveniles (< 1 year of age) or adults (> 1 year of age). The adults were further classified into the following age groups: 1–2, 3–4, 5–6 or > 6 years of age. All the dogs were asymptomatic (no signs suggestive of disease), and none had received any prophylaxis or treatment for *T. gondii* or *L. infantum*. Blood samples were collected from the saphenous veins of the dogs into plain sterile tubes and were allowed to clot at room temperature for 3 h. The samples were then centrifuged at 1,000 g for 10 min, and the separated sera were stored at -20 °C until analysis. All blood samples were collected using new disposable tubes, syringes and needles. The study was approved by the Animal Ethics Committee of the Shanghai Veterinary Research Institute of the Chinese Academy of Agricultural Sciences (No. SYXK<HU> 2011–0116).

Determination of *T. gondii* and *L. infantum* antibodies. All the serum samples were tested for the presence of antibodies to *T. gondii* and *L. infantum*. The *T. gondii* seroprevalence was detected using a commercially available ID Screen® Toxoplasmosis indirect multi-species enzyme linked immunosorbent assay (ELISA) kit (IDVET, Grabels, France), whereas the *L. infantum* seroprevalence was determined using a commercially available Ingezim Leishmania indirect immunoenzymatic assay for the detection of specific antibodies to *L. infantum* in dog serum (Ingenasa, Spain). Each kit comprised a microtitration strip plate coated with a special antigen. Positive and negative control sera were provided in the kits. All of the serum samples were diluted appropriately and tested according to the manufacturers' instructions. Briefly, a 96-well ELISA plate was coated with a specific antigen; after incubation of a diluted serum sample in the test well and subsequent washing, a conjugate was added. The plate was then washed again, and a chromogenic enzyme substrate added. The optical density (OD) was then recorded at 450 nm using a photometer (BioTek, Gene Company Ltd., USA). A serum sample was considered positive if the OD reading was higher than the positive cut-off value according to the instructions provided by the manufacturer of the kit. Each sample was tested in duplicate in two repeat assays.

Statistical analysis. Differences in the prevalences of *T. gondii* and *L. infantum* between male and female dogs and between dogs of different age classes were tested using the Chi-Square Test program in SPSS for Windows (Release 16.0 standard version, SPSS Inc., Chicago, IL, USA). Differences were considered statistically significant if $P < 0.05$.

RESULTS

The seroprevalence of *L. infantum* infection and *T. gondii* infection in dogs from Shanghai was 5.9% (24/408) and 9.1% (37/408), respectively (Tab. 1). The co-infection with these two important parasites was found in 1.7% (7/408) of the surveyed dogs.

Higher exposure rates for *L. infantum* were observed in male (7.5%, 16/212) dogs compared with female (4.1%, 8/196) dogs. The seroprevalence of *T. gondii* infection was 9.4% (20/212) in males and 8.7% (17/196) females (Tab. 1). No significant influence ($P > 0.05$) of such factors was found on the prevalence of these two parasites.

The seroprevalence of *L. infantum* infection was 7.0% (6/86) in crossbred dogs and 5.6% (18/322) in purebred dogs (Tab. 1). The prevalence of *T. gondii* was 10.5% (9/86) in mixed-breed dogs and 8.7% (28/322) in purebred dogs. However, no significant association ($P > 0.05$) was observed between breeds for both *L. infantum* and *T. gondii* infection.

The 408 dogs studied were grouped into five age groups: <1, 1–2, 3–4, 5–6 or > 6 years of age, as shown in Table 1.

Table 1. Seroprevalence of *L.infantum* and *T. gondii* in household dogs, according to gender, age and breed

Dog group \ Antibodies	Total	Anti- *L. infantum*		Anti-*T. gondii*	
Gender		Positive		Positive	
	No.	*No.*	*%*	*No.*	*%*
Male	212	16	7.5	20	9.4
Female	196	8	4.1	17	8.7
Age (yr)					
<1	75	1	1.3	1	1.3
1–2	97	1	1.0	4	4.1
3–4	68	6	8.8	5	7.4
5–6	77	7	9.1	10	13.0
>6	91	9	9.9	17	18.7
Breed					
Purebred	322	18	5.6	28	8.7
Crossbred	86	6	7.0	9	10.5
Total	**408**	**24**	**5.9**	**37**	**9.1**

