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RESEARCH ARTICLE

Besnoitiosis: An Emerging Parasitic Disease in Yaks (*Bos grunniens*) and Tibetan Sheep (*Ovies aries*) on the Qinghai Tibetan Plateau, China

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A B S T R A C T

Besnoitiosis is an emerging disease in Tibetan sheep and yaks in Qinghai Tibetan plateau (QTP) of China. That is why a survey was conducted for the seroprevalence and detection of associated risk factors of this disease in Tibetan sheep and yaks in QTP of China. A total of 1092 and 1449 serum samples were collected from Tibetan sheep and yaks respectively and checked by employing commercial ELISA kits. The seroprevalence of besnoitiosis in Tibetan sheep was found to be 3.21% ranging from 0 to 5.6% in different counties. The male and female sheep were found positive with 3.15 and 3.25% cases respectively for this disease. A total of 16/1449 (1.10%) yaks were found to be positive for besnoitiosis ranging from 0-2.03% from different counties. In years of 2011-17, the seroprevalence has been ranged from 0-4.10%. Based on conditional stepwise logistic regression analysis, it can be speculated that the varying climatic changes in QTP region and age are the influencing risk factors in causing besnoitiosis in Tibetan sheep and yaks. The survey herein has highlighted the risk and rising potential for this parasitic disease in yaks and Tibetan sheep on the remote plateau.

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INTRODUCTION

The emerging parasitic disease of besnoitiosis is mainly caused by the obligate intracellular apicomplexan, cyst-forming coccidia species (Maksimov *et al*., 2016; Lee *et al*., 2017; Gutiérrez-Expósito *et al*., 2017; Jiménez-Meléndez *et al*., 2017). Among those, *Besnoitia* (*B.*) *besnoiti* and *B. caprae* are considered pathogenically infective for bovine and ovine respectively. These parasites have been reported to cause alopecia, hyperkeratosis and tissue cysts in mucous membranes of the host animals (Gutiérrez-Expósito *et al*., 2017). Bovine besnoitiosis was recognized as an extremely important disease in several countries in Asia, Africa, Middle East, South America and Europe due to the major economic losses in the cattle industry in terms of low milk production, poor health, male sterility and death (Gazzonis *et al*., 2014; Waap *et al*., 2014; García-Lunar *et al*., 2015; Gollnick *et al*., 2015; Alshehabat *et al*., 2016; Verma *et al*., 2017).

The indigenous breed of Tibetan sheep (*Ovies aries*) is mainly living at high altitudes of 3000 m on Qinghai Tibetan Plateau (QTP) with approximately a population of 50 million (Jing *et al*., 2017, 2018). The nutritious meat and valuable fur of this animal have become an economically important aspect for the native herdsmen in this area (Jing *et al*., 2018). Infectious diseases have become a serious threat to the health of livestock animals on QTP (Li *et al*., 2017), especially in the case of besnoitiosis, which not only causes economic losses but brings difficulties to the local nomadic population because heavily damaged skins of Tibetan sheep can hardly be employed for making fur-jackets to resist the bitterly cold environment at such high altitude.

The QTP iconic symbol, long-haired bovine animal and famous as "boat of the plateau"; the yaks are mostly found throughout the Himalayan area of Southern Asia at the high altitudes of 3000 m (Li *et al*., 2015a; Li *et al*., 2017; Li *et al*., 2018a). With 14 million of population, the yaks account for 90% of the global yak population. They are of great importance for the local Tibetans raising their economics and livelihood in terms of nutritious milk & meat, fuel-based dung, precious wool and as conveyance animal (Li *et al*., 2015b; Li *et al*., 2017; Li *et al*., 2018b). Reportedly, any infectious disease poses a great threat in this region leading to ultimately affect both local nomads and the yaks (Li *et al*., 2018a).

Serological studies are very common in the research of infectious diseases contributing to their prevention and control (Waap *et al*., 2014). To the best of our knowledge, no study has been conducted on besnoitiosis in China especially in yaks and sheep on highlands of QTP. Therefore, a survey was conducted to investigate the seroprevalence and detect risk factors of besnoitiosis in Tibetan sheep and yaks on QTP, China. The survey herein may highlight the risk and rising potential for this parasitic disease in these animals on the remote plateau.

MATERIALS AND METHODS

Ethics statement: The samples collection and other procedures were adopted under the permission of the Laboratory Animals Research Centre of Hubei, Qinghai, Sichuan, Gansu, Tibet and Zhejiang in P. R. China.

