

## Seroprevalence of *Toxoplasma gondii* infection in domestic cats in central China

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**Abstract** – To investigate the seroprevalence of *Toxoplasma gondii* infection in domestic cats in central China, 843 serum samples were collected in Henan province between March 2015 and May 2016 and tested for IgG antibodies against *T. gondii* using the enzyme-linked immunosorbent assay (ELISA). The overall seroprevalence of *T. gondii* was 21% (178/843). No significant difference was observed based on the sex of cats ( $p > 0.05$ ). Significantly higher seroprevalence ( $p < 0.05$ ) was observed in mixed-breed cats (24%) compared to purebred cats (17%). Seroprevalence in rural cats (29%) was significantly higher ( $p < 0.01$ ) than in urban cats (16%), and increased significantly ( $p < 0.01$ ) with age. These results showed that *T. gondii* was highly prevalent in domestic cats in Henan province, central China, which might have important implications for public health.

**Key words:** *Toxoplasma gondii*, Cat, Seroprevalence, ELISA, Central China.

**Résumé** – Séroprévalence d'infection par *Toxoplasma gondii* chez les chats domestiques en Chine centrale. Pour étudier la séroprévalence de l'infection par *Toxoplasma gondii* chez les chats domestiques en Chine centrale, 843 échantillons de sérum ont été prélevés dans la province du Henan entre mars 2015 et mai 2016 et testés pour l'anticorps IgG contre *T. gondii* par méthode immuno-enzymatique (ELISA). La séroprévalence globale de *T. gondii* était de 21 % (178/843). Aucune différence significative n'a été observée selon le sexe des chats ( $p > 0.05$ ). Une séroprévalence significativement plus élevée ( $p < 0.05$ ) a été observée chez les chats de race mixte (24 %) par rapport aux chats de race pure (17 %). La séroprévalence chez les chats en milieu rural (29 %) était significativement ( $p < 0.01$ ) plus élevée que chez les chats urbains (16 %), et augmentait de façon significative ( $p < 0.01$ ) avec l'âge. Ces résultats ont montré que *T. gondii* était très répandu chez les chats domestiques dans la province du Henan, en Chine centrale, ce qui pourrait avoir des implications importantes pour la santé publique.

### Introduction

*Toxoplasma gondii* is an obligate intracellular protozoan parasite that has a worldwide distribution and infects a wide range of warm-blooded vertebrates, including humans [3]. The ingestion of undercooked meat containing tissue cysts and the ingestion of food or drink contaminated with sporulated *T. gondii* oocysts are important modes of transmission of *T. gondii*. As definitive hosts of this parasite, cats play an important role in the life cycle of *T. gondii*, facilitating the genetic recombination between strains, as well as environmental contamination [12]. Antibodies to *T. gondii* have been

reported in cats worldwide and seroprevalence in cats was recently summarized [2, 4, 8, 9]. The objective of the present survey was to determine the seroprevalence of *T. gondii* in domestic cats in Henan province, central China, and to evaluate the main associated risk factors related to exposure to *T. gondii* in this region [6, 14].

### Materials and methods

#### Ethics statement

The study was reviewed and approved by the Ethics Review Committee of the Xinxiang Medical University (Reference No. 2015016).

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## Study site

The study was conducted in Henan province in the central part of mainland China, covering an area of 167,000 km<sup>2</sup> and having a population of approximately 106 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 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 843 blood samples of domestic cats were collected from these five cities in Henan province between March 2015 and May 2016. Cat owners were asked for details of the animal's age, sex, source, and breed using a structured questionnaire. Blood samples were centrifuged and sera were recovered and transferred to 1.5 mL Eppendorf tubes. All the sera were then 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 (enzyme-linked immunosorbent assay) Kit (Combined Company, Shenzhen, Guangdong Province, China) according to the manufacturer's instructions [5, 13]. The manufacturer's test report showed that the sensitivity and specificity of this ELISA kit were 95% and 100%, respectively. Positive and negative control sera were provided in the kit. Briefly, the *T. gondii* specific antigen was used to coat 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 the chromogenic enzyme substrate was added. Finally, the optical density (OD) values of each well were measured using an ELISA plate reader (Multiskan MK3; Thermo Scientific, Waltham, MA, USA) at 450 nm.

A relative rate percent (IRPC) value was obtained using the following formula:

$$\text{IRPC} = \frac{\text{OD}_{450}(\text{sample}) - \text{mean OD}_{450}(\text{blank control})}{\text{mean OD}_{450}(\text{negative control}) - \text{mean OD}_{450}(\text{blank control})}$$

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

## Statistical analysis

Differences in *T. gondii* prevalence for different variables such as age, breed, and sex 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

Antibodies to *T. gondii* were detected in 21.12% (178/843) of cats (Table 1). Seropositivity percentages from different cities were: 21.84% of 174 from Anyang, 14.56% of 158 from Sanmenxia, 30.54% of 167 from Zhengzhou, 17.68% of 181 from Xinyang, and 20.86% of 163 from Shangqiu.

