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Seroprevalence and risk factors of *Toxoplasma gondii* infection in domestic sika deer (*Cervus nippon*) in northeastern China

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ABSTRACT

Toxoplasmosis is a worldwide zoonosis caused by *Toxoplasma gondii*, which can infect warm-blooded animals and humans. A serological survey was undertaken to examine the seroprevalence and risk factors associated with *T. gondii* infection in sika deer in northeastern China. 114 (13.46%, 95% CI 11.16–15.76) out of 847 serum samples were positive to *T. gondii* by modified agglutination test (MAT) at a 1:25 cut-off, with titers of 1:25 in 44, 1:50 in 32, 1:100 in 17, 1:500 in 11, 1:1500 or higher in 10. These samples were collected between November 2012 and October 2013 from Inner Mongolia, Jilin and Heilongjiang provinces in China. However, statistically significant differences were not observed between *T. gondii* seroprevalence and genders or regions of sika deer in the logistic regression analysis ($P > 0.05$) and left out of the final model. Seroprevalence of *T. gondii* infection in male sika deer was 14.07% (95% CI 11.14–17.01), slightly higher than that in the female (12.38%) (95% CI 8.69–16.06) and seroprevalence of *T. gondii* infection in Harbin, Changchun city, Jilin city and Chifeng city were 12.02% (95% CI 7.60–16.44), 15.51% (95% CI 11.52–19.50), 12.27% (95% CI 7.23–17.31) and 12.50% (95% CI 7.38–17.63), respectively. Seasons of sampling were considered as main risk factors associated with *T. gondii* infection, autumn (15.32%) were more than two times (OR = 1.98, 95% CI = 1.18–3.33, $P = 0.01$) at risk of acquiring *T. gondii* infection compared to winter (8.37%). Our results indicated a widespread exposure to *T. gondii* among sika deer in China. To our knowledge, this is the first report of *T. gondii* seroprevalence in sika deer in China.

1. Introduction

Toxoplasma gondii is an intracellular protozoan parasite. The life cycle of *T. gondii* includes three infectious stages, the cats and other felids are the only recognized definitive hosts and a large variety of homeothermic animals are intermediate hosts, including humans and white-tailed deer, mule deer and other deer (Dubey, 2010). It is transmitted to humans and animals via ingesting *T. gondii* tissue cysts from undercooked meat and consuming water or food contaminated with *T. gondii* oocysts, or vertical spread to posterities through transplacental transmission (Dubey and Jones, 2008; Yu et al., 2013). Toxoplasmosis is one of the serious diseases related to abortion, fetal death and neonatal death and also associated with HIV/AIDS (Djurkovic-Djakovic, 2002; Elmore et al., 2010; Gao et al., 2012). Approximately 1/3 of the world population and 7.88% of population in China have been infected with *T. gondii* (Dubey, 2010; Tian et al., 2012; Zhou et al., 2011). Toxoplasmosis can be asymptomatic acquired by most patients (Dubey, 2010; Montoya and Liesenfeld, 2004), and no vaccine was available to prevent human infection with *T. gondii*.

Sika deer (*Cervus nippon*) represents the most ancient and primitive members of the genus *Cervus*. At present, the number of domesticated sika deer in China is approximately 550,000 head. Velvet antlers are one of the main products from sika deer, and are used in traditional Chinese medicine (Li et al., 2013). In addition, sika deer constitutes a considerable part of the meat consumption in northeast China. In China, the prevalence of *T. gondii* infection is continuously increasing in recent years (Zhou et al., 2011). Public health concerns related to *T. gondii* clearly shown that epidemiological investigations of *T. gondii* infection in animals are an important task, particularly those that being consumed by humans as food.

There are some surveys about seroprevalence of *T. gondii* in sika deer around the world. (Matsumoto et al., 2011; Omata et al., 2005), But little is known about the seroprevalence of *T. gondii* infection in sika deer in China. In this survey, we investigated the

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Fig. 1. Map showing Heilongjiang Province, Jilin Province and Inner Mongolia Autonomous Region shadowed in different color (red, yellow and green) in northeastern China, where sika deer serum samples were collected for detection of *Toxoplasma gondii* antibodies. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

seroprevalence of *T. gondii* infection in sika deer in northeastern China and assessed its associated risk factors.

