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The prevalence of antibodies to *Toxoplasma gondii* in horses in Changji Hui Autonomous Prefecture, Xinjiang, northwestern China

Prevalência de anticorpos contra *Toxoplasma gondii* em cavalos na Região de Uygur, Xinjiang, Noroeste da China

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Abstract

Toxoplasmosis is a worldwide zoonosis caused by *Toxoplasma gondii*, which can infect warm-blooded animals and humans. The present study was performed to investigate the seroprevalence of *T. gondii* in horses in Xinjiang, northwestern China. A total of 637 blood samples were collected from seven regions in Changji Hui Autonomous Prefecture, Xinjiang in 2011 and assayed for *T. gondii* antibodies using the modified agglutination test (MAT). Risk factors (age, gender, and region) related to seroprevalence were determined by a multivariate logistic regression analysis. A total of 200 horses (31.4%, 95% CI 27.79–35.00) were seropositive for *T. gondii*. Age, gender, and region present no association with seroprevalence (p>0.05) in the logistic regression analysis. The results indicated that *T. gondii* is widely prevalent in horses in Xinjiang, northwestern China, representing a serious threat to animal and human health. Therefore, more careful measures should be performed to control and prevent *T. gondii* infection in horses from Xinjiang, northwestern China.

Keywords: *Toxoplasma gondii*, horse, seroprevalence, modified agglutination test (MAT), China.

Resumo

A toxoplasmose é uma zoonose global causada pelo *Toxoplasma gondii,* o qual pode infectar animais de sangue quente e seres humanos. Este estudo foi realizado com o objetivo de investigar a soroprevalência em cavalos para *T. gondii,* na região de Xinjiang, no Noroeste da China. Em 2011, foram recolhidas 637 amostras de sangue em sete distritos da Prefeitura Autônoma de Changji Hui do Xinjiang, as quais foram testadas para a presença de anticorpos, utilizando-se o teste de aglutinação modificado (MAT). Foram estimados fatores de risco relacionados com a soroprevalência (idade, sexo e distrito), através de uma análise de regressão logística multivariada. Um total de 200 equinos (31,4%, 95% IC 27,79 – 35,00) foi positivo para *T. gondii*. Idade, sexo e região estudada não apresentaram associação com a soroprevalência (p>0,05) na análise de regressão logística. Os resultados revelam que a infecção por *T. gondii* tem uma prevalência generalizada em todo o território de Xinjiang, no Noroeste da China, constituindo uma séria ameaça à saúde de animais e de humanos. Consequentemente, propõe-se que sejam adotadas medidas reforçadas para o controle e prevenção da infecção de cavalos por *T. gondii*, no Xinjiang, Noroeste da China.

Palavras-chave: *Toxoplasma gondii*, cavalo, soroprevalência, teste de aglutinação modificado (MAT), China.

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Introduction

Toxoplasma gondii is an important zoonotic intracellular apicomplexan protozoan that can infect humans and nearly all warm-blooded animals worldwide (DUBEY, 2010; MONTOYA & LIESENFELD, 2004; TENTER et al., 2000; SOUSA et al., 2014; CERRO et al., 2014; BARROS et al., 2014). Humans and animals can be infected mainly through the ingestion of tissue cysts from undercooked or raw meat or through the consumption of food or water contaminated with sporulated oocysts shed by infected felids (KIJLSTRA & JONGERT, 2008). Around the world, approximately one-third of the human population has been infected with *T. gondii*, and it is estimated that nearly 7.88% of the population in China has been exposed to *T. gondii* (DUBEY, 2010; ZHOU et al., 2011). Toxoplasmosis is typically asymptomatic in healthy individuals but can cause mortality and morbidity in congenitally infected and immunocompromised patients, such as individuals with AIDS (MONTOYA & LIESENFELD, 2004; BELANGER et al., 1999).

