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Seroprevalence of *Toxoplasma gondii* in horses and donkeys in Yunnan Province, Southwestern China

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Abstract

Background: *Toxoplasma gondii* is an intracellular protozoan parasite that infects almost all warm-blooded animals, including humans, with a worldwide distribution. There have been limited reports about the seroprevalence of *T. gondii* infection in equids around the world and little is known about the seroprevalence of *T. gondii* in equids in southwestern China, in particular in Yunnan Province. The objective of the present investigation was to estimate the seroprevalence of *T. gondii* infection in equids in this area.

Methods: A total of 399 serum samples (266 from horses and 133 from donkeys) were collected in 2012, and assayed for *T. gondii* antibodies by Indirect Haemagglutination (IHA) test using a commercially available kit.

Results: A total of 108 (27.1%) equids, including 81 (30.5%) horses and 27 (20.3%) donkeys were positive for *T. gondii* antibodies, and the seroprevalence ranged from 18.8% to 37.5% among different sampling areas. The seroprevalence was 27.4% and 26.8% for male and female equids, respectively, and the difference was not statistically significant ($P > 0.05$). The seroprevalence ranged from 21% to 32.9% among different age groups, and the difference was not statistically significant ($P > 0.05$).

Conclusions: The results of the present survey indicated the existence of high *T. gondii* seroprevalence in Yunnan Province, southwestern China, which has significant public health concern. Therefore, it is imperative that improved integrated measures be carried out to prevent and control *T. gondii* infection in equids in the studied region.

Background

Toxoplasma gondii is an important zoonotic parasite, which can infect humans and almost all warm-blood animals, with a worldwide distribution [1-5]. Toxoplasmosis is not only of great importance for livestock and causes huge economic loss to the livestock industry, it is also a public health problem owing to its transmission to humans by ingestion of uncooked meat containing tissue cysts, or consuming food or drink contaminated with oocysts, or accidental ingestion of sporulated oocysts from the environment [1-5]. Although *T. gondii*

infection rarely displays obvious clinical symptoms in adults, it may lead to severe consequences in an immunodeficient person such as an AIDS patient, and infection in pregnant women may lead to abortion, still birth, or other serious congenital consequences in newborns [1-3].

Horses and donkeys are important and useful animals to humans in many ways, such as sport competitions, police work, carriage, and so on. Horse and donkey meat are also the popular and delicate food for people worldwide. Infection of *T. gondii* is subclinical in horses, atypical clinical signs includes fever, ataxia, retinal degeneration and encephalomyelitis, and abortion or still-birth in pregnant equids. Human toxoplasmosis cases associated with consumption of horse meat have been reported in some countries [6,7].

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Yunnan Horse and Yunnan Donkey are fine equine breeds in China, they play very important roles in agriculture and tourism, and meat of horse and donkey are considered as special delicacy dishes in local restaurants in Yunnan Province. However, data on horse and donkey infection with *T. gondii* is limited in southwestern China. The objective of the present survey was to examine the seroprevalence of *T. gondii* infection in horses and donkeys in Yunnan Province, southwestern China, and the results would provide fundamental data for prevention and control of *T. gondii* infection in equine animals, and also will provide base-line information on potential risk factors associated with public health.

Methods

Ethics statement

The collection of serum samples from equids in the present study was consented by owners of equids, and all horses and donkeys were handled in strict accordance with good animal practice according to the Animal Ethics Procedures and Guidelines of the People's Republic of China.

Sampled regions

The serum samples were collected from Diqing, Lijiang, Dehong, Baoshan, Yuxi and Zhaotong prefectures and municipalities of Yunnan Province, southwestern China (Figure 1).



Figure 1 Map showing Yunnan Province (YN), shadowed in southwestern China, where horse and donkey serum samples were collected for detection of *Toxoplasma gondii* antibodies. The specific locations of sampling areas were marked in the enlarged map of Yunnan Province (YN), including Diqing state (DQ), Lijiang City (LJ), Dali state (DL), Baoshan city (BS), Dehong state (DH), Yuxi City (YX) and Zhaotong City (ZT).

