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Brief Communication

Seroprevalence and risk factors of *Toxoplasma gondii* infection in pet dogs in Hunan Province, subtropical China

Xing-Xing Wen, Zhuying Liu*, Ping-Yuan Xu*

Hunan Biological and Electromechanical Polytechnic, Changsha 410127, China

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***Correspondence**
zl, liuzhuying@hnbemc.edu.cn
pyx, xupingyuan@hnbemc.edu.cn

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Abstract

Toxoplasma gondii infections are ubiquitous in both animals and humans. Although seroprevalence data exist for pet dogs across multiple Chinese provinces, limited epidemiological information is available for *T. gondii* infection in pet dogs in subtropical China's Hunan Province. We examined *T. gondii* antibodies in pet dogs from Hunan Province using the indirect hemagglutination test. Logistic regression analyses were used to identify factors associated with *T. gondii* infection (season, sex, age, breed, and location). The overall seroprevalence was 10.8% (95% confidence interval (CI)=9.0–12.6) (118/1,092), with regional variations ranging from 8.0% (95% CI=4.2–11.8) to 21.1% (95% CI=8.1–34.0). Antibody titers followed a descending distribution: 42.3% (1:64), 30.5% (1:128), 20.3% (1:256), 5.1% (1:512), and 1.7% (1:1,024). The multivariate analysis identified the season (highest in summer: odds ratio=2.0, 95% CI=1.2–3.4) and age (>3 years: odds ratio=2.8, 95% CI=1.5–5.3) as factors independently associated with the outcome (P<0.05). These finding revealed the high seroprevalence of *T. gondii* infection in pet dogs in Hunan Province, subtropical China, highlighting the risk of zoonotic transmission. Therefore, effective measures should be taken to prevent and control toxoplasmosis in pet dogs in this province.

Keywords: Dogs, seroprevalence, risk factors, *Toxoplasma gondii*

Toxoplasma gondii, an obligate intracellular protozoan, poses significant clinical risks for immunocompromised individuals and congenital infections [1]. Human transmission primarily occurs through the ingestion of oocyst-contaminated environmental substrates or undercooked meat containing tissue cysts [2]. Despite its global health burden, the prevention of toxoplasmosis remains challenging because of the unavailability of commercial vaccines and the paucity of therapeutic options [3].

T. gondii infection is also highly prevalent in domestic animals, causing substantial economic losses worldwide [4]. Globally, the seroprevalence of *T. gondii* in dogs varies significantly by region—reflecting differences in climate, husbandry practices, and diagnostic methods. In China, the pet dog population has experienced a rapid expansion, surpassing 52 million in 2023, with an annual growth rate of 2%. A few surveys have indicated that *T. gondii* infection in pet dogs can cause health problems in these animals and threaten humans [5]. *T. gondii* oocysts ingested by dogs can pass through the digestive tract and remain infectious [6]. Dogs may indirectly carry these oocysts from contaminated environments [7]. Thus, *T. gondii* infection in dogs can serve as an indicator of human environmental contamination levels for humans [8]. Recent investigations into the worldwide seroprevalence of *T. gondii* infection in dogs (including pet dogs) have shown significant variability depending on the geographical region and study design, making comparisons

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Investigation: Wen XX
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Project administration: Liu Z, Xu PY
Resources: Xu PY
Software: Xu PY
Supervision: Liu Z, Xu PY
Validation: Liu Z, Xu PY
Visualization: Liu Z, Xu PY
Writing – original draft: Wen XX
Writing – review & editing: Xu PY

Conflict of interest
The authors declare no conflict of interest related to this study.

ORCID
Xing-Xing Wen
https://orcid.org/0009-0000-6140-1690
Zhuying Liu
https://orcid.org/0000-0002-4267-3700
Ping-Yuan Xu
https://orcid.org/0009-0004-7741-9819

challenging but demonstrating the wide geographical distribution of this parasite [9]. In recent years, several reports of *T. gondii* infection in pet dogs in China have emerged [10]. However, these studies were mostly published in local Chinese journals, making them inaccessible to foreign scholars. Moreover, limited epidemiological information is available on *T. gondii* infection in pet dogs in subtropical China's Hunan Province.

This study aimed to determine the seroprevalence of *T. gondii* in pet dogs in Hunan Province. The results of this study should provide a basis for the implementation of control strategies against *T. gondii* infection in pet dogs in this province and elsewhere.

All procedures involving animals in the present study were approved by the Animal Ethics Committee of Hunan Biological and Electromechanical Polytechnic (approval No. 202211). A total of 1,092 blood samples were collected from pet dogs visiting 24 veterinary clinics (6 per zone) across 4 geographical zones (east, south, west, and north) in Hunan Province, China (Table 1). The inclusion criteria were complete records of season, sex, age, breed, and location; dogs with incomplete records or recent antiparasitic treatment (2 weeks) were excluded. One blood sample was collected from each dog, and all blood samples were labeled individually and cooled during transport to the laboratory at Hunan Biological and Electromechanical Polytechnic (Changsha, Hunan Province). There, these samples were centrifuged at 1,000 g for 10 min, after which the supernatant serum was obtained, frozen, and stored at -20°C until use.