Felids are the only recognised definitive hosts of *T. gondii*, but humans and virtually all warm-blooded species, including horses, can be intermediate hosts (ELMORE et al., 2010). Horses are widely distributed in the Xinjiang Uygur Autonomous Region, serving as an important means of transportation for the local population. Humans may acquire *T. gondii* infections by consuming improperly cooked horse meat containing tissue cysts (POMARES et al., 2011). In horses, *T. gondii* infection is generally subclinical; however, atypical clinical symptoms, including fever, ataxia, retinal degeneration and encephalomyelitis, sometimes appear (DUBEY et al., 1999). In China, although there are several serological surveys for *T. gondii* infection in horses and some Chinese reports were recently summarised (MIAO et al., 2013; YANG et al., 2013), information on horses remains limited in northwestern China. To the best of our knowledge, there has been only one survey performed in Xinjiang, northwestern China, and it took place more than 10 years ago. In that survey, *T. gondii* antibodies were assayed by IHA, and no positive sample was observed in the 60 serum samples. The aim of the present survey was to examine the prevalence of antibodies against *T. gondii* in horses in Xinjiang, northwestern China, providing fundamental data for understanding the main transmission routes between animals and humans, as well as preventing and controlling this disease.

Materials and Methods

Region and samples

Xinjiang Uygur Autonomous Region is located in northwest China, covering an area of approximately 1,660,000Km^2 and occupying approximately one-sixth of the area of China. The climate in this region is typically temperate and monsoonal continental with an annual precipitation of 150 mm, temperature differences between day and night, and abundant sunshine (yearly sunlight exposure of 2500–35000 h). A total of 637 blood samples were collected from August to December in 2011 in Changji Hui Autonomous Prefecture, Xinjiang. In total, 84, 103, 80, 91, 94, 93 and 92 blood samples were obtained from Miao'ergou, Jimusa'er, Hutubi, Qitai, Manasi, Fukang, and Mulei Kazakh Autonomous County, respectively. Information regarding the age, gender and geographic origin of each horse were obtained. The ages of the horses were classified into four groups: foal (0<age≤1, 129 samples), adolescent (1<age≤5, 118 samples), middle-aged (5<age≤10, 326 samples) and elderly (age>10, 64 samples). In total, 248 of the sampled animals were male, and 389 were female. Blood samples were centrifuged at 3000 × g for 5 min. The separated serum samples were stored at –20°C until use.

Serological assay

T. gondii antibodies were detected in serum samples by the modified agglutination test (MAT) as described previously (DUBEY & DESMONTS, 1987). In brief, serum samples were added to 96-well "U" bottomed polystyrene plates and then diluted twofold from 1:25 to 1:3200. Positive and negative control serums were included in each test, and controversial serums were re-tested. Horse sera with MAT titres of 1:25 or higher were considered positive for *T. gondii* antibodies based on previous studies (YANG et al., 2013; GARCÍA-BOCANEGRA et al., 2012; ALVARADO-ESQUIVEL et al., 2012).

Statistical analysis

Statistical analysis was performed using SAS [Version 8.0] and SPSS [Release 18.0 standard version]. The risk factors (age, gender and region) were analysed using multivariable logistic regression models in SPSS. Differences in the seroprevalence of *T. gondii*-infected male and female horses, among various age groups and regions, were analysed using a *Chi square* test with SPSS. A value of $P<0.05$ was considered statistically significant.

Results

A total of 637 horses from seven regions in Changji Hui Autonomous Prefecture, Xinjiang, were assayed by MAT for *T. gondii* antibodies. In total, 200 of 637 horses (31.4%, 95% CI 27.79–35.00) were seropositive for *T. gondii* with titres of 1:25 in 86 individuals, 1:50 in 55, 1:100 in 37, 1:200 in 11, 1:400 in 2, 1:800 in 3, and 1:1600 or higher in 6. The seroprevalence of *T. gondii* infection from seven regions ranged from 27.7% in Manasi to 37.5% in Hutubi. Infected horses were observed in all age groups, ranging from 26.6–35.7%. Prevalence in female was 33.1% and in male horses 28.9% (Table 1).