Table 1 Seroprevalence of *Toxoplasma gondii* infection in equids in Yunnan province, southwestern China

Factor	Category	Sample size	Positive no. (Seroprevalence %)	Positive no. in different titers				
				1:64	1:128	1:256	1:512	1:1024
Species	Horse	266	81 (30.5)	38	20	13	2	8
	Donkey	133	27 (20.3)	13	5	2	1	6
Age (year)	0 < yr ≤ 1	73	24 (32.9)	12	5	3	1	3
	1 < yr ≤ 5	108	34 (31.5)	14	5	9	2	4
	5 < yr ≤ 10	138	29 (21)	12	11	2	0	4
	yr > 10	80	21 (26.3)	13	4	1	0	3
Gender	Male	190	52 (27.4)	25	12	6	1	8
	Female	209	56 (26.8)	26	13	9	2	6
Regions	Diqing	16	6 (37.5)	0	1	2	0	3
	Lijiang	83	23 (27.7)	16	7	0	0	0
	Dali	116	36 (31)	31	5	0	0	0
	Baoshan	39	9 (23.1)	2	5	2	0	0
	Dehong	16	3 (18.8)	1	1	1	0	0
	Yuxi	93	20 (21.5)	1	5	2	1	11
	Zhaotong	36	11 (30.6)	0	1	8	2	0
	Total	399	108 (27.1)	51	25	15	3	14

Blood samples

A total of 399 blood samples (266 from horses and 133 from donkeys) were collected via jugular vein of animals in 2012 in Yunnan Province. Whenever possible, data regarding species/breed, geographic origin, age and gender of each animal were collected. The ages of animals were

classified into four categories according to their growth cycle: foal (0 < yr ≤ 1, 73 samples), adolescent (1 < yr ≤ 5, 108 samples), middle age (5 < yr ≤ 10, 138 samples) and elderly age (yr > 10, 80 samples), 190 were males and 209 were females. All samples were sent to the laboratory in Kunming and centrifuged (3,000 rpm) for 5 min,

Table 2 Seroprevalence of *Toxoplasma gondii* infection in equids in China

Species	Provinces	No. tested	Prevalence (%)	Serological test	Cut-off value	Time tested	References
Horses	Liaoning	711	25	MAT ^a	1:25	2012	[9]
	Sichuan	242	4.55	IHA ^b	1:64	1982	[10]
	Liaoning	76	1.32	IHA	1:64	1989-1990	[11]
	Shangdong	147	0	IHA	1:64	1991-1993	[12]
	Gansu	149	2.7	IHA	1:64	unknown	[13]
	Qinghai	100	6	IHA	1:64	2000	[14]
	Xinjiang	60	0	IHA	1:64	unknown	[15]
	Hebei	43	2.33	IHA	1:64	unknown	[16]
Donkeys	Liaoning	738	23.6	MAT	1:25	2012	[9]
	Shangdong	17	5.88	IHA	1:64	1991-1993	[12]
	Xinjiang	30	0	IHA	1:64	unknown	[15]
	Hebei	33	26.06	IHA	1:64	unknown	[16]
Equids*	Ningxia	945	0.95	IHA	1:64	unknown	[17]
	Shanxi	1108	0.09	IHA	1:64	1986	[18]
	Henan	230	0.43	IHA	1:64	1988-1993	[19]
	Guangdong	149	2.7	IHA	1:64	1982	[20]

* Data for horses and donkeys were not separately reported in these references.

^a MAT: Modified Agglutination test.

^b IHA: Indirect Haemagglutination test.

and the serum samples were kept at -20°C until assayed for antibodies to *T. gondii*.

Serological assay

Antibodies to *T. gondii* were detected in serum samples by an indirect hemagglutination antibody (IHA) test using a commercially available kit (Veterinary Research Institute, Jiangsu Academy of Agricultural Sciences, Nanjing, China) according to the recommended protocol of the manufacturer for the detection of antibodies to *T. gondii* in animals. The serum sample was considered as positive when a layer of agglutinated erythrocytes was observed in wells with dilutions of 1:64 or higher.

