First Report of *Neospora caninum* Seroprevalence in Free-Range Chickens *(Gallus domesticus)* in Central China

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ABSTRACT

Neospora caninum is a protozoan parasite of livestock and companion animals that can cause serious disease in dogs and cattle. Free-range chickens have been used as indicators of soil contamination with *N. caninum* oocysts because they feed from the ground. In the present study, we investigated the seroprevalence of *N. caninum* in free-range chickens from different cities in Henan province, central China. A seroprevalence study of a total of 1,389 free-range chickens was conducted to detect anti-*N. caninum* antibodies using the indirect fluorescent antibody test (IFAT). The overall seroprevalence of *N. caninum* in free-range chickens in Henan province, central China was 19.37% (269/1,389). The highest *N .caninum* seroprevalence was detected in Sanmenxia (27.84%), followed by Xinyang (24.01%), Zhengzhou (17.11%), Anyang (16.20%), and the lowest in Shangqiu (11.03%). In conclusion, the present study documents the first survey of *N. caninum* seroprevalence in free-range chickens in China, revealing 19.37% seropositivity. The high prevalence of *N. caninum* antibodies in free-range chickens indicate that soil contamination with *N. caninum* oocysts is substantial in central China.

Keywords: *Neospora caninum*; Seroprevalence; Free-Range; Chicken; Central China.

INTRODUCTION

Neospora caninum (*N. caninum*) is an apicomplexan parasite, the etiologic agent of neosporosis, a devastating infectious disease regarded as a major cause of reproductive loss in cattle and neuromuscular disease in dogs worldwide (1-3).

N. caninum infects a large spectrum of domestic and wild mammals. Its definitive hosts include dog and the dingo, coyote, and the gray wolf (4-6). Many mammals, including dogs, cattle, water buffalo, horses, sheep, goats, bison, and white-tailed deer can serve as natural intermediate hosts for *N. caninum* (7, 8). Recent studies have broadened the list of known intermediate hosts to include birds such as chickens (9), pigeons (10) and sparrows (11).

In recent years, seroprevalence studies of *N. caninum* in chickens have been conducted extensively in various parts of the world (9, 12, 13). In 2008, antibodies to *N. caninum* were found in 23.5% of 200 free-range and 1.5% of 200 indoor chickens from Brazil, and *N. caninum* DNA was detected in 6 of 10 seropositive chickens (9). Then subsequently, antibodies to *N. caninum* were found in sera of free-range chickens from different countries in the Americas. The seropositive rate of chickens was 7.2% to 18.5% in North America, 39.5% to 83.6% in Central America, and 11.2% to 62.3% in South America (12). In 2013, Ibrahim reported that the overall seroprevalence of *N. caninum* in chicken from Delta, Egypt was 15.51% (13).

However, little is known about the prevalence of *N. caninum* in domestic chickens in China. Moreover, free-range chickens have been used as indicators of soil contamination

with *N. caninum* oocysts because they feed from the ground. Therefore, this study was performed for the first time to determine the prevalence of *N. caninum* infection in free-range chickens in Henan province, central China.

MATERIALS AND METHODS

Ethical statement

The study was reviewed and approved by the ethical review committee of the Xinxiang Medical University (reference no. 2015018).

Free-range chickens and sampling locations

The study was conducted in Henan province, 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 between longitude and latitude, 110°21' - 116°39' and 31°23' - 36°22', respectively. 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, Sanmenxia, Zhengzhou, Xinyang and Shangqiu, located in the northern, western, central, southern and eastern parts of Henan province, were selected for sample collections.

Blood samples

A total of 1389 blood samples from free-range chickens were collected from the above five cities in Henan province between January and December 2015. Data regarding gender, age, and location of these chickens were also recorded. All free-range chickens were raised on the ground, and allowed to roam freely without fencing during the daytime and only housed at night.

Sera preparation

Blood samples were centrifuged and sera were recovered and transferred to 1.5 ml Eppendorf tubes. Thereafter, all the sera were stored at -80°C until tested for *N. caninum* antibodies.

Indirect fluorescent antibody test (IFAT)

The reference method for the detection of antibodies directed to *N. caninum* is the indirect fluorescent antibody test (IFAT) (14-16). The IFAT was performed to detect anti-*N. caninum* antibodies in chicken sera as previously described by Stuart *et al*. (2012) (17) and Martins *et al*. (2011) (12) with some modifications. Briefly, Teflon-masked slides were coupled with *N. caninum* NC-1 tachyzoites maintained in Vero cells (*Neospora caninum* FA Substrate Slide, Catalog no. SLD-IFA-NC, VMRD Inc, Washington, USA). Complete *N. caninum* tachyzoites were used as an antigen and goat anti-Chicken IgY(H+L)-FITC Conjugate (Catalog no. 6100-02, SouthernBiotech, Birmingham, USA) was used as secondary antibody. Serum from a specific-pathogen-free (SPF) chicken experimentally infected with 10^4 tachyzoites of the strain of NC-1 was used as positive control serum. A negative control serum was obtained from a SPF chicken. Both positive and negative controls were used on each slide. Each serum sample was screened using 1:50 dilution (cut-off value) and only a bright fluorescence of the whole tachyzoite surface was considered as a positive result (12).

