Prevalence of *Toxoplasma gondii* Antibodies in Dogs in Central China

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ABSTRACT

To investigate the seroprevalence of *T. gondii* infection in dogs in central China, 1,176 serum samples were collected from domestic dogs in Henan province, central China between March 2015 and February 2016 and tested for IgG antibody against *T. gondii* using the enzyme linked immunosorbent assay (ELISA). The overall seroprevalence of *T. gondii* was 18.20% (214/1176). No significant difference was observed between this seroprevalence according to gender and breed of dogs ($p>0.05$). The infection rate in rural dogs (22.22%) was higher than in urban dogs (14.45%), and the difference was statistically significant ($p<0.01$). Significantly higher seroprevalence was observed in watchdogs (22.36%) compared to pet dogs (15.38%) ($p<0.01$). The prevalence of *T. gondii* antibodies in dogs increased significantly ($p<0.01$) with the increase of age. The results of the present study indicated the high exposure of *T. gondii* in dogs in Henan province, central China, which might have important implications for public health.

Keywords: *Toxoplasma gondii*; dog; Seroprevalence; ELISA; Central China

INTRODUCTION

Toxoplasma gondii (*T. gondii*) is an obligate intracellular protozoan parasite that has a worldwide distribution and infects a wide range of warm-blooded vertebrates, including humans and dogs (1). The sexual part of the life cycle occurs in feline carnivores, which excrete the oocysts in their faeces. After a 2-5 day long sporulation process, the oocysts become infectious and can be transmitted to other hosts through inadvertent ingestion. Asexual multiplication in the intermediate host leads to the formation of tissue cysts in muscle tissue and organs, which are infectious for hosts that consume them (2). Humans become infected by ingesting tissue cysts from undercooked meat, or by consuming food or drink contaminated with *T. gondii* oocysts (3, 4).

T. gondii infection can cause serious illness in young dogs, especially in those co-infected with canine distemper virus (5). The clinical signs of toxoplasmosis in dogs are usually characterized by ataxia, diarrhea, and respiratory distress (6-8).

Although only Felids are known to produce *T. gondii* oocysts, dogs can act as mechanical vectors in the transmission of *T. gondii* oocysts to humans because of their habit of eating cat feces and also rolling over in cat excreta (9). If they ingest feces of infected cats, some of the oocysts can pass unchanged through the dog intestine, appear in feces and remain viable (10). It has been hypothesized that dogs roll in cat feces and thus their hair become contaminated with oocysts. Humans might acquire *T. gondii* infection by patting dogs that have rolled over in infected cat feces (9). Additionally, viable *T. gondii* has been isolated from tissues of dogs in many countries (11-13). *T. gondii* can be transmitted to humans by consumption of undercooked meat from infected dogs.

Based on the above reasons, understanding the prevalence

of *T. gondii* exposure in dogs is of economic and public health importance.

Antibodies to *T. gondii* have been reported in dogs worldwide (14-17). In recent years, there have also been some surveys of *T. gondii* antibodies in dogs in some provinces of China (18-20). However, little is known of *T. gondii* seroprevalence in dogs in central China. Therefore, the objective of the present survey was to determine the seroprevalence of *T. gondii* in domestic dogs in Henan province, central China, and to evaluate the main associated risk factors relating to exposure to *T. gondii* in this region.

MATERIALS AND METHODS

Ethical statement

The study was reviewed and approved by the ethical review committee of the Xinxiang Medical University (reference no. 2015016).

The study site

The study was conducted in Henan province, which is located in the central part of the mainland China, covering an area of 167,000 km^2 and a population of approximately 106.01 million. Its geographical position is at east longitude 110°21′-116°39′ and at north latitude 31°23′-36°22′. The Yellow River passes through central Henan. The area has a continental monsoon climate, with four distinctive seasons. The average annual temperature is 12.1-15.7 °C, with a mean annual rainfall of 532.5-1380.6 mm.