Collection of Serum Samples and Examination of Antibodies: To investigate the antibodies of ovine and bovine besnoitiosis, a total of 1092 and 1449 blood samples were collected respectively through convenience sampling from Tibetan sheep and yaks from different counties on QTP of China. Detailed information was documented regarding the gender and age of animals. The serum was harvested from blood samples as described previously by Li *et al*. (2015a), which were examined for antibodies to bovine and caprine besnoitiosis by piloting commercial enzyme-linked immunosorbent assay (ELISA) kits (Bovine/sheep besnoitiosis-Ag ELISA Kit, Potenov Technology Co., Ltd, Beijing, China) according to manufacturer's instructions. The examiner value was according to the optical density (OD) values of OD 450. To ensure validity, the average OD 450 of positive controls was ≥1.00; the average OD 450 of negative controls was ≤0.15. The results were interpreted as positive when the OD 450≥ cut off value; negative when the OD 450< cut off value. The critical (cut off) value was calculated by the following formula:

Cut off value = the average OD 450 of negative controls + 0.15.

Statistical analysis: Multivariable logistic regression model (MLRM) was carried out to discover the risk factor of variable related to besnoitiosis infection in yaks and Tibetan sheep, as the previous study depicted (Li *et al*., 2017). SPSS (Statistics 24.0, Somers, NY) was employed for the statistical analyses of the results. The results were considered significant when P≤0.5.

RESULTS

Prevalence and risk factors of *Besnoitiosis* in Tibetan sheep: The seroprevalence of besnoitiosis in Tibetan sheep was found to be 3.21% (95% CI: 2.2-4.4) ranging from 0% (95% CI: 0.0-1.3) to 5.6% (95% CI: 3.6-8.4) in different counties. The seroprevalence was observed 3.15% (95% CI: 1.8-5.1) and 3.25% (95% CI: 2.0-5.0) in male and female sheep respectively ranging from 1.53% (95% CI: 0.6-3.3) to 6.51% (95% CI: 3.6-10.7) in different years (Table 1).

Region and age were revealed to be more influencing risk factors in the present study basing on the conditional stepwise logistic regression (CSLR) results. Seroprevalence of besnoitiosis in Tibetan sheep in Qinglong (5.20%) and Baingoin (5.60%) counties had respectively, two times (OR=2.978, 95% CI=0.981-9.040, P=0.044<0.05) and three times (OR=3.180, 95% CI=1.184-8.541, P=0.016<0.05) easier to infect with this condition as compared to the sheep in Nyainrong county (1.83%). Tibetan sheep in age >2 years (6.51%) were found to have four times (OR=4.493, 95% CI=1.701-11.868, P=0.001<0.01) more likely to be infected with besnoitiosis in comparison with sheep of age not more than 1 year (1.53%), while no obvious difference was discovered in sheep at the age of 1<year≤2 and 0<year≤1 (Table 1). Though we did not found gender to be a risk factor according to CSLR results, a noteworthy difference was found in different gender Tibetan sheep in Qinglong (P=0.023<0.05; $\chi2$=5.143) (Fig. 1), age of 0<year≤1 (P=0.022<0.05; $\chi2$=5.248) and 1<year≤2 (P=0.002<0.01; $\chi2$=9.346) (Fig. 2), respectively.

Prevalence and risk factors of *Besnoitiosis* in yaks: A total of 16 yaks out of 1449 (1.10%, 95% CI: 0.6-1.8) were found to be positive to *besnoitiosis*. The seroprevalence were ranged from 0 (95% CI: 0.0-1.4) to 2.03% (95% CI: 0.7-4.4) in different provinces. The seroprevalence of *Besnoitiosis* in different gender of yaks was 0.85% (95% CI: 0.3-1.7) and 1.44% (95% CI: 0.7-2.7) in male and female respectively. The prevalence of besnoitiosis was ranged from 0 (95% CI: 0.0-2.2) to 2.53% (95% CI: 1.0-5.1) in different growing years; and ranged from 0 (95% CI: 0.0-1.8) to 4.10% (95% CI: 1.3-9.3) from 2011 to 2017 (Table 2).