Discussion

Age, gender and region were not significant in the logistic regression analysis ($P > 0.05$), suggesting that age, gender and region were not crucial factors for *T. gondii* infection, a finding that was consistent with the results of previous studies (MIAO et al., 2013; YANG et al., 2013). The present study showed that all the regions had *T. gondii* positive horses, and there were no significant

differences among the various regions in Changji Hui Autonomous Prefecture, Xinjiang (P > 0.05). Seroprevalence to *T. gondii* in male horses (28.6%, 95% CI 23.00–34.26) was lower than that in female horses (33.1%, 95% CI 28.48–37.84). Difference was not significant (p>0.05). Similar findings were reportedin a previous study in Portugal (LOPES et al., 2013). Regarding age group, also no association was observed, however the highest prevalence (35.7%, 95% CI 27.39–43.93) was observed in foals. A relatively lower prevalence was observed in older horses (26.6%, 95% CI 15.74–37.38), which was contrary to the result reported in a previous survey in Tunisia (BOUGHATTAS et al., 2011).

Globally, a few surveys have previously reported the prevalence of *T. gondii* in horses (Table 2). In the present study, the overall average *T. gondii* seroprevalence in Changji Hui Autonomous Prefecture, Xinjiang was 31.4%, which was similar to that observed in Yunnan Province (30.5%) but higher than that in Liaoning province (25.0%) (MIAO et al., 2013; YANG et al., 2013). Horses are considered clinically resistant to *T. gondii*, and the prevalence of *T. gondii* in horses was generally lower in most countries, such as 1% in Sweden (JAKUBEK et al., 2006); 1.8% in Greece (KOUAM et al., 2010); 2.6% in Jeju Island, South Korea (GUPTA et al., 2002); 6.1% in Mexico (ALVARADO-ESQUIVEL et al., 2012); 6.9% in North America (DUBEY et al., 1999); 7.2% in Turkey (KARATEPE et al., 2010); 10.8% in southern Spain (GARCÍA-BOCANEGRA et al., 2012); and 13.3% in Portugal (LOPES et al., 2013). However, higher prevalences included 31.6% in Saudi Arabia (ALANAZI & ALYOUSIF, 2011); 34% in Costa Rica (DANGOUDOUBIYAM et al., 2011); 52.6% in Egypt (SHAAPAN & GHAZY, 2007); and 71.2% in Iran (HAJIALILO et al., 2010). These differences in seroprevalence are most likely caused by differences in ecological and geographical factors, serological test methods, living styles and the number of infected cats.

The results of the present study indicated a high prevalence of *T. gondii* in horses in Changji Hui Autonomous Prefecture, Xinjiang, which may represent a potential source of human infection with *T. gondii*. Therefore, a more targeted approach to address this problem should be executed to control and prevent *T. gondii* infection in horses from Xinjiang, northwestern China.

Table 1. Seroprevalence of *Toxoplasma gondii* infection in horses in Changji, Xinjiang Uygur Autonomous Region, northwestern China.

