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Seroprevalence of *Toxoplasma gondii* infection in free-range chickens in Jilin Province, northeastern China

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Abstract. The prevalence of *Toxoplasma gondii* infection in free-range chickens in Jilin province, northeastern China was investigated. A total of 1095 serum samples were collected from nine administrative regions from July to October, 2012, and antibodies to *T. gondii* were examined by an in-house enzyme-linked immunosorbent assay (ELISA) using *Toxoplasma* lysate antigen (TLA). The detection results were confirmed by Western blot. The overall seroprevalence of *T. gondii* in free-range chickens was 17.6% (95% confidence interval [CI], 15.4–19.4%), ranging from 13.3% (95% CI, 6.3–20.4%) in Siping to 23.6% (95% CI, 15.7–31.6%) in Liaoyuan. There was no significant difference in *T. gondii* infection among different regions in Jilin province ($P > 0.05$). The widespread presence of *T. gondii* infection in free-range chickens of Jilin province implies the wide contamination with *T. gondii* oocysts in the living environment of people, and free-range chickens might be an important source of infection for humans.

INTRODUCTION

Toxoplasma gondii is an obligate intracellular protozoan with an extremely broad host range with the ability to infect almost all mammals and birds. The infection with *T. gondii* may cause abortion in pregnant women, or serious disease in fetuses and in immunocompromised individuals (Montoya & Liesenfeld, 2004). It is estimated that about 25% of the world population is carrying the parasite (Petersen, 2007). The infections are usually acquired by ingesting undercooked or raw meat containing tissue cysts, or ingesting oocyst-contaminated food or water (Tenter *et al.*, 2000).

Chickens play an important role in the epidemiology of *T. gondii* infection, as they are an efficient infection source for cats that excrete the environmentally resistant oocysts, and humans may become infected after eating undercooked *T. gondii*-infected chicken meat (Dubey, 2010a). The free-range chickens are considered as an important indicator of soil contamination with *T. gondii* oocysts (Dubey, 2010b). Though *T. gondii* can rarely cause clinical disease in chickens, tissues of infected chickens are considered an important infection source for humans, cats, and other animals (Dubey, 2010a).

Several sero-epidemiological surveys on *T. gondii* infection in free-range chickens have been carried out in China, showing a prevalence of 11.2% in Shenyang (Yang *et al.*, 2012), 10.1% in Lanzhou (Cong *et al.*, 2012), 11.4% in Guangdong (Yan *et al.*, 2009), 18.8% in Jinzhou (Xu *et al.*, 2012), and 34.7% in a few villages in northeastern China (Zhu *et al.*, 2008). Zhao *et al.* (2012) investigated *T. gondii* infection in free-range chickens in 13 provinces/municipalities in China, with the overall prevalence of 19.3%, ranging from 0% in Anhui to 35.2% in Jiangsu. Here, we report seroprevalence of *T. gondii* in free-range

chickens from Jilin province of northeastern China.

MATERIALS AND METHODS

Toxoplasma lysate antigen (TLA) preparation

Toxoplasma lysate antigen (TLA) was prepared as described by Zhu and colleagues (Zhu *et al.*, 2008). Briefly, *T. gondii* tachyzoites were maintained in African Green Monkey Kidney (Vero) cells. Parasites from freshly lysed host cells were harvested, washed twice in phosphate buffered saline (PBS) and sonicated 3×30 s at 5 kHz. The cell debris was spun down, and the supernatant with TLA was collected. The TLA was diluted to a final concentration of 1 mg/ml in PBS.

Studied areas and sample collection

Jilin province lies in the central part of northeastern China. The province has a total area of 190,000 km^2 and a total population of 27.3 million, with a northerly continental monsoon climate with long, cold winters and short, warm summers. Average January temperatures range from -20 to -14°C. Rainfall averages at 350 to 1000 mm.

The blood samples were collected by venipuncture from 1095 free-range chickens in 9 administrative regions of Jilin Province (Fig. 1). The number of serum samples from each region is shown in Table 1. Sera were separated by centrifugation at 1,500×g for 5 min and stored at -20°C until use.