2. Materials and methods

2.1. The investigation site

The present study was carried out in four cities of northeastern China (Fig. 1). Changchun city (43°05'–45°15'N, 124°18'–127°05'E) is the capital of Jilin province and the climate in Changchun is a semi-wet, monsoon type and the average annual temperature is 4.8 °C. Jilin city (42°31′–44°40′N, 125°40′–127°56′E) was also located in Jilin province and the climate of Jilin city is northerly continental monsoon type with the average annual temperature of 3.9 °C. Harbin (44°04'–46°40'N, 125°42'–130°10'E) is the capital of Heilongjiang province and the tenth most populous city nationally. The climate in Harbin is a temperate continental monsoon type with the average annual temperature of 3.5 °C. Chifeng city (41°17'–45°24'N, 116°21'–120°58'E) is located in the upper reaches of the Xiliao River. The prevailing climate in Chifeng is a local steppe climate with the average annual temperature of 7.6 °C. Sika deer is an important sector of livestock industry in Changchun, Chifeng and Harbin cities.

2.2. Serum samples

A total of 847 serum samples were collected from 10 sika deer herds of the farms over 100 head registered from four locations of Chifeng city in Inner Mongolia, Harbin city in Heilongjiang province and Changchun and Jilin cities in Jilin province, northeastern China, from November 2012 to October 2013. Serum was separated by centrifugation at 1000 × *g* for 10 min and serum was obtained, frozen, and stored at −20 °C until detected. Information about species, geographic origin and gender were acquired from farm administrators.

2.3. Serological examination

Antibodies to *T. gondii* were examined using the modified agglutination test (MAT). The detection procedures were carried out as described previously (Dubey and Jones, 2008; Gamarra et al., 2008). *T. gondii* whole cell antigen was purchased from KeraFAST, Inc. (USA). This antigen was prepared using the RH strain of *Toxoplasma* cultivated via human foreskin fibroblast cells in culture. In brief, two-fold dilutions of serum samples were performed using the serum diluting buffer, and agglutination was performed in U-bottom 96-well microtiter plates using a mixture of 50 μL antigen and 50 μL diluted serum. The plates were incubated at 37 °C overnight. The test was considered positive when a layer of agglutinated parasites was formed in wells at dilutions of 1:25 or higher. Each serum sample was tested at dilutions of 1:25, 1:50, 1:100 and 1:500, and positive and negative control were included in each test (Gamarra et al., 2008). Although the specificity and sensitivity of MAT have not been evaluated for the diagnosis of toxoplasmosis in sika deer, it is the most evaluated and specific test for the diagnosis of toxoplasmosis in animals, particularly in pigs (Dubey, 1997; Dubey et al., 1995).

2.4. Statistical analysis

Positive samples were assessed among the 847 sika deer samples, as well as all factors (gender, season and geographical origin of deer). Exploratory analysis was performed to explore variables potentially associated with exposure to *T. gondii* infection, all the factors were studied in a multivariable logistic regression model, and probability (P) value <0.05 was considered as statistically significant between levels within factors and interactions. Odds-ratios (OR) with 95% confidence intervals based on likelihood ratio statistics are reported. All statistical analyses were performed using SPSS (Release 18.0 standard version, SPSS Inc., Chicago, Illinois).

3. Results

In the present study, a total of 114 (13.46%, 95% CI 11.16–15.76) out of 847 serum samples from sika deer were seropositive for *T. gondii* infection by MAT at a 1:25 cut-off, with titers of 1:25 in 44, 1:50 in 32, 1:100 in 17, 1:500 in 11, 1:1500 or higher in 10 (Table 1). As shown in Table 2, the prevalence of *T. gondii* infection in sika deer from Harbin city (n = 208), Changchun city (n = 316), Jilin city (n = 163) and Chifeng city (n = 160) were 12.02% (95% CI 7.60–16.44), 15.51% (95% CI 11.52–19.50), 12.27% (95% CI 7.23–17.31) and 12.50% (95% CI 7.38–17.63), respectively.