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Original Article

Seroprevalence and Risk Factors of *Toxoplasma gondii* Infection in Buffaloes, Sheep and Goats in Yunnan Province, Southwestern China

Fengcai ZOU [1, 2], Xin YU [2], Yan YANG [2, 3], Shuang HU [2], Hua CHANG [2], *Jianfa YANG [2], Gang DUAN [2]

1. *School of Life Sciences, Yunnan University, Kunming, Yunnan Province, 650091, PR China*
2. *College of Animal Science and Technology, Yunnan Agricultural University, Kunming, Yunnan Province, 650201, PR China*
3. *State Key Laboratory of Veterinary Etiological Biology, Key Laboratory of Veterinary Parasitology of Gansu Province, Lanzhou Veterinary Research Institute, CAAS, Lanzhou, Gansu Province, 730046, PR China*

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***Correspondence**
Email:
jsc315@163.com

Abstract

Background: The seroprevalence of *Toxoplasma gondii* infection in buffaloes, sheep and goats in Yunnan Province, southwestern China was conducted between May 2012 and December 2013.

Methods: A total of 973 (427 buffaloes, 154 sheep and 392 goats) serum samples were collected from seven administrative regions of Yunnan Province, and examined for *T. gondii* antibodies by indirect hemagglutination (IHA) test. Some risk factors related to species, age, gender and geographical origin were determined using a multinomial logistic regression.

Results: The overall seroprevalence of *T. gondii* in ruminant species was estimated at 11.9%. The final logistic regression model demonstrated that host species and geographical origin were the main risk factors associated with *T. gondii* infection ($P< 0.05$).

Conclusion: Taken together, the results of the present study revealed a high exposure to *T. gondii* in ruminant species in Yunnan Province, which has an important implication for public health.

Introduction

Toxoplasmosis is one of the most widespread foodborne parasitic zoonoses caused by the obligate intracellular protozoan *Toxoplasma gondii*. The parasite causes early abortion, stillbirths and reproductive diseases. Humans may become infected with *T.*

gondii by direct contact with *Toxoplasmosis*-positive ruminants or through the ingestion of the uncooked meat of such ruminants (1). However, data on flocks and herds infection with *T. gondii* is limited in Yunnan Province, therefore, it is difficult to evaluate the risk of human *T. gondii* infection in relation to beef and mutton consumption in Yunnan.

This study aimed to evaluate the seroprevalence of *T. gondii* infection in buffaloes, sheep and goats, and to identify certain risk factors associated with the prevalence of *T. gondii* infection in Yunnan Province, southwestern China.

Materials and Methods

A total of 973 blood samples were collected from the jugular veins of 427 buffaloes, 154 sheep and 392 goats reared in seven administrative regions of Yunnan Province, southwestern China, between May 2012 and December 2013. All serum samples were obtained by centrifuging the blood samples at 3000 rpm for 5 min, after which they were stored at -20 °C until antibody testing.

T. gondii antibodies were evaluated by indirect hemagglutination (IHA) test using a commercially available kit, according to the manufacturer's instructions (2, 3). In brief, sera were added to a 96-well V-bottomed polystyrene plate. Then, IHA dilution solution was added, and the samples were diluted twofold serially starting from 1:16 to 1:1024. Then, the *T. gondii*-sensitized erythrocyte antigen was added to each well, gently shaken for 2 min, and incubated at 37 °C for 2 h. The assay result was considered positive for Toxoplasmosis when a layer of hemocyte agglutination occurs in the wells with sample dilutions of 1:64 or higher. Positive, negative and blank controls were included in each test.

Data of the investigated factors (species, gender, age and geographical origin) were analyzed using multinomial logistic regression in PASW Statistics version 18.0, where positive samples were coded as 1 and negative samples were coded as 0. A probability (*P*) value of less than 0.05 was considered to show statistically significant association with *T. gondii* infection. Odds ratios (OR) and 95% confidence intervals (95% CI) were recorded to quantify the relationship between different levels of *T. gondii* infection and the associated risk factors.

Results

Out of 973 samples, 116 (11.9%) were seropositive with the following distributions: 69/392 (17.6%) for goats, 15/154 (9.7%) for sheep and 32/427(7.5%) for buffaloes. The antibody titers were 1:64 in 35 samples, 1:128 in 38 samples, 1:256 in 18 samples, 1:512 in 7 samples and 1:1024 in 19 samples. The seropositivity of females (12.3%, 84/683) was slightly higher than that of males (11.0%, 32/290). The seropositivities of young animals (age, ≤2 yr) and adults were 14% and 10.2%, respectively. The *T. gondii* seroprevalence with respect to geographical origin varied among the different administrative districts of Yunnan Province, ranging from 5.9% in Dehong to 47.7% in Zhaotong (Table 1).