Factor	Category	No. tested	No. positive	Prevalence (%)	95% CI	OR (95%)	*p*-value
Gender	Male	248	71	28.6	23.1–34.7	Reference	*P* = 0.255
	Female	389	129	33.2	28.5–38.1	1.24 (0.87–1.75)	
Age	0<age≤1	129	46	35.7	27.4–44.6	Reference	*P* = 0.587
	0<age≤5	118	35	29.7	21.6–38.8	0.76 (0.45–1.30)	
	5<age≤10	326	102	31.3	26.4–36.7	0.82 (0.53–1.26)	
	age>10	64	17	26.6	16.3–39.1	0.65 (0.34–1.26)	
Region	Fukang	93	28	30.1	21.0–40.5	Reference	*P* = 0.829
	Jimusa'er	103	34	33.0	24.1–43.0	1.14 (0.63–2.10)	
	Hutubi	80	30	37.5	26.9–49.0	1.39 (0.74–2.62)	
	Qitai	91	27	29.7	20.5–40.2	0.98 (0.52–1.84)	
	Mulei	92	31	33.7	24.2–44.3	1.17 (0.63–2.20)	
	Manasi	94	26	27.7	18.9–37.8	0.89 (0.47–1.67)	
	Miao'ergou	84	24	28.6	19.2–39.5	0.93 (0.49–1.78)	
Total		637	200	31.4	27.8–35.3		

No. represents Number; CI is confidence intervals; OR is Odds-ratios.

Table 2. The seroprevalence of *Toxoplasma gondii* infection in horses globally.

Region	Sample size	Prevalence (%)	Serological test	Cut off	Reference
Ankara Province ,Turkey	100	28.0	SFDT	1:16	(GÜÇLÜ et al., 2007)
Curitiba, Paraná, Brazil	100	17.0	IFAT	1:64	(FINGER et al., 2013)
Costa Rica	315	34.0	MAT	1:25	(DANGOUDOUBIYAM et al., 2011)
Czech Republic	522	22.6	LAT	/	(BÁRTOVÁ et al., 2010)
Durango State, Mexico	495	6.1	MAT	1:25	(ALVARADO-ESQUIVEL et al., 2012)
Fernando de Noronha, Brazil	16	43.7	MAT	1:25	(COSTA et al., 2012)
Giza-Zoo Egypt	150	52.6	Bioassays in mice	/	(SHAAPAN & GHAZY, 2007)
Greater Cairo, Egypt	100	25.0	ELISA	/	(HARIDY et al., 2009)
Greece	753	1.8	ELISA	/	(KOUAM et al., 2010)
Jeju Island, South Korea	191	2.6	IFAT	1:100	(GUPTA et al., 2002)

MAT: modified agglutination test; IHA: indirect hemagglutination test; ELISA: enzyme-linked immunoabsorbent assay; SFDT: Sabin Feldman dye test; LAT: latex agglutination test; DAT: direct agglutination test; IFAT: indirect fluorescence antibody test.

Table 2. Continued...

Region	Sample size	Prevalence (%)	Serological test	Cut off	Reference
Liaoning Province, China	711	25.0	MAT	1:25	(YANG et al., 2013)
New Caledonia	25	16.0	ELISA	/	(ROQUEPLO et al., 2011)
Niğde Province, Turkey	125	7.2	SFDT	1:16	(KARATEPE et al., 2010)
North America	1788	6.9	MAT	1:20	(DUBEY et al., 1999)
Portugal	173	13.3	MAT	1:20	(LOPES et al., 2013)
Qazvin, Iran	52	71.2	MAT	1:20	(HAJIALILO et al., 2010)
Riyadh Province, Saudi Arabia	266	31.6	SFDT	1:16	(ALANAZI & ALYOUSIF, 2011)
Southern Spain	454	10.8	MAT	1:25	(GARCÍA-BOCANEGRA et al., 2012)
Swedish	414	1.0	DAT	1:40	(JAKUBEK et al., 2006)
Tunisia	158	17.7	MAT	1:20	(BOUGHATTAS et al., 2011)
Urmia, Northwest Iran	26	11.5	MAT	1:20	(RAEGHI et al., 2011)
Yunnan Province, China	266	30.5	IHA	1:64	(MIAO et al., 2013)

MAT: modified agglutination test; IHA: indirect hemagglutination test; ELISA: enzyme-linked immunoabsorbent assay; SFDT: Sabin Feldman dye test; LAT: latex agglutination test; DAT: direct agglutination test; IFAT: indirect fluorescence antibody test.

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