Statistical analysis

Statistical analysis of *T. gondii* seroprevalence in different regions, ages and genders of animals were performed using Generalized Linear Model (GLM) test in the SPSS software (Release 18.0 standard version, SPSS Inc., Chicago, Illinois), and $P < 0.05$ was considered statistically significant.

Results and discussion

108 of 399 examined equids were seropositive for *T. gondii* antibodies, the overall seroprevalence was 27.1%, and the seroprevalence ranged from 18.8% in Dehong to 37.5% in Diqing. 81 (30.5%) of the examined horses and 27 (20.3%) of the examined donkeys were seropositive, but the difference was not statistically significant ($P > 0.05$). The antibody titers were 1:64 in 38 horses and 13 donkeys, 1:128 in 20 horses and 5 donkeys, 1:256 in 13 horses and 2 donkeys, 1:512 in 2 horses and 1 donkey, 1:1024 in 8 horses and 6 donkeys. Seroprevalence in male and female equids was 27.4% and 26.8%, respectively, and the difference was not statistically significant ($P > 0.05$). Seroprevalence in animals of four different age groups were not significantly different ($P > 0.05$) (Table 1).

Under natural conditions, *T. gondii* seroprevalence in horses may vary from 0% to 90% [8]. In the present study, the overall average *Toxoplasma* seroprevalence was 27.1% in horses and donkeys in Yunnan Province, which was the highest among reported studies in China [9-20] (Table 2). *T. gondii* seroprevalence in horses was 30.5% in this survey, which was the second highest so far reported in the world. Previously reported *T. gondii* seroprevalence in horses were: 31.6% in Saudi Arabia by DT [21], 28.8% in Iran by MAT [22], 25% in Egypt by ELISA [23], 17.7% in Tunisia by MAT [24], 11.6% in Brazil by IFAT [25], 10.8% in Spain by MAT [26], 7.2% in Turkey by DT [27], 6.9% in North America by MAT [6], 6.1% in Mexico by MAT [28], 2.6% in South Korea by IFAT [29], and 1% in Sweden by DAT [30].

The different seroprevalence results may due to differences in hygiene conditions, climates, and the prevalence of *T. gondii* in cats, as well as the sensitivity of the serological methods. Our preliminary survey showed that the prevalence of *T. gondii* oocysts in the faeces of stray cats in Yunnan was 25.6% (unpublished data), indicating a high risk as a source of *T. gondii* infection for equids, other animals and humans. In the present study, we used IHA to detect antibodies to *T. gondii* in horse and donkey serum samples because IHA is considered one of the most sensitive and specific serological methods for detecting *T. gondii* antibodies in equids, other animals and humans [31-35], and it have been extensively used in China (Table 2, [32-35]). The cutoff value of 1:64 was used according to the national standard (GB/T 18448.2-2008) of China for detection of *T. gondii* antibodies in humans and animals.

Due to the population size of donkeys in Yunnan Province, the sampled number (No = 133) of donkeys in the present study was small, and the results of the present survey may not reflect the accurate *T. gondii* seroprevalence in donkeys. Nevertheless, the present investigation revealed that horses and donkeys in Yunnan Province had a high *T. gondii* seroprevalence, indicating a potential threat to public health in this province, which is one of the most famous tourist destinations in China.

Conclusions

The results of the present study indicate that *T. gondii* seroprevalence in horses and donkeys in Yunnan Province is quite high, consumption of horse or donkey meat is likely to be a risk factor for human infection with *T. gondii*. Therefore, it is imperative to take prevention and control measures to reduce *T. gondii* prevalence in equine animals in this province.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

FCZ and XQZ conceived and designed the study, and critically revised the manuscript. QM, XW, and JFY performed the experiments, analyzed the data and drafted the manuscript, NLS, FZY and YTF helped in study design, study implementation and manuscript revision. All authors read and approved the final manuscript.

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