

Full Length Research Paper

Seroprevalence of *Toxoplasma gondii* infection in dogs in Jinzhou City, Northeast China

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To investigate the prevalence of dogs exposed to *Toxoplasma gondii* in Jinzhou City, Liaoning Province, northeast China, 304 dogs were screened for specific antibodies to *T. gondii* by enzyme-linked immunosorbent assay (ELISA) using a commercially-marketed kit. The overall prevalence of *T. gondii* IgG antibodies were found in 82 of 304 (26.97%) dogs with regional variations, but these differences were not statistically significant ($P > 0.05$). The study also showed that prevalence increased significantly with age ranging from 18.49 to 45.20%, of which, the prevalence of dogs >3 years was significantly higher than those <1 year ($P < 0.01$). The results of our study indicated that dogs in Jinzhou had a relatively high *T. gondii* seroprevalence, which has implications for public health in this region.

Key words: *Toxoplasma gondii*, Toxoplasmosis, dog, enzyme-linked immunosorbent assay (ELISA), seroprevalence, Jinzhou, Northeastern China.

INTRODUCTION

Toxoplasmosis caused by the obligate intracellular protozoan, *Toxoplasma gondii* is an important parasitic zoonosis with a world-wide distribution (Zhou et al., 2008; Dubey, 2010; Zhou et al., 2011). Although, toxoplasmosis is mainly benign or associated with mild non-specific clinical symptoms in most patients, toxoplasmosis in congenitally infected children and immunocompromised persons such as AIDS and organ transplant recipients can cause high rates of morbidity and mortality (Montoya and Liesenfeld, 2004; Dubey, 2010). Humans can be infected postnatally by ingesting tissue cysts from undercooked

meat of intermediate hosts, consuming food or drink contaminated with oocysts, or by accidentally ingesting oocysts in the environment (Dubey, 2010; Pereira et al., 2010).

Cats and wild felids as the only definitive hosts play a crucial role in the epidemiology of this parasitic disease due to shedding oocysts in their faeces directly (Dubey, 2010). Although, dogs that become infected by ingestion of infective oocysts from soil contaminated with *T. gondii* or by ingestion of tissue cysts from infected small animals such as birds, rodents do not produce *T. gondii*

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oocysts like cats (Lindsay et al., 1997). In addition, free living dogs such as stray dogs are exposed without any protection from pathogens in the environment, roam freely and contact the same contaminated environment which humans are also exposed to, especially in the urban areas where free-range chickens are not available, so stray dogs could be used as sentinels of environmental spreading with *T. gondii* in densely built urban areas; the prevalence of *T. gondii* antibodies in stray dogs might be a good model for indicating environmental contamination and the risk of toxoplasmosis to humans (Meireles et al., 2004; Salb et al., 2008; Dubey, 2010; Yan et al., 2012).

There has been some published epidemiological studies of *T. gondii* prevalence in dogs in China (Wu et al., 2011; Yan et al., 2012), but little is known about *T. gondii* infection in northeastern China. The aim of our study was to determine the seroprevalence of *T. gondii* in dogs in Jinzhou, Liaoning Province, Northeast China.

MATERIALS AND METHODS

The investigated city

The survey was conducted in Jinzhou City (41°07'N, 121°08'E), western Liaoning Province, which covers an area of 10,301 square kilometers with a population of approximately 3 million. Jinzhou City has a low elevation of 20 to 400 m lying to the north of China's Bohai Sea, and has a typical monsoon-influenced humid continental climate with an annual rainfall of 540 to 640 mm, and an average annual temperature of 8 to 9°C.