There are 17 provincial cities distributed in the Henan province, with the city of Zhengzhou as its capital. Five cities including Anyang (35°13′-36°22′N, 113°37′-114°58′E), Sanmenxia (33°31′-35°05′N, 110°21′-112°01′E), Zhengzhou (34°16′-34°58′N, 112°42′-114°13′E), Xinyang (31°46′-31°52′N, 114°01′-114°06′E) and Shangqiu (33°43′-34°52′N, 114°49′-116°39′E), located in the northern, western, central, southern and eastern parts of Henan province, were selected for sample collections.

Sample collection

A total of 1,176 blood samples of domestic dogs was collected from the above five cities in Henan province between March 2015 and February 2016. Dog owners were asked for details of the animals' age, sex, source (where were the animals originated), breed, use and rearing conditions using a structured questionnaire. Blood samples were centrifuged at 5000 rpm for 5 min at 4 ℃ and sera were recovered and transferred to 1.5 ml Eppendorf tubes. The sera were stored at -80°C until tested for anti-*T.gondii* antibodies.

Determination of antibodies to *T. gondii*

Antibodies to *T. gondii* were determined using the commercial *T. gondii* IgG ELISA Kit (Combined Company, Shenzhen, Guangdong Province, China) according to the manufacturer's instructions (21, 22). Positive and negative control sera were provided in the kit. Briefly, the *T. gondii* specific antigen was coated on a 96-well ELISA plate. After incubation of the diluted serum sample (1:100) in the test well and subsequent washing, a conjugate was added. The plate was washed again and then a chromogenic enzyme substrate was added. The optical density (OD) at 450 nm was read using a photometer (BIO-RAD, Hercules, CA, USA). A relative rate percent (IRPC) value was obtained using the following formula:

$$\text{IRPC} = \frac{\text{OD450(sample)} - \text{mean OD450(blank control)}}{\text{mean OD450(negative control)} - \text{mean OD450(blank control)}}$$

The sera were considered negative to *T. gondii* if the IRPC<2.1, and positive if IRPC >2.1.

Statistical analysis

Differences in *T. gondii* prevalence for different variables such as age, breed and gender were analyzed using a Chi square test. Statistical analysis was performed using SPSS 20 software for Windows (SPSS Inc, Chicago, Illinois, USA). The differences were considered statistically significant if $p < 0.05$.

RESULTS

In this study, 1176 dogs were tested for the presence of antibodies against *T. gondii* using the ELISA. As shown in Table 1, an overall recorded seroprevalence of *T. gondii* in dogs in Henan province, central China was 18.20% (214/1176). Seropositive dogs from different cities were: 24.68% of 235 from Sanmenxia, 20.25% of 242 from Xinyang, 17.97% of 256 from Zhengzhou, 15.53% of 219 from Shangqiu, and 12.05% of 224 from Anyang.

The seroprevalence of *T. gondii* in males was 18.96% (117/617) and in females was 17.35% (97/559) (Table 1). Although the seroprevalence in males was higher than the females, the difference was not significant ($p>0.05$). The

Table 1: Seroprevalence of *Toxoplasma gondii* infection in dogs in Henan province, central China according to the breed, age and gender of the dogs.

Variable	No. examined	No. of positive	Prevalence (%)	X^2	*P*-value
Region					
Anyang	224	27	12.05	14.059	0.007
Sanmenxia	235	58	24.68		
Zhengzhou	256	46	17.97		
Xinyang	242	49	20.25		
Shangqiu	219	34	15.53		
Gender					
Male	617	117	18.96	0.511	0.475
Female	559	97	17.35		
Breed					
Purebred	742	142	19.14	1.194	0.275
Mixed-breed	434	72	16.59		
Area					
Urban	609	88	14.45	11.916	0.001
Rural	567	126	22.22		
Purpose					
Watchdog	474	106	22.36	9.256	0.002
Pet dog	702	108	15.38		
Age (years)					
≤3	318	39	12.26	15.281	<0.001
3~6	573	105	18.32		
≥6	285	70	24.56		
Total	1176	214	18.20		

seroprevalence of *T. gondii* infection was 19.14% (142/742) in purebred dogs and 16.59% (72/434) in mixed-breed dogs, showing no significant difference by breed (p>0.05).