The seroprevalence of *T. gondii* was higher in males (22.16%, 92/416) than in females (20.14%, 86/427) but the difference was not significant ( $p > 0.05$ ). The seroprevalence of *T. gondii* infection was 16.67% (54/324) in purebred cats and 23.89% (124/519) in mixed-breed cats, showing a significant difference by breed ( $p < 0.05$ ). Moreover, significantly higher seroprevalence was found in rural cats (29.26%), compared to that of cats raised in urban area (16.35%) ( $p < 0.01$ ).

The prevalence of *T. gondii* infection in cats increased significantly ( $p < 0.01$ ) with the increase in age.

## Discussion

The present survey showed that *T. gondii* seroprevalence in cats in rural areas was significantly higher compared to urban cats ( $p < 0.01$ ), which is consistent with reports by other authors [1, 10]. The difference in seroprevalence in urban and rural areas might be explained by differences in local reservoirs of the parasite, both in prey animals and in the environment, which serve as local infection sources for the cats. In Henan, domestic cats are usually kept outdoors in rural areas and often roam more freely with greater access to parasites. In addition, the number of rodents in rural areas is much higher than that in urban areas. Higher *T. gondii* seroprevalence found in cats in rural areas probably results from the carnivorous behavior of cats living outdoors and eating prey animals such as rodents and birds. The higher seropositivity with increasing age of the cats supports the hypothesis that most cats acquire *T. gondii* infection after weaning [1, 7, 13].

In order to protect public health, more measures should be taken. The proper disposal of cat litter, keeping cats indoors to minimize their acquisition of infection from prey or the environment, and reducing the feral cat population are recommended. Reducing close contact with cats and protecting the play areas of children might potentially reduce the oocyst burden [11]. What is more, children, pregnant women, and immunocompromised people should adhere to hygiene principles after contact with soil and cats. Given the high seroprevalence of *T. gondii* IgG antibodies in cats, performing a screening test and determination of the IgG antibody titer in high-risk populations such as pregnant women in Henan province, central China is recommended.

**Table 1.** Seroprevalence of *Toxoplasma gondii* infection in domestic cats in Henan province, central China.

| Variable           | No. examined | No. of positive | Prevalence (%) | 95% CI      | $\chi^2$ | <i>p</i> -Value |
|--------------------|--------------|-----------------|----------------|-------------|----------|-----------------|
| <b>Region</b>      |              |                 |                |             |          |                 |
| Anyang             | 174          | 38              | 21.84          | 15.70–27.98 | 14.327   | <0.001          |
| Sanmenxia          | 158          | 23              | 14.56          | 9.06–20.06  |          |                 |
| Zhengzhou          | 167          | 51              | 30.54          | 23.55–37.52 |          |                 |
| Xinyang            | 181          | 32              | 17.68          | 12.12–23.24 |          |                 |
| Shangqiu           | 163          | 34              | 20.86          | 14.62–27.10 |          |                 |
| <b>Gender</b>      |              |                 |                |             |          |                 |
| Male               | 416          | 92              | 22.16          | 18.13–26.10 | 0.493    | 0.482           |
| Female             | 427          | 86              | 20.14          | 16.34–23.94 |          |                 |
| <b>Breed</b>       |              |                 |                |             |          |                 |
| Purebred           | 324          | 54              | 16.67          | 12.61–20.72 | 6.252    | 0.012           |
| Mixed-breed        | 519          | 124             | 23.89          | 20.22–27.56 |          |                 |
| <b>Area</b>        |              |                 |                |             |          |                 |
| Urban              | 532          | 87              | 16.35          | 13.21–19.50 | 19.630   | <0.001          |
| Rural              | 311          | 91              | 29.26          | 24.20–34.32 |          |                 |
| <b>Age (years)</b> |              |                 |                |             |          |                 |
| ≤1                 | 214          | 15              | 7.01           | 3.59–10.43  | 86.813   | <0.001          |
| 1–3                | 276          | 41              | 14.86          | 10.66–19.05 |          |                 |
| 3–6                | 198          | 53              | 26.77          | 20.60–32.93 |          |                 |
| ≥6                 | 155          | 69              | 44.52          | 36.69–52.34 |          |                 |
| Total              | 843          | 178             | 21.12          | 18.36–23.87 |          |                 |

## Conflict of interest

The authors declare that they have no conflict of interest.

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