Serum samples

A total of 304 blood samples were randomly collected from dogs in nine regions of Jinzhou City between August and October, 2011, including 141 urban pet dogs from municipal districts and Taihe District, 136 rural dogs from towns or villages of Bezhen City, Heishan County, Linghai City, Yi County, Linghe District, Guta District, Songshan New District and 27 dogs from the Experimental Animal Center of Liaoning Medical University. Data on gender, geographic origin and age of each dog were collected. About 5 ml blood was collected from the saphenous veins of dogs into a sterile, plain test tube without anticoagulant. The blood samples were sent to the Parasitology Laboratory, College of Animal Science and Veterinary Medicine, Liaoning Medical University and centrifuged at 3,000 g for 10 min, and then the serum was collected and stored at -20°C until assayed.

Serological examination

Toxoplasma-specific IgG antibodies were measured by enzyme-linked immunosorbent assay (ELISA) using a commercially-marketed kit (Zhuhai S.E.Z Haitai Biological Pharmaceuticals Co., Ltd., Zhuhai, China) according to the manufacturer's recommendations. Positive, negative and critical control sera were provided in the kit. Briefly, the *T. gondii*-specific antigen was coated on a 96-well ELISA microplate. Positive control, negative control and three critical controls were added in each plate, respectively. After the diluted serum sample (1:100) was added to

each well, the plate was incubated at 37°C for 30 min. The plate was washed three times and a conjugate was then added with an incubation at 37°C for 30 min. A chromogenic enzyme substrate was then added followed by three washings, after dark incubation at 37°C for 10 min, the reaction was stopped with stop solution. The optical density (OD) at 450 nm was measured in a microplate reader (Bio Tek Instrument Inc, Vermont, USA). The serum was considered as positive if the OD450 (sample) > mean OD450 (critical control) multiplied by 1.1.

Statistical analysis

Differences in the seroprevalence of *T. gondii* infected dogs between male and female, the sources of dogs and different age groups were analyzed using a Chi square (χ^2) test in Predictive for Analytics Software (PASW®) Statistics 18 (Release 18.0 standard version, SPSS Inc., Chicago, America). The *P*-value < 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

A total of 304 sera of dogs were examined by ELISA for *T. gondii* antibodies (Table 1). Antibodies to *T. gondii* were found in 82 (26.97%) dogs. The seroprevalence in male dogs was 27.29% (27 of 99), while the seroprevalence in female dogs was 26.83% (55 of 205), but no significant difference in *T. gondii* prevalence was found between male and female dogs (*P* > 0.05). In general, seroprevalence increased with age and the seropositivity was higher in older dogs, indicating postnatal infection (Lin et al., 1998; Dubey, 2010). Our results showed that a significantly higher (45.20%) prevalence of *T. gondii* infection was detected in > 3-year-old of dogs, when compared with a seropositive rate of 27.40% in dogs of < 1-year-old (*P* < 0.01), suggesting that the risk of exposure to *T. gondii* may increase with age. The present survey demonstrated that the prevalence of *T. gondii* in rural dogs (29.41%) was higher than that in urban dogs (25.00%) and experiment dogs (25.93%) (Table 2), which is consistent with reports by others (Lin et al., 1998; Dubey, 2010), but the difference in this study was not statistically significant (*P* > 0.05). Varied *T. gondii* seroprevalence was detected for dogs in different districts of Jinzhou City, the differences may be attributed to the hunting habits, living conditions of dogs and the density of stray cats (the probable disseminator of *T. gondii* in this area).

The overall seroprevalence of *T. gondii* infection in dogs in Jinzhou was 26.97% (82 of 304). This result was similar to that of Hebei province (26.92%) (Yuan et al., 2004), lower than the seroprevalence of 33.3% in stray dogs in Guangzhou (Zhang et al., 2010) and 40.3% in Xuzhou dogs (Yan et al., 2012), but was higher than that in other regions of China, such as Beijing (13.21%) (Yu et al., 2006), Lanzhou (10.8%) (Wu et al., 2011), Xinjiang (10.5%) (Wang et al., 2011), Haikou (20.7%) (Huang et al., 2008) and Inner Mongolia (12.6%) (Lu et al., 2010). These differences may be related to dietary habits, geographic