ELISA

ELISA was conducted as described previously (Zhu *et al.*, 2008). Briefly, the microtiter plates were coated with 50 µl of TLA at a concentration of 5 µg/ml, and incubated with chicken sera (1:100 dilution). After washing, anti-chicken IgY peroxidase-labeled conjugates (Thermo Scientific, USA), (1:20000 dilution) were added to each well.

Figure 1. Map of Jilin province, Northeastern China. Shadowed areas were the sampling locations for the present survey

Table 1. Seroprevalence of *T. gondii* infection in FR chickens in Jilin province, northeastern China

Location	No. tested	No. positive	Prevalence, % (95%, CI[a])
Changchun	110	16	14.6 (8.0, 21.1)
Jinlin	90	14	15.6 (8.8, 24.7)
Tonghua	130	28	21.5 (14.5, 28.6)
Siping	90	12	13.3 (6.3, 20.4)
Liaoyuan	110	26	23.6 (15.7, 31.6)
Baishan	170	25	14.7 (9.4, 20.0)
Yanbian	180	31	17.2 (11.7, 22.7)
Songyuan	105	21	20.0 (12.4, 27.7)
Baicheng	110	20	18.2 (11.0, 25.4)
Total	1095	193	17.6 (15.4, 19.9)

[a] CI, confidence interval.

The enzymatic activity was revealed by addition of tetramethylbenzidine chromogenic substrate (TMB, Thermo Fisher Scientific, USA), and the color development reaction was stopped by adding 50 µl of 1 M sulfuric acid and the color intensity was measured in a microtiter plate reader (BioTek ELx800) at 450 nm. Each sample was tested twice and the result was determined by calculating the mean value of the optical density (OD) for duplicate wells. A serum sample was considered positive if the OD of the sample/the OD of the negative control was $\geq$2.0.

Western blot assay

To further confirm the detection results of the ELISA method, several positive and negative serum samples were tested by Western blot assays (Zhu *et al.*, 2008). Briefly, TLA was separated by SDS-PAGE gel, and transferred to a polyvinylidene fluoride membrane. The membrane was cut into strips and incubated with positive and negative sera (1:500 dilution) identified in the ELISA assays. The membrane strips were further incubated with an anti-chicken IgY peroxidase-labeled conjugates (Thermo Scientific) antibody (1:5000 dilution) after washing in TBST buffer (10 mM Tris, 150 mM NaCl, pH 8.0 and 0.05% Tween 20). Eventually, the strips were incubated with DAB substrate solution to visualize the protein bands that were recognized by the specific antibodies.

Data analysis

The difference of *T. gondii* prevalence in chickens from different regions was compared by the chi-square test using SAS statistical software (v. 9.3; SAS Institute Inc., Cary, NC), and p values less than 0.05 were considered statistically significant.

RESULTS AND DISCUSSION

As free-range chickens get food from the ground or the soil, they are recognized as an important indicator of soil contamination with the environmentally resistant oocysts of *T. gondii* (Dubey, 2010b). Many investigations of *T. gondii* infection in free-range chickens have been conducted in a number of countries, showing various seroprevalences ranging from 12.5% to 90% (Dubey *et al.*, 2005a; 2005b; 2006; 2008; de Oliveira *et al.*, 2009; Harfoush & Tahoon Ael, 2010; Beltrame *et al.*, 2012; Chumpolbanchorn *et al.*, 2013; Tilahun *et al.*, 2013). In China, the prevalence of *T. gondii* infection in free-range chicken were found to be 10-35%, significantly lower than other countries, such as Brazil and Australia, where the prevalence could reach above 60% (de Oliveira *et al.*, 2009; Chumpolbanchorn *et al.*, 2013).