The highest prevalences of *L. infantum* (9.9%) and *T. gondii* (18.7%) infection were detected in dogs > 6 years of age, followed by intermediate prevalences in the age groups of 5–6 (9.1% for *L. infantum* and 13.0% for *T. gondii*) and 3–4 (8.8% for *L. infantum* and 7.4% for *T. gondii*) years, whereas the lowest prevalences were found in dogs in the 1–2 (1.0% for *L. infantum* and 4.1% for *T. gondii*) and <1 (1.3% for *L. infantum* and 1.3% for *T. gondii*) year age groups.

DISCUSSION

Knowledge of the distributions of multi-pathogens, such as *T. gondii* and *L. infantum,* is important for understanding their ecology and epidemiology, and to facilitate public health interventions. In this study, 408 household dogs from Shanghai were included in an investigation of the occurrence of antibodies against two important pathogens. The survey revealed that the seroprevalence of *L. infantum* infection in dogs from Shanghai was only 5.9%, a value that is significantly lower than that found in endemic areas of Wenchuan, Heishui and Jiuzhaigou Counties in China [19, 20]. This different may be explained by the fact that household dogs from Shanghai receive better care from their owners and are therefore less likely to come into contact with the insect vector. Dogs infected with *L. infantum* can exhibit clinical signs of leishmaniasis [21]. However, it has been estimated that more than 50% of seropositive dogs are asymptomatic [22] and can remain free of clinical symptoms for several years or even throughout their lifetimes [23]. Because all the dogs tested in this study were asymptomatic, the results obtained show that dogs with no external clinical signs can also harbour *L. infantum*, although at a lower rate than dogs presenting external clinical signs. Similar results have been described previously [20, 24].

In the current study, the observed prevalence of *T. gondii* in dogs was 9.1%, a value that is higher than the total *T. gondii* seroprevalence (3.2%) found in a previous study conducted by the authors in 2009, but similar to the *T. gondii* seroprevalence in dogs found in the outskirts of Shanghai (town dogs – 6.0%; countryside dogs – 9.8%) [16]. This result may have been obtained because most of the 408 dogs selected in this study were from homes in the outskirts of Shanghai. In this area, most of the owners allow their dogs to roam outside the home, which presumably places them in closer contact with other sources of animal products and allows exposure to *T. gondii* oocytes from freely roaming cats. Nevertheless, it was found that the seroprevalence of *T. gondii* in dogs in this area was lower than that in most other parts of China [25], perhaps because since 2002 the owners of dogs in Shanghai have been supplied a sulpha drug free of charge by the animal health authorities [16].

The rates of exposure to both *L. infantum* and *T. gondii* were higher in males (7.5% for *L. infantum* and 9.4% for *T. gondii*) than in females (4.1% for *L. infantum* and 8.7% for *T. gondii*); although this association was not statistically significant, this finding indicates that male dogs are more likely to be infected by *L. infantum* and *T. gondii*. Similar results have been obtained in previous studies [26], which have shown that higher exposure rates to *L. infantum* infection are found in male dogs; however, gender remains unlikely to be a determining factor for infection with *L. infantum*. Similar to the prevalence of *L. infantum*, no significant difference was found in the prevalence of *T. gondii* between genders. Similar findings have been reported by other researchers [27, 28]; thus, gender is probably not a determining factor for infection with *T. gondii*. In the presented study, breed-related factors were also evaluated. Relatively higher exposure rates for *L. infantum* and *T. gondii* were observed in crossbred dogs compared with purebred dogs; however, no significant influence of such factors was found on the prevalence of these two parasites. The results obtained indicate that breed does not significantly affect the seroprevalence of these two parasites.