Serum samples were analyzed using a validated indirect hemagglutination test kit (NY/T 573-2002, Lanzhou Veterinary Research Institute, Lanzhou, China) as per the manufacturer's recommendations and previous descriptions [11,12]. This kit has been extensively used for detecting specific antibodies to *T. gondii* in dogs in China for many years [13]. Samples with titers of ≥1:64 were considered positive.

Data were analyzed with PASW Statistics version 18 (SPSS Inc., Chicago, IL, USA). Continuous variables were presented as mean values with standard deviations for normally distributed data or median values with interquartile ranges for non-normally distributed data. Categorical variables were presented as frequencies with percentages. Continuous data were compared among the groups using the Kruskal-Wallis test or the one-way ANOVA

Table 1. Seroprevalence of *Toxoplasma gondii* in pet dogs in Hunan Province, subtropical China

Region	No. of tested	No. of positive	Prevalence (%) (95% CI)
Changde	150	13	8.7 (4.2–13.2)
Changsha	200	16	8.0 (4.2–11.8)
Chenzhou	98	12	12.2 (5.8–18.7)
Hengyang	102	12	11.8 (5.5–18.0)
Huaihua	66	7	10.6 (3.2–18.0)
Loudi	103	9	8.7 (3.3–14.2)
Shaoyang	38	8	21.1 (8.1–34.0)
Xiangxi	51	8	15.7 (5.7–25.7)
Yiyang	74	8	10.8 (3.7–17.9)
Yongzhou	45	6	13.3 (3.4–23.3)
Yueyang	85	9	10.6 (4.0–17.1)
Zhuzhou	80	10	12.5 (5.3–19.7)
Total	1,092	118	10.8 (9.0–12.6)

CI, confidence interval.

for non-normally or normally distributed data, respectively. Categorical data were compared among groups using chi-square tests. The measure of association in this study was the odds ratio with its 95% confidence interval (CI). Factors associated with *T. gondii* infection were identified using univariate and multivariate logistic regression analyses. Factors with $P<0.2$ in the univariate analysis were selected for the multivariate analysis model, which identified factors independently associated with *T. gondii* infection. The best model was judged using Fisher's scoring algorithm. A *P*-value of <0.05 was considered statistically significant.

Antibodies against *T. gondii* were detected in 10.8% (95% CI=9.0–12.6) (118/1,092) of the pet dogs, with titers of 1:64 in 50, 1:128 in 36, 1:256 in 24, 1:512 in 6, and 1:1,024 in 2. This finding indicates that *T. gondii* infection is widespread among pet dogs in Hunan Province. This finding is similar to that of a recent study showing that the seroprevalence of *T. gondii* in cats in Hunan Province is 6.4%, which is higher than that in most other provinces of China [14]. The seroprevalence of *T. gondii* in pet dogs from different regions ranged from 8.0% (95% CI=4.2–11.8) to 21.1% (95% CI=8.1–34.0) (Table 1), with statistically significant differences ($P<0.01$). The seroprevalence of *T. gondii* in pet dogs from 7 out of the 12 representative administrative regions in Hunan Province exceeded 10.8% (95% CI=9.0–12.6) (the average value), with the highest seroprevalence (21.1%) (95% CI=8.1–34.0) observed in Shaoyang (Table 1). One plausible reason for this finding is that Shaoyang harbors a relatively higher density of stray cats (the cat is the definitive host of *T. gondii*). In this study, the seroprevalence of *T. gondii* in pet dogs was higher than that in Henan (0.8%) ($P<0.05$) [15] and Liaoning Provinces (10.0%) ($P<0.05$) [16] but lower than that in Anhui (18.6%) ($P<0.05$) [17], Shandong (15.6%) ($P<0.05$) [18], and Yunnan (21.6%) ($P>0.01$) Provinces [13]. These differences may be attributed to detection methods, geographical environments, overall sample sizes, and animal husbandry practices.

The *T. gondii* seroprevalence in pet dogs was higher in summer (15.2%) (95% CI=13.0–17.3), followed by autumn (11.2%) (95% CI=9.3–13.1), and lower in spring (8.7%) (95% CI=7.0–10.4) and winter (8.3%) (95% CI=6.6–9.9) (Table 2). This result suggests that *T. gondii* is prevalent throughout the year, with peaks in summer and autumn. Our multivariate analysis revealed that the odds of being seropositive in summer was 2 times higher than that in winter ($P<0.05$). This seasonal pattern likely reflects the influence of climatic conditions on oocyst survival and transmission dynamics. Hunan Province has a subtropical moist monsoon climate, with an average annual temperature of 16°C–18°C and high humidity in summer and autumn, which is conducive to oocyst survival [19]. The peaks in *T. gondii* seroprevalence in summer and autumn suggest that controlling toxoplasmosis during these 2 seasons is critical. The seroprevalence of *T. gondii* in pet dogs aged >3 years (16.5%; 95% CI=14.3–18.7) was higher than that in pet dogs aged ≤1 year (8.6%; 95% CI=6.9–10.3) (Table 2), and these differences were statistically significant ($P<0.05$). Our multivariate analysis also showed that dogs aged >3 years had 3 times the odds of seropositivity compared to those aged ≤1 year. This suggests cumulative exposure over time, as older dogs have had more opportunities to encounter contaminated environments or ingest infected prey.

