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# Seroepidemiology of *Toxoplasma gondii* and *Neospora caninum* infections in goats in Hubei province, China

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**Abstract.** Scarce information is available about the seroprevalence of *Toxoplasma gondii* (*T. gondii*) and *Neospora caninum* (*N. caninum*) infections in goats in Hubei province, China. In the present study, the prevalence of *T. gondii* and *N. caninum* infections in goats were investigated in Hubei province, China between 2014 and 2015. A total 2007 serum samples were collected from 6 counties of Hubei province, China and were tested for antibodies to *N. caninum* and *T. gondii* by an enzyme-linked immunosorbent assay (ELISA) and an indirect agglutination test (IAT), respectively. Antibodies against *T. gondii* and *N. caninum* were detected in 13.4% and 3.9%, respectively in goats. 2% (41) serum samples were positive to both parasites. There was no apparent association of *T. gondii* and *N. caninum* infection with gender of the animals. There were significant differences of *T. gondii* ($p < 0.01$), *N. caninum* ($p < 0.05$) and both parasites ($p < 0.01$) infection with season. This is the first time that antibodies to *T. gondii* and *N. caninum* have been detected in goats in Hubei province, China.

## INTRODUCTION

*Toxoplasma gondii* (*T. gondii*) and *Neospora caninum* (*N. caninum*) are two closely related intracellular protozoan parasites with worldwide distributions (Liu *et al.*, 2015). The two parasites have indirect life cycles with carnivores as their definitive hosts and both protozoa can infect a wide range of animal species and cause clinical diseases in domestic and wild animals (Liu *et al.*, 2008). *N. caninum* and *T. gondii* both are considered as close relatives sharing many common morphological and biological features (Dubey *et al.*, 1988; 2002); however, they are subsequently differentiated based on host preferences, etiology and genetic differences. (Dubey *et al.*, 1988; 2002).

Toxoplasmosis caused by *T. gondii* is one of the most prevalent worldwide zoonotic parasites and the parasite may be transmitted to other animals and humans by providing infected uncooked meat and raw milk (Tenter, 2009; Li *et al.*, 2014). It is estimated that *T. gondii* infects up to one-third of the human population in the world (Dubey & Jones, 2008). Neosporosis caused by *N. caninum* known to cause abortions in ruminants (Anastasia *et al.*, 2013). Although this protozoan parasite has not been demonstrated as a zoonosis, antibodies to

*N. caninum* have been reported in humans (Liu *et al.*, 2015).

The seroprevalence of *T. gondii* and *N. caninum* infection in goats have been investigated worldwide and found to vary greatly (Anastasia *et al.*, 2013; Gebremedhin *et al.*, 2014; Liu *et al.*, 2015). However, limited information is available about the seroprevalence of *T.gondii* and *N. caninum* in goats. Therefore, the objective of the present survey herein was to estimate the seroepidemiology of *T. gondii* and *N. caninum* infections in goats in Hubei province, China for the first time.

## MATERIALS AND METHODS

**Samples Collection:** Blood samples were collected from the jugular vein by local veterinary practitioners from 2007 goats in 2014 and 2015 in 6 counties in Hubei province (Table 1). After collection, each of the samples was centrifuged at 1000 × g for 10 min, and serum was separated and stored at -20 till further analysis.

**Determination of antibodies against *T. gondii*:** All serum samples were tested for antibodies to *T. gondii* using a commercial indirect agglutination test (IAT, Lanzhou Veterinary Research Institute of Chinese Academy of Agricultural Sciences) according to the manufacturer's instructions. The test was considered positive when a layer of agglutinated erythrocytes was formed in wells when using serum dilutions of 1:64 or higher, and positive and negative controls were included in each test (Li *et al.*, 2014).

**Determination of antibodies against *N. caninum*:** Blood samples were tested by means of a commercial enzymelinked immunosorbent assay (ELISA) (IDEXX Neospora X2) according to the manufacturer's instructions. The S/P value was calculated based on the optical density (OD) values according to the formula: S/P = (OD 650 of sample - average OD 650 of negative controls) / (average OD 650 of positive controls - average OD 650 of negative controls). To ensure validity, the difference of average OD 650 of positive controls and negative controls must ≥0.150, also average OD 650 of negative controls must ≤ 0.200. The results were interpreted as positive when the S/P value ≥0.50.