The 408 dogs were classified as juveniles (< 1 year of age) or adults (> 1 year of age), and it was found that the prevalences in adult dogs were significantly higher than that in juveniles, both for *L. infantum* infection and *T. gondii* infection. Similar observations have been reported by other researchers who found that the prevalence of *L. infantum* infection was lower among dogs <2 years [20, 29]. In this study, the adult dogs were further classified into the following age groups: 1–2, 3–4, 5–6 or > 6 years of age. For both *L. infantum* and *T. gondii* infection, the highest prevalence was detected in dogs > 6 years of age, and the prevalence was increasing while the infection rate was found to increase with age. The effect of age on *T. gondii* infection coincides with the findings obtained in other studies, including one by the authors of the current study [16, 27, 28]. This association may be explained by the progressively longer exposure time of older dogs to potential sources of *T. gondii* infection. The higher frequency of *L. infantum* positivity among older dogs may be due to the fact that adult dogs tend to remain outside the home for much of the time, thereby increasing their chances of coming into contact with insect vectors. This finding may also be related to the nature of the serological response: a long serological latency may occur after infection, and animals can remain seropositive for long periods [30].

Dogs can be infected with several types of pathogens, including simultaneous infection with *L. infantum* and *T. gondii* [31, 32]. In the presented study, co-infection with these two important parasites was found in 1.7% (7/408) of the surveyed dogs. In dogs, *T. gondii* generally does not cause primary infection unless preceded by an immunosuppressive disease [33]. A significant association has previously been found in several studies between *L. infantum* and *T. gondii* in dogs, suggesting that the immunosuppression caused by *L. infantum* may enhance the susceptibility of dogs to *T. gondii* [31, 32, 34]. However, this hypothesis lacks scientific corroboration. Nevertheless, it is important to emphasize the finding that dogs that were positive for both *T. gondii* and *L. infantum* shared the same habitat, i.e., their owners allowed them to roam outside the home, presumably placing the dogs in closer contact with other sources of animal products and animals that are positive for various parasitic diseases, thereby increasing the dogs' exposure to sand flies and coccidian oocytes; thus, these dogs were exposed to many infection risk factors.

Epidemiologically, dogs are the main reservoirs of *L. infantum*, which is transmitted among animals and to humans by phlebotomine sand flies [2]. Dogs have also been implicated as a potential risk factor for *T. gondii* infection in humans through the mechanical transmission of oocytes [13]. The presence of *L. infantum* and *T. gondii* in dogs can cause local outbreaks of leishmaniasis and toxoplasmosis, and the prevalence of these diseases is related to the trends of these diseases in humans in these regions. In this study, it was found that although more than half of the surveyed dog

owners had some knowledge of toxoplasmosis and knew of the important role of dogs in the transmission of *T. gondii*, approximately only one-in-five knew of the important role of dogs in the transmission of *L. infantum*. To reduce the risk of *L. infantum*, the following preventive measures can be adopted: housing dogs in closed kennels during periods of intense vector activity, reducing microenvironmental factors that favour the development of the vector in houses, and encouraging the use of insecticide-impregnated collars [6]. In China, standard measures to control leishmaniasis generally focus on the control of infected dogs, vector control and the treatment of patients; these measures have played important roles in preventing the rapid spread of these diseases in China [20]. Furthermore, since 2002, dog owners in Shanghai have been supplied with a sulpha drug free of charge by the animal health authorities to control canine toxoplasmosis.

In summary, dogs can be infected by a variety of widely-distributed parasites. Leishmaniasis and toxoplasmosis remain major human health issues worldwide, including in China [35, 36]. The current investigation revealed that the seropositive rates of these two important parasites in Shanghai dogs were lower than those in dogs from other parts of China, and other areas worldwide. This finding may be explained by the fact that dogs in Shanghai, as one of the most developed cities in China, receive better care from their owners than dogs in other areas. The rates of *T. gondii* and *L. infantum* infection in dogs from Shanghai were similar, and co-infection with *T. gondii* and *L. infantum* was also found. The effect of age on the observed single prevalences of both parasites was stronger than the effects of gender and breed. A higher frequency of positivity was found among older dogs for both *T. gondii* and *L. infantum* infection. To reduce the transmission of these parasites from dogs to humans (which is important due to the severity of the associated diseases), special attention should be paid to the improvement of diagnostic assays and control measures against these parasites. Moreover, the implementation of such measures depends not only on the degree of awareness exhibited by dog owners, but also mainly on socio-economic issues and the degree of attention paid by the government, which has the power to reinforce the importance of frequent and continuous examination of dogs through disease control programmes (e.g., treating dogs with anti-*leishmanial* and anti-*T. gondii* drugs) that can interrupt the transmission of these diseases soon after infection.

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