Gender and age were not significant in the conditional stepwise logistic regression analysis ($P > 0.05$) and were excluded from the final model. The other two factors, namely, species and region, were considered risk factors that significantly influence seroprevalence. Statistically, buffaloes had lower frequency to show significant seropositivity compared to that for goats (OR = 0.153, 95% CI = 0.025–0.958, $P = 0.045$), while no statistically significant differences in the seroprevalence were observed for sheep and goats. Qujing (OR = 0.087, 95% CI = 0.010–0.730, $P = 0.024$), Yuxi (OR = 0.067, 95% CI = 0.008–0.597, $P = 0.015$) and Zhaotong (OR = 0.019, 95% CI = 0.002–0.161, $P=0.000$) showed significantly lower seropositive samples compared to those from Xishuangbanna ($P<0.05$). However, no statistically significant regional differences in seroprevalence were found among the other three administrative regions compared to that in Xishuangbanna (Table 2).

Table 1: Seroprevalence and antibody titers of *Toxoplasma gondii* infection in ruminant species in Yunnan Province, Southwest China by indirect hemagglutination (IHA) test

Factors	Category	No. tested	No. positive (%)	Antibody titers				
				1:64	1:128	1:256	1:512	1:1024
Species	Buffaloes	427	32 (7.5)	10	12	5	2	3
	Sheep	154	15 (9.7)	1	3	2	0	9
	Goats	392	69 (17.6)	23	23	11	5	7
Gender	Male	290	32 (11.0)	12	11	4	1	4
	Female	683	84 (12.3)	22	27	14	6	15
Age groups	yr≤2	436	61 (14.0)	14	11	3	0	3
	yrs> 2	537	55 (10.2)	11	13	6	3	5
Regions	Kunming	106	10 (10.2)	0	1	0	0	9
	Qujing	262	35 (13.4)	15	1	3	2	4
	Yuxi	81	16 (19.8)	6	9	0	0	1
	Zhaotong	44	21 (47.7)	2	4	10	3	2
	Baoshan	152	13 (8.6)	4	4	3	1	1
	Dehong	286	17 (5.9)	6	7	1	1	2
	Xishuangbanna	42	4 (9.5)	1	2	1	0	0
Total		973	116 (11.9)	35	38	18	7	19

Table 2: Parameter estimates for species and zones of ruminant (n=973) as risk factors for *Toxoplasma gondii* infection

Factors	Category	Prevalence (%)	OR[a]	95%CI[b]	*P*-value
Species	Buffaloes	7.5	0.153	0.025-0.958	0.045
	Sheep	9.7	0.552	0.216-1.411	0.215
	Goats	17.6	Reference		
Zones	Kunming	9.4	0.281	0.039-2.047	0.210
	Qujing	13.4	0.087	0.010-0.730	0.024
	Yuxi	19.8	0.067	0.008-0.597	0.015
	Zhaotong	47.7	0.019	0.002-0.161	0.000
	Baoshan	8.6	0.850	0.254-2.847	0.792
	Dehong	5.9	1.821	0.575-5.769	0.309
	Xishuangbanna	9.5	Reference		

a OR: odds ratio, b CI: confidence interval

Discussion

The average seroprevalence of *T. gondii* infection was 11.9% in the present survey. This value was slightly higher than that of recent studies of *T. gondii* infection in flocks and herds in Jiangsu Province (7.8%, n = 51) (4), Qinghai Province (3.4%, n = 206) (5), Xinjiang Province (5.1%, n = 495) (6) and Shanghai City (2.5%, n = 477) (7). Several factors may be responsible for the differences in prevalence, including geographical, ecological, feeding conditions and animal welfare, in addition to the number of tested samples.

Host species represent one of the main risk factors for *T. gondii* seroprevalence. For instance, in this study the seroprevalence in buffaloes was lower than that of sheep and goats ($P < 0.05$). Thus, buffaloes have lower susceptibility as hosts to *T. gondii* than that by sheep and goats, as evidenced by previous studies from various countries. For instance, the seroprevalences of goats and sheep were 24.9% in Ethiopia (8), 24.5% in Greece (9), 30.5% in Ghana (10) and 11.8% in Spain (11). In contrast, seroprevalences in buffaloes were 8.8% in Iran (12), 3.9% in Brazil (13). The present study showed that geographical origin repre-

sents another risk factor. The samples collected from Qujing, Yuxi and Zhaotong were less likely to show seropositivity compared to those collected from Xishuangbanna, Kunming, Baoshan and Dehong ($P < 0.05$). This difference may be related the number of cats present in these regions. For instance, nearly 80,000 cats are present in Xishuangbanna, Kunming, Baoshan and Dehong, whereas only about 50,000 cats are present in Qujing, Yuxi and Zhaotong.

Conclusion

To our knowledge, this is the first systematic study of *T. gondii* infection in ruminants, with the results showing that the seroprevalence of *T. gondii* is high in Yunnan Province. Host species and geographical origin are the main risk factors for *T. gondii* seroprevalence, suggesting important implications for public health. Integrated and efficient measures are required to prevent and control *T. gondii* infection in buffaloes, sheep and goats in Yunnan Province.

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