Table 1: Prevalence and risk factors of Besnoitiosis infection in Tibetan sheep on the QTP

Variable	Category	No. tested	No. positive	% (95% CI)	P-value	OR (95% CI)
Region	Sog	273	0	0 (0.0-1.3)		
	Nyainrong	273	5	1.83 (0.6-4.2)		Reference
	Qinglong	171	9	5.20 (2.4-9.8)	0.044	2.978 (0.981-9.040)
	Baingoin	375	21	5.60 (3.6-8.4)	0.016	3.180 (1.184-8.541)
Gender	Male	476	15	3.15 (1.8-5.1)		Reference
	Female	616	20	3.25 (2.0-5.0)	0.929	1.031 (0.522-2.037)
Age	0<year≤1	393	6	1.53 (0.6-3.3)		Reference
	1<year≤2	484	15	3.10 (1.7-5.1)	0.130	2.063 (0.793-5.368)
	year>2	215	14	6.51 (3.6-10.7)	0.001	4.493 (1.701-11.868)
Total		1092	35	3.21 (2.2-4.4)		

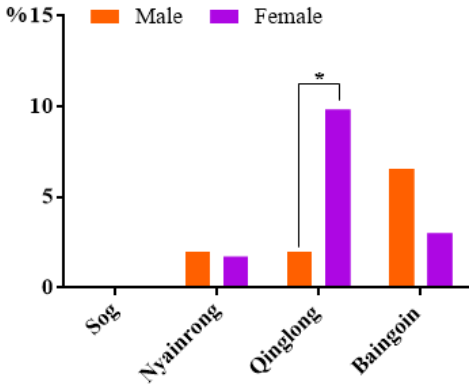

Fig. 1: Prevalence of Besnoitiosis infection in male and female Tibetan sheep in different regions.

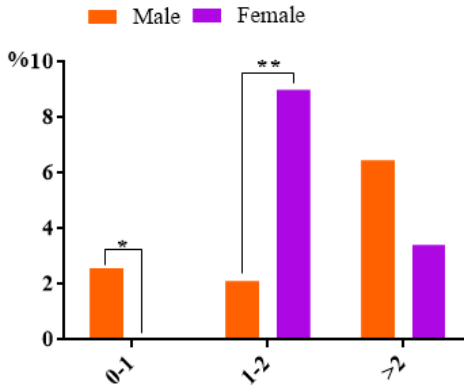

Fig. 2: Prevalence of Besnoitiosis infection in male and female Tibetan sheep in different ages.

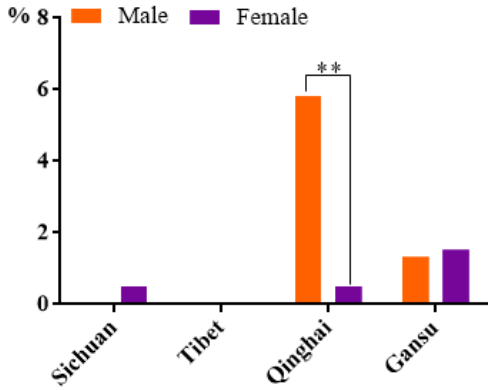

Fig. 3: Prevalence of Besnoitiosis infection in male and female yaks in different provinces.

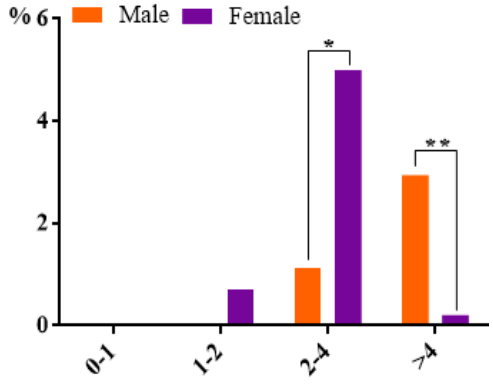

Fig. 4: Prevalence of Besnoitiosis infection in male and female yaks in different ages.

Table 2: Prevalence and Risk Factors of Besnoitiosis infection in yaks on the QTP

Variable	Category	No. tested	No. positive	% (95% CI)	P-value	OR (95% CI)
Region	Sichuan	299	1	0.33 (0.0-1.8)		Reference
	Tibet	255	0	0 (0.0-1.4)		
	Qinghai	295	6	2.03 (0.7-4.4)	0.055	6.187 (0.740-51.708)
	Gansu	600	9	1.50 (0.7-2.8)	0.116	4.538 (0.572-35.988)
Gender	Male	824	7	0.85 (0.3-1.7)		Reference
	Female	625	9	1.44 (0.7-2.7)	0.287	1.705 (0.632-4.604)
Age	0<year≤1	169	0	0 (0.0-2.2)		
	1<year≤2	275	1	0.36 (0.0-2.0)		Reference
	2<year≤4	277	7	2.53 (1.0-5.1)	0.033	7.104 (0.868-58.127)
	4<year	728	8	1.10 (0.5-2.2)	0.271	3.044 (0.379-24.455)
Year	2011	200	0	0 (0.0-1.8)		
	2012	187	1	0.53 (0.0-2.9)		Reference
	2013	169	1	0.59 (0.0-3.3)	0.943	1.107 (0.069-17.840)
	2014	122	5	4.10 (1.3-9.3)	0.026	7.949 (0.917-68.887)
	2015	188	2	1.06 (0.1-3.8)	0.565	2.000 (0.180-22.247)
	2016	183	1	0.55 (0.0-3.0)	0.988	1.022 (0.063-16.462)
	2017	400	7	3.83 (1.6-7.7)	0.237	3.313 (0.405-27.123)
Total		1449	16	1.10 (0.6-1.8)		