Statistical analysis

Statistical analysis was performed using SPSS 20 software for Windows (SPSS Inc, Chicago, Illinois, USA). Statistical analyses of *N. caninum* prevalence using different categorical variables were performed by X^2 test. The differences were considered statistically significant when $p < 0.05$.

RESULTS

In the present study, a total of 1,389 blood samples from free-range chickens was collected and examined for antibodies against *N. caninum* using the IFAT.

As shown in Table 1, overall recorded seroprevalence of *N. caninum* in free-range chickens in Henan province, central China, was 19.37% (269/1,389). Seropositive chickens from different cities were: 16.20% of 284 from Anyang, 27.84% of 291 from Sanmenxia, 17.11% of 263 from Zhengzhou, 24.01% of 279 from Xinyang, and 11.03% of 272 from Shangqiu.

The seroprevalence was 18.30% (123/672) and 20.36% (146/717) in males and females, respectively (Table 1). Although the seroprevalence in females was higher than males, the difference was not significant ($p>0.05$).

The prevalence of *N. caninum* seropositivity in chickens increased significantly ($p<0.01$) with age. The highest prevalence of *N. caninum* infection was 27.64%, which was detected in ≥120 day old chickens (Table 1).

Table 1. Seroprevalence of *Neospora caninum* infection in free-range chickens in Henan province, central China.

Variable	No.examined	No. positive	Prevalence (%)
Region			
Anyang	284	46	16.20
Sanmenxia	291	81	27.84[a]
Zhengzhou	263	45	17.11[b]
Xinyang	279	67	24.01[ac]
Shangqiu	272	30	11.03[bcd]
Gender			
Male	672	123	18.30
Female	717	146	20.36
Age (days)			
≤60	353	39	11.05
60~120	667	128	19.19[A]
≥120	369	102	27.64[AB]
Total	1389	269	19.37

[a] represent $p<0.05$ when compared with Anyang;
[b] represent $p<0.01$ when compared with Sanmenxia;
[c] represent $p<0.05$ when compared with Zhengzhou;
[d] represent $p<0.01$ when compared with Xinyang;
[A] represent $p<0.01$ when compared with ≤60 days group;
[B] represent $p<0.01$ when compared with 60~120 days group.

DISCUSSION

Many serological surveys of *N. caninum* infections in different animals have been conducted using diverse methods in different regions in the world. However, very few studies have investigated the distribution of *N. caninum* infections in China (18-22). Meng *et al.* reported that 13.6% (in 2013) and 15.8% (in 2014) of the serum samples collected from farmed sika deer in Liaoning, Jilin and Heilongjiang provinces were positive for *N. caninum*, respectively (18). Specific IgG antibodies for *N. caninum* were detected in 10.33% and 7.23% of the sheep and goats in China, respectively (19). Twenty-eight of 103 (27.2%) sera from farm-bred blue foxes were positive for *N. caninum* (20). Antibodies to *N. caninum* also were found in 6 (6.25%) of 96 dogs from Jilin, Henan and Anhui provinces of China with titers of 1:25 in 2, 1:50 in 3, and 1:100 in one dog (21). In 2007, Yu *et al.* reported that the overall seroprevalence of *N. caninum* in dairy cattle was 17.2% (45/262), and the herds seroprevalence of *N. caninum* was 88.9% (8/9) in China (22). Until now, nothing was known about the prevalence of this parasite in chickens in China.

The present survey showed that the overall seropositivity for *N. caninum* infection in free-range chickens was 19.37 %, which was higher than that observed in Colombia (11.2%) and USA (7.2%) (12), similar to that in Egypt (21.43%) (13), Mexico (18.5%) and Venezuela (21.7%) (12), but lower than that in the Brazil (23.5%) (9), Argentina (58.1%) and Chile (62.3%) (12). These differences may be due to differences in the sensitivities of the various serological tests used, chicken breeds examined, or climatic factors of each region, or due to a combination of these conditions. The high seroprevalence of neosporosis in chickens indicated that soil contamination due *N. caninum* oocysts was widespread in China.

Age-related prevalence data in dogs indicate that most animals become infected after birth; higher prevalences have been documented in older versus younger dogs (23). Similarly, in the present study, the prevalence of *N. caninum* infection in chickens increased significantly ($p<0.01$) with an increase in age. This may be due to the increased opportunities over time that chickens have had to be exposed to *Neospora* or due to the long lasting serological immunologic memory of specific anti-*N. caninum* IgG antibodies antibodies in affected birds.

This is the first survey to evaluate the seroprevalence of *N. caninum* in free-range chickens in China. Our results indicated a widespread exposure of free-range chickens to *N. caninum* in China. We have highlighted an alarming situation indicating possible soil contamination with *N. caninum* oocysts widespread in central China.

ACKNOWLEDGEMENTS

This study was supported by the Doctoral Scientific Research Activation Foundation of Xinxiang Medical University (No. XYBSKYZZ201504), the National Natural Science Foundation of China (No. 81502313) and the Key Scientific and Technological Project of Xinxiang City (No. ZG15014).

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