Significantly higher seroprevalence was found in rural dogs (22.22%), compared to that of in dogs raised in urban area (14.45%) (p<0.01). Significantly higher seroprevalence was observed in watchdogs (22.36%) compared to pet dogs (15.38%) (p<0.01) (Table 1).

The prevalence of *T. gondii* infection in dogs increased significantly (p<0.01) with the increase of age. The highest prevalence of infection (24.56%) was detected in six-year-old or older dogs, followed by intermediate prevalence (18.32%) in the 3-6 years age group, while the prevalence found in dogs in the ≤3 years age groups was 12.26% (Table 1).

DISCUSSION

ELISA is among the most commonly used methods for investigation of IgG antibody. IgG antibodies usually appear within 1-2 weeks of *T. gondii* infection, peaking within 1-2 months and declining at various rates but and usually persisting for life (23). Because of its high sensitivity and specificity, low cost, and ease of use, ELISA is widely used for diagnosis of *T. gondii* infection (24).

The present investigation showed that the overall seropositivity for *T. gondii* exposure was 18.20% in dogs in Henan. Compared with other provinces in China, the prevalence of 18.20% was lower than the values of 20.56% in dogs in a study performed in Guizhou (20), 21.5% in Jiangsu (19), and 24.0% in Beijing (25), but higher than those observed in Shanghai (3.2%), Jilin (15.72%), Inner Mongolia (13.55%) and Liaoning (13.48%) (26, 27). Among these regions, the difference in *T. gondii* seroprevalence may be due to ecological and geographical factors, serological technique used as well as feeding and animal welfare which refers to how animals adapted to their environment and satisfied their basic natural needs for dogs in these areas.

Statistical analysis showed that differences in *T. gondii* infection between female and male dogs were not significant (p >0.05), suggesting that gender of the host is not a crucial factor for *T. gondii* infection. These findings agree with other previous studies (17, 28, 29).

In the present study, although the seroprevalence in purebred dogs was slightly higher than the mixed-breed dogs, the difference was not significant (p>0.05), which is in agreement with previous reports (14, 30). In contrast, Raimundo *et al.* observed significantly higher seropositivity in mixed-breed than in purebred dogs (31). The role of breeds in the epidemiology of canine toxoplasmosis is not well established, and requires further research.

The present survey also showed that *T. gondii* seroprevalence in rural dogs was higher than that in urban dogs (p<0.01), which is consistent with reports by others (31, 32). These differences may be attributed to differences in the living conditions of dogs including feeding and environment, the density of stray cats and the degree of environmental contamination with *T. gondii* oocysts between rural and urban area.

Significantly higher seropositivity was found in watchdogs compared to pet dogs in the present study. The higher occurrence of *T. gondii* in watchdogs reflects more opportunities to ingest *T. gondii* infected tissues of animals or oocysts from the environment (33). In Henan, watchdogs are usually kept outdoors in rural areas, which often roam more freely

with greater access to parasites than pet dogs usually kept indoors.

Our findings of a gradual increase of seroprevalence of *T. gondii* with dog's age are in agreement with other reports (19, 34, 35), suggesting that the increasing age was a risk factor for *T. gondii*. Recently, Lopes *et al*. observed that for each year increase in age, the risk of a dog being found seropositive significantly increased by an Odds Ratio (OR) of 1-18 (CI95%:1.02-1.36) (30). The higher seroprevalence in older animals reflects a cumulative likelihood for exposure to *T. gondii* and lifelong persistence of antibodies. These results suggest that horizontal transmission is the main route of infection in dogs.

In addition, the meat of dog is readily consumed in China and the Far East. Thus, our findings have important implications for the prevention of *T. gondii* infection by eating dog meat.

In conclusion, this study revealed a high prevalence of *T. gondii* infection in dogs in Henan province, central China. Integrated measures, such as strengthening the management of the dogs feeding and reducing the contact between dogs and cats, should be taken to prevent and control toxoplasmosis in dogs in this area for public health concerns.

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