Table 1. Seroprevalence of *T. gondii* infection in dogs by gender and age in Jinzhou City, Northeastern China.

| Statistical characteristic | Male | | Female | | P- value | Odds ratio (95% CI) | Total | |
|----------------------------|-------------------------|-------------------|-------------------------|---------------------|----------|---------------------|------------------------|--------------------|
| | No. positive/ total no. | (95% CI) | No. positive/ total no. | (95% CI) | | | No. positive/ total no | (95% CI) |
| Types | | | | | | | | |
| Urban pet dogs | 11/40 | 27.50(15-44) | 24/101 | 23.76(15.46-32.06) | 0.643 | 1.217(0.530-2.795) | 35/141 | 24.82(17.69-31.95) |
| Rural dogs | 11/48 | 22.92(12-37) | 29/88 | 32.95(23.15-42.77) | 0.220 | 0.605(0.270-1.355) | 40/136 | 29.41(21.75-37.07) |
| Experimental dogs | 5/11 | 45.45(17-77) | 2/16 | 12.50(2-38) | 0.141 | 5.833(0.874-38.936) | 7/27 | 25.93(11-46) |
| Total | 27/99 | 27.27(18.5-36.04) | 55/205 | 26.83(20.76-32.90) | 0.935 | 1.023(0.596-1.754) | 82/304 | 26.97(21.98-31.96) |
| Age (years) | | | | | | | | |
| < 1 year | 6/41 | 14.63(6-29) | 16/78 | 20.51(11.55-29.47) | 0.432 | 0.664(0.238-1.853) | 22/119 | 18.49(11.51-25.47) |
| 1-3 | 12/36 | 33.33(19-51) | 17/77 | 22.08(12.82-31.34) | 0.202 | 1.765(0.734-4.244) | 29/113 | 25.67(17.62-33.72) |
| >3 | 9/22 | 40.91(21-64) | 22/50 | 44(30-59) | 0.807 | 0.881(0.319-2.436) | 31/72 | 43.06(31.62-54.50) |
| Total | 27/99 | 27.27(18.5-36.04) | 55/205 | 26.83(20.76-32.90) | 0.935 | 1.023(0.596-1.754) | 82/304 | 26.97(21.98-31.96) |

Table 2. Seroprevalence of *T. gondii* infection of dogs in different residences of Jinzhou City, Liaoning province.

| Residence area of dogs | No. tested | No. positive | Prevalence (%) |
|----------------------------------------|------------|--------------|----------------|
| Urban areas | | | |
| Jinzhou municipal districts | 97 | 29 | 29.89 |
| Taihe district | 44 | 6 | 13.64 |
| Total | 141 | 35 | 25.00 |
| Rural areas (towns or villages) | | | |
| Lvyang town, Beizhen city | 2 | 0 | 0 |
| Bailaohu village, Guta district | 7 | 3 | 42.86 |
| Villages of Taihe district | 55 | 12 | 21.82 |
| Villages of Yi county | 48 | 18 | 37.50 |
| Daling village, Songshan new district | 11 | 3 | 27.27 |
| Villages of Heishan county | 9 | 4 | 44.44 |
| Daye town, Linghai city | 4 | 0 | 0 |
| Total | 136 | 40 | 29.41 |
| Experimental animal center | 27 | 7 | 25.93 |
| Total | 304 | 82 | 26.97 |

variation, climate changes, animal welfare conditions in these areas, as well as the various diagnostic techniques of different sensitivity and specificity.

Conclusion

The results of the present survey showed that *T. gondii* infection in dogs in Jinzhou was relatively high, especially in older dogs, which raises public health concern. Therefore, it is imperative for the public health authorities, dog owners and veterinarians in Jinzhou to pay more attentions to this concern, and further investigations of *T. gondii* infection in other animals in this city need to be performed.

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