The high seroprevalence of *T. gondii* in pet dogs in Hunan Province, along with the identified season and age as risk factors, can be further interpreted in the context of environ-

Table 2. Multivariate analysis of risk factors and seroprevalence of *Toxoplasma gondii* in pet cats in Hunan Province, subtropical China

Risk factor	Category	No. of positive/ No. of tested	Prevalence (%) (95% CI)	Adjusted odds ratio	*P*-value
Season	Spring	24/276	8.7 (7.0–10.4)	1.056 (0.577–1.934)	0.879
	Summer	40/264	15.2 (13.0–17.3)	1.981 (1.142–3.436)	0.015
	Autumn	32/286	11.2 (9.3–13.1)	1.397 (0.790–2.472)	0.256
	Winter	22/266	8.3 (6.6–9.9)	Reference	
Sex	Female	70/578	12.1 (10.2–14.0)	1.338 (0.907–1.972)	0.144
	Male	48/514	9.3 (7.6–11.1)	Reference	
Age group	≤1	5/76	6.6 (5.1–8.0)	Reference	
	1< years ≤2	28/326	8.6 (6.9–10.3)	1.392 (0.560–3.974)	0.509
	2< years ≤3	53/496	10.7 (8.9–12.5)	1.699 (0.657–4.395)	0.315
	>3	32/194	16.5 (14.3–18.7)	2.696 (0.928–6.710)	0.048
Breed	Cross-breed	93/876	10.6 (8.8–12.4)	Reference	
	Pure breed	25/216	11.6 (9.7–13.5)	1.102 (0.689–1.761)	0.714
Location	Central Hunan	37/317	11.7 (9.8–13.6)	1.113 (0.742–1.668)	0.600
	Eastern Hunan	26/280	9.3 (7.6–11.0)	Reference	
	Southern Hunan	18/143	12.6 (10.6–14.6)	1.407 (0.743–2.663)	0.314
	Western Hunan	15/117	12.8 (10.8–10.8)	1.437 (0.731–2.824)	0.284
	Northern Hunan	22/235	9.4 (7.6–11.1)	1.009 (0.556–1.832)	1.000

CI, confidence interval.

mental and host-related factors. The subtropical moist monsoon climate of Hunan Province plays a crucial role as an environmental factor. The warm and humid conditions in summer and autumn not only promote the survival and development of *T. gondii* oocysts in the environment but also facilitate the activities of potential intermediate hosts, such as rodents and birds. These intermediate hosts can carry and spread *T. gondii*, increasing the risk of pet dog exposure. For example, rodents may contaminate food and water sources with *T. gondii* oocysts, and pet dogs that come into contact with these contaminated sources are more predisposed to infection.

Regarding host-related factors, the age-dependent increase in seroprevalence can be explained by the immune status and exposure history of pet dogs. Younger dogs, especially those less than a year old, may have relatively immature immune systems; however, they also have less long-term exposure to the parasite. As dogs age, their immune systems mature; however, they accumulate exposure experiences over time. Additionally, older dogs may have different behavioral patterns than younger ones. They may be more active outdoors, exploring more areas, and coming into contact with various potential sources of infection, further increasing their *T. gondii* infection risk.

Pet dogs are the most common human companion animals, yet they can carry zoonotic pathogens such as *T. gondii*, raising public health concerns, as dogs can mechanically transmit *T. gondii* oocysts to humans via contaminated fur, paws, or oral contact. Although dogs are not definitive hosts, their role as sentinels for environmental oocyst contamination is well-documented. Some surveys have demonstrated that owners of pet dogs have a significantly higher *T. gondii* seroprevalence than people who do not own such animals [20]. Pet dogs infected with *T. gondii* may exhibit similar clinical manifestations to canine distemper, making misdiagnosis common in some pet hospitals. Currently, there are approximately 4

million pet dogs in Hunan Province, and most pet hospitals do not offer *T. gondii* infection detection services for pet dogs, indicating a potential threat to public health in Hunan Province, one of China's renowned tourist destinations.

Nevertheless, this study has several limitations that should be acknowledged. First, the cross-sectional design employed prohibits temporal trend analyses. Second, there was inherent sampling bias toward urban veterinary clinics, underrepresenting tendencies in rural areas. Third, there was no genetic typing to characterize *T. gondii* strains. In view of these limitations, future studies should employ longitudinal study designs and molecular methods across diverse habitats.

In conclusion, this study demonstrates a high seroprevalence of *T. gondii* infection in pet dogs in subtropical China's Hunan Province, with season and age identified as independent risk factors for the infection. However, this serious situation has received little attention in the past. Therefore, effective measures should be taken to prevent and control toxoplasmosis in pet dogs in this province. These measures could include strengthening environmental sanitation management (especially during summer and autumn), promoting regular *T. gondii* testing for pet dogs (especially older ones), and enhancing public awareness of the zoonotic risks of *T. gondii* infection in pet dogs.

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