According to CSLR results, age and year were found to be RFs significantly affecting the seroprevalence. Yaks at the age of 2<year≤4 (2.53%) have been revealed seven times (OR=7.104, 95% CI=0.868-58.127, P=0.033<0.05) easier to get an infection of besnoitiosis as compared with the yaks of the age of 1<year≤2 (0.36%). Yaks, in 2014 (4.10%) were uncovered to be seven times (OR=7.949, 95% CI=0.917-68.887, P=0.026<0.05) more likely to be infected by besnoitiosis in relation to the yaks in the year of 2012 (0.53%) (Table 2); while, region and gender were not presented to be the risk factor from the results of CSLR (Table 2). The conspicuous difference was demonstrated in the gender of yaks living in Qinghai province (P=0.007<0.01; $\chi 2$=7.334) (Fig. 3), age of 2<year≤4 (P=0.049<0.05; $\chi 2$=3.885) and 4<year (P=0.001<0.01; $\chi 2$=10.785) (Fig. 4) respectively.

DISCUSSION

With growing economic and social reforms in China, the meat products demands especially of beef and mutton types have increased during the last decade (Li *et al*., 2016; Li *et al*., 2017). According to census 2016, about 106 and 16 million heads of cattle and sheep respectively have been counted in China (National Bureau of Statistics of China: http://data.stats.gov.cn/easyquery.htm?cn=C01). The infectious diseases are severely constraining the development of pastures; so, it is of great importance to

carry out epidemiological studies for the survey of diseases having strong impacts on farm animals (Li *et al*., 2017).

Since the first discovery of *B. besnoiti* in France (Waap *et al*., 2014), it has been reported in many countries (Waap *et al*., 2014; Langenmayer *et al*., 2015; Liénard *et al*., 2015; Talafha *et al*., 2015). The current study herein is aimed to report the prevalence of besnoitiosis in yaks (1.10%), which is in line with previous studies. Although the seropositive results of *B. besnoiti* in yaks in China is less in other countries including Jordan (6.0%) (Talafha *et al*., 2015), France (5.1%) (Waap *et al*., 2014); and Korea (3.4%) (Lee *et al*., 2017); however, it is much lesser with the bovine in Ireland (68%) (Ryan *et al*., 2016) and Italy (22.7%) (Gazzonis *et al*., 2014). The seroprevalence of besnoitiosis infection in Tibetan sheep was 3.21%, which was in concomitant with the results reported in sheep (4.68%) in Spain (Gutiérrez-Expósito *et al*., 2017). In current research, area, year and age were found to be associated risk factors of besnoitiosis in Tibetan sheep and yak, which was in accordance with the previous studies (Talafha *et al*., 2015; Gazzonis *et al*., 2017). Sex, in the present study was not shown to be a risk factor both in yaks and Tibetan sheep which contradicted with previous results in which had presented male cattle at higher risk of infection (Gazzonis *et al*., 2017). However, obvious differences were found in animals of different gender in current study (Fig. 1-4).

The social animals of yaks and Tibetan sheep share the high plateau with numerous domestic and wild lives on the QTP (Li *et al*., 2014). The infected yaks and Tibetan sheep may contaminate this parasitic disease to other animals (Gutiérrez-Expósito *et al*., 2016). Bloodsucking arthropods and mucosal contact by licking among the animals have been demonstrated the ways of transmission of these protozoans (Ryan *et al*., 2016). Until now, neither effective treatment nor safe vaccine is available; so, culling of the animals was employed as the best way to deal with infected animals (Waap *et al*., 2014).

Conclusions: The current research reveals for the first time the seroprevalence and associated RFs of besnoitiosis in two highland animals of yaks (*Bos grunniens*) and Tibetan sheep on the QTP, China. The survey herein may highlight and rise essential public concern for this parasitic disease in yaks and Tibetan sheep on the remote plateau.

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Authors contribution: KL and JKL designed the study. KL, LHZ, HQL, KM and MM performed the experiments and KL, MUN and SN analyzed the data. KL wrote the manuscript. All authors read and approved final manuscript.

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