**Statistical analysis:** Statistical analysis of *T. gondii* and *N. caninum* prevalence were performed by chi-square test with SPSS (Statistical Analysis System, Version 17.0). The differences were considered statistically significant at 5% level of significance ($P < 0.05$).

## RESULTS

The results showed that anti-*Toxoplasma* antibodies were detected in all of the 6 counties (100%), the prevalence were ranged from 3.2% to 19.1% and there were significant difference in the different counties ($p < 0.01$); anti-*Neospora* antibodies were detected in 5 counties (83.3%), the seroprecalence were ranged from 0 to 11.7% and there were significant difference in the different counties ($p < 0.01$); antibodies to both parasites were found in 4 counties (66.7%), prevalence were ranged from 0 to 6.1% and there were significant difference in the different counties ($p < 0.01$) (Table 1).

In different genders, the seroprevalence of *T. gondii*, *N. caninum* and both parasites were 12.5% and 14.2%, 3.5% and 4.2%, 1.6% and 2.4% in male and female goats, respectively. The prevalence of *T. gondii*, *N. caninum* and both parasites were all not statistically significant ($p \geq 0.05$) (Table 1).

In different seasons, the prevalence of *T. gondii* were ranged from 2.5% to 16.6% and there was significant difference in the different seasons ($p < 0.01$). Anti-*Neospora* antibodies were ranged from 1.0% to 4.9% and there was significant difference in the different seasons ($p < 0.05$). The prevalence of both parasites were ranged from 0 to 2.9% and there was significant difference in the different seasons ($p < 0.01$) (Table 1).

Table 1. Seroprevalence of *Toxoplasma gondii* and *Neospora caninum* in goats in different counties, genders and seasons in Hubei province, China

| County[abc] | *T. gondii* | | *N. caninum* | | *T. gondii* + *N. caninum* | |
|---|---|---|---|---|---|---|
| | Positive/ examined | Prevalence (%) | Positive/ examined | Prevalence (%) | Positive/ examined | Prevalence (%) |
| A | 41/291 | 14.0 | 11/291 | 3.8 | 6/291 | 2.1 |
| B | 61/450 | 13.6 | 23/450 | 5.1 | 17/450 | 3.8 |
| C | 11/348 | 3.2 | 3/348 | 0.9 | 0/348 | 0 |
| D | 37/213 | 17.4 | 25/213 | 11.7 | 13/213 | 6.1 |
| E | 113/591 | 19.1 | 16/591 | 2.7 | 5/591 | 0.8 |
| F | 6/114 | 5.3 | 0/114 | 0 | 0/114 | 0 |
| Gender | | | | | | |
| Male | 117/934 | 12.5 | 33/934 | 3.5 | 15/934 | 1.6 |
| Female | 152/1073 | 14.2 | 45/1073 | 4.2 | 26/1073 | 2.4 |
| Season[def] | | | | | | |
| Spring | 37/356 | 10.4 | 9/356 | 2.5 | 2/356 | 0.6 |
| Summer | 92/571 | 16.1 | 27/571 | 4.7 | 16/571 | 2.8 |
| Autumn | 133/799 | 16.6 | 39/799 | 4.9 | 23/799 | 2.9 |
| Winter | 7/281 | 2.5 | 3/281 | 1.0 | 0/281 | 0 |
| Total | 807/6021 | 13.4 | 234/6021 | 3.9 | 123/6021 | 2.0 |

[a]There were significant difference in the prevalence of *T. gondii* in different counties ($p < 0.01$ x2=57.619)
[b]There were significant difference in the prevalence of *N. caninum* in different counties ($p < 0.01$ x2=52.291)
[c]There were significant difference in the prevalence of both parasites in different counties ($p < 0.01$ $\chi^2 = 38.183$)
[d]There were significant difference in the prevalence of *T. gondii* in different seasons ($p < 0.01$ $\chi^2 = 42.455$)
[e]There were significant difference in the prevalence of *N. caninum* in different seasons ($p < 0.05$ $\chi^2 = 10.936$)
[f]There were significant difference in the prevalence of both parasites in different counties ($p < 0.01$ $\chi^2 = 14.196$)