In the present survey, of the 1095 serum samples tested, 193 (17.6%; 95% CI, 15.4–19.9%) were seropositive for *T. gondii* by

ELISA, with the following distributions: 14.6% (95% CI, 8.0–21.1%) in Changchun, 15.6% (95% CI, 8.8–24.7%) in Jilin, 21.5% (95% CI, 14.5–28.6%) in Tonghua, 13.3% (95% CI, 6.3–20.4%) in Siping, 23.6% (95% CI, 15.7–31.6%) in Liaoyuan, 14.7% (95% CI, 9.4–20.0%) in Baishan, 17.2% (95% CI, 11.7–22.7%) in Yanbian, 20.0% (95% CI, 12.4–27.7%) in Songyuan, 18.2% (95% CI, 11.0–25.4%) in Baichen (Table 1). There was no significant difference of the prevalence of *T. gondii* among different regions ($P > 0.05$). A previous survey showed a higher prevalence (34.7%) of *T. gondii* infection in free-range chickens in northeastern China (Zhu *et al.*, 2008). Due to the relative small sample size of the study, the results could not probably reflect the infection rates of *T. gondii* in free-range chickens in the region. The overall prevalence of 19.3% in free-range chickens from 13 provinces/municipalities in China was similar to the results (17.6%) of present study (Zhao *et al.*, 2012).

There are many serological tests available to detect *T. gondii* antibodies in chickens. The dye test is one of the most sensitive and specific tests for the diagnosis of toxoplasmosis in humans, but it does not work well in chickens (Dubey *et al.*, 1993). The indirect hemagglutination test is an insensitive test, its sensitivity and specificity may be less than 50% in experimentally infected chickens (Frenkel, 1981; Dubey *et al.*, 1993). The modified agglutination test (MAT) is widely used to detect *T. gondii* infection in chickens (Dubey *et al.*, 2008; Tilahun *et al.*, 2013). However, the MAT titer specific for the diagnosis of toxoplasmosis in poultry has not been determined because *T. gondii* could sometimes be isolated from chickens with a MAT titer of only 1:5 (Dubey, 2010a). The ELISA test can be automated and is convenient for large-scale surveys. Its sensitivity may be higher and could perform better than the MAT (Hill *et al.*, 2006; Garcia *et al.*, 2008). Thus, we used ELISA methods to detect the infection with *T. gondii* in chickens in the present study.

Western blot can be used as an aid to confirm the results of serological tests. In the present study, the ELISA results, including 12 positive and 78 negative samples, were further verified by western blot assay using TLA. Results demonstrated only the positive sera by the ELISA assay reacted with TLA, while no reaction was seen with the negative sera (Fig. 2), suggesting that the ELISA data obtained from sera of free-range chickens reflected the prevalence of *T. gondii* infection in the studied samples.

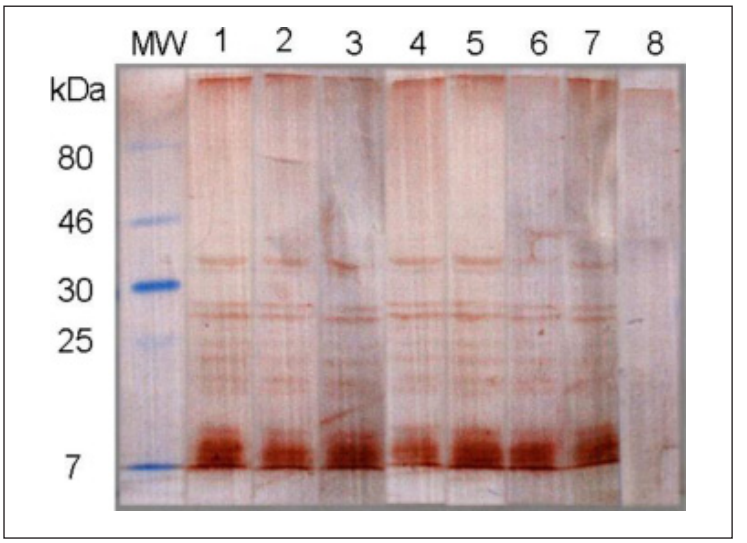

Figure 2. The representative positive and negative samples confirmed by Western blot. MW, molecular weight. Lanes 1–7, the ELISA-positive samples showing reaction with TLA; Lane 8, the ELISA-negative sample showing no reaction with TLA

Although the disease caused by *T. gondii* infection in chickens is rare, the parasite has been isolated from free-range chickens, and consuming undercooked infected chicken is an important source of *T. gondii* infection for humans and other animals (Boyer *et al.*, 2005). The results of the present and other studies indicated that soil contamination with *T. gondii* oocysts may be an important infection source of *T. gondii* for humans.

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