## DISCUSSION

Since the first discovery of complete life cycle of *T. gondii* in 1970, numerous of wild and domestic animals have been identified as intermediate hosts world widely. Previously, Anastasia *et al*. (2013) has reported the presence of *T. gondii* in sheep and goats in Greece with the prevalence of 53.71% and 61.3% in sheep and goats, respectively; Gebremedhin *et al*. (2014) reported that the prevalence of *T. gondii* infection in goats in Central Ethiopia was 15.48%; Jung *et al*. (2014) reported that the prevalence of *T. gondii* infection in Native Korean Goats was 5.1%. In domestic China, the prevalence of *T. gondii* infection in sheep and goats in Qinghai province, China were 21.33% and 29.54%, respectively (Liu *et al*., 2015). In the present study herein, the seroprevalence of *T. gondii* infection in goats were 13.4%, which was between previous study. The differences maybe due to different investigative methods (Liu *et al*., 2015). A significant difference ($p < 0.01$) was observed among different counties in the region due to the hygienic conditions and variations in cat population, a definitive host of *T. gondii*. No significant difference ($p < 0.01$) was seen between different gender because of having the same opportunity to be infested by this protozoan. In different seasons, there were significant difference ($p < 0.01$) and the prevalence were much higher in summer and

autumn, the reason may because of the wild climate and more activities of animals in these time.

After *N. caninum* was first discovered and fully described in 1988, natural *N. caninum* infection has been detected in a number of species including goat and serological evidence of *N. caninum* infection has been found in wild ruminants (Liu *et al*., 2008). The seroprevalence of *N. caninum* in goats was found 3.9% in present survey which is in accordance with the previous studies (Iovu *et al*., 2012; Anastasia *et al*., 2013; Topazio *et al*., 2014; Unzaga *et al*., 2014; Liu *et al*., 2015). The different diagnostic methods and cutoffs used by each author are some of the factors that influences that broad frequency range (Silva *et al*., 2015). There was significant difference in the different counties ($p < 0.01$), the reason may because of difference in presence of dogs, hygiene conditions. In different genders, the prevalence was 3.5% and 4.2% in male and female goats, respectively. There were not statistically significant ($p \geq 0.05$) in the prevalence in different genders, which may due to the two genders have same chance infecting this parasite. In different seasons, there were significant difference in the different seasons ($p < 0.05$) and the prevalence were much higher in summer and autumn, which maybe due to the wild climate and increasing activity of dogs in these seasons.

Both *T. gondii* and *N. caninum* with same morphological and biological properties are reported to affect the ruminants resulting in fetal mortality, abortion and nervous system disorders (Liu *et al*., 2008) causing tremendous economic losses in goat production. In present study, 2% (41) serum samples were found positive for both protozoa which was lower than that in Qinghai (Liu *et al*., 2015). The higher frequency of seropositive animals among the *T. gondii*-affected goats may be related to the management practice and their grazing habits directly on the ground making them more likely to ingest the food contaminated with *T. gondii* oocysts (Silva *et al*., 2015). As Li *et al.* (2016) reported that the prevalence of *T. gondii* in pet dogs in Wuhan of Hubei province, Chinas was 13.3%. The infected goats may have potential threat to transmit this protozoan to other animals, even human beings which can be infected by *T. gondii* through water or undercooked meat and meat-derived products (Li *et al*., 2014; Liu *et al*., 2015). Though, raw milk is less related to *T. gondii* transmission than other foods, human toxoplasmosis outbreaks with transmission of *T. gondii* by raw milk have been reported previously (Silva *et al*., 2015). The low prevalence of antibodies to *N. caninum* may reflect low rates of infection in other hosts such as dogs, goats or wild animals in this area, this is yet to be confirmed by epidemiology studies (Liu *et al*., 2008).

In the present study, the prevalence of *N. caninum* was found to be significantly lower than *T. gondii*, consistent with similar surveys in previous studies (Anastasia *et al*., 2013; Liu *et al*., 2015). The results of this survey showed that *T. gondii* infection is highly prevalent in goats, but *N. caninum* infection is not widespread in this species in this area. Therefore, it is imperative to take integrated control strategies and measures to prevent and control *T. gondii* infections in goats in Hubei and further epidemiological studies are required to determine the distribution of these parasites in a wider area. This is the first time that infection with *T. gondii* and *N. caninum* in goats have been reported in Hubei province, China.

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**Conflict of interest:** The authors declare that they have no competing interests.

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