Tropical Biomedicine 35(3): 736–743 (2018)

Epidemiological investigation and risk factors of *Peste des petitis ruminants* (PPR) in yaks (*Bos grunniens*) and cattle in five regions of China

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Received 2 June 2017; received in revised form 22 May 2018; accepted 27 May 2018

Abstract. To investigate the prevalence of *Peste des petitis ruminants* in yaks and cattle in poorly studied areas of China. A total of 1202 and 560 blood samples were collected from yaks and cattle, respectively and processed using a commercial ELISA kit. Multivariable logistic regression model was piloted to find the variables, potentially associated with exposure of *PPR* infection in yaks and cattle. Results indicated that the overall prevalence of *PPR* in yaks was 11.2%. A total 66 (11.8%) out of 560 were examined out to be positive to *PPR* in cattle in Jiangxi province. According to conditional stepwise logistic regression, region, age and gender were found to be the more influencing risk factors in yaks, while region and age were found to be the potential risk factors in cattle. The current results reported the prevalence and associated risk factors of *PPR* in bovine for the first time in China.

INTRODUCTION

Peste des petitis ruminants (PPR) agent *Peste des petitis ruminants virus (PPRV)* is a member of the genus Morbillivirus within the family Paramyxoviridae (Gibbs *et al.*, 1979). *PPR* is a highly contagious and devastating disease of small ruminants, which mainly affect sheep and goats; and occasionally wild small ruminants (Li *et al.*, 2016a). The world organization for animal health has identified *PPR* as a noticeable and economically important trans-boundary viral disease of sheep and goats, which is associated with high morbidity and mortality (Karim *et al.*, 2016). Previously, *PPRV* has been reported in large ruminants like cattle and buffaloes in the Punjab, Pakistan (Abubakar *et al.*, 2017), cattle in Sudan (Intisar *et al.*, 2017), and camels in Iran and Sudan (Zakian *et al.*, 2016; Intisar *et al.*, 2017).

The long haired wild and domestic yak (*Bos grunniens*) are physiologically adapted to high cold altitude (3000 m) throughout the Himalayan region of the South Central Asia (Li *et al.*, 2014; Li *et al.*, 2016b). Almost 90% (about 14 million) of the world yaks' population inhabit on the Qinghai-Tibetan Plateau in China (Li *et al.*, 2015; Li *et al.*, 2017). This animal is especially important to native herdsmen for the usefulness and economic value of its milk, meat, dung, and wool (Li *et al.*, 2014; Li *et al.*, 2018). Infectious diseases are serious threat for animal health and productivity in developing countries (Durrani *et al.*, 2017; Mehmood *et al.*, 2017; Saleem *et al.*, 2018; Wang *et al.*, 2018; Zhang *et al.*, 2017). So, any yak disease may cause heavy losses to the herdsman, as the herds survive mainly on yaks in such remote plateaus (Li *et al.*, 2014; Li *et al.*, 2017).

Peste des petitis ruminants virus is endemic in most of Saharan and sub-Saharan Africa, Turkey, the Middle East, and Indian subcontinent, it has recently been reported in areas previously thought to be free of the disease including China (Abubakar *et al.*, 2017). Qinghai Tibetan plateau in China is adjoining with India, and it is estimated that one third of small ruminants are seropositive to *PPRV* with an economic losses US$ 39 million every year (Singh, 2011). The *PPRV*-infected domestic cases had been reported in Tibet in 2007 in domestic (Wang *et al.*, 2009) and wild small ruminants, like Tibetan antelope (*Pantholops hodgsonii*), Tibetan gazelle (*Procapra picticaudata*) and bharal (*Pseudois nayaur*) in Tibet from 2007 to 2008 (Bao *et al.*, 2011 & 2012). The *PPR* outbreak occurred in more than 20 provinces and cities of Xinjiang (Wu *et al.*, 2016). The *PPRV* was also reported in wild ibexes in Bazhou, Xinjiang (Xia *et al.*, 2016), which is adjoined to Qinghai, Tibet and Gansu provinces. However, scarce information is known about the epidemic condition of *PPR* in yaks and cattle in China. Therefore, the current survey was piloted to investigate the current situation of seroprevalence of *PPR* in yaks and cattle in areas of the country which were poorly studied so far.

MATERIALS AND METHODS

Serum samples

A total 1202 and 560 blood samples were collected from yaks and cattle on the Qinghai Tibetan plateau (Qinghai 225; Tibet 317; Sichuan 209 and Gansu 451) and Jiangxi province (12 areas) of China, respectively (Fig. 1, Table 1). The gender and age information were recorded on a prescribed performa. Each of the yak and cattle blood samples was centrifuged at 1000 × g for 10 min, respectively, and serum was separated and stored at -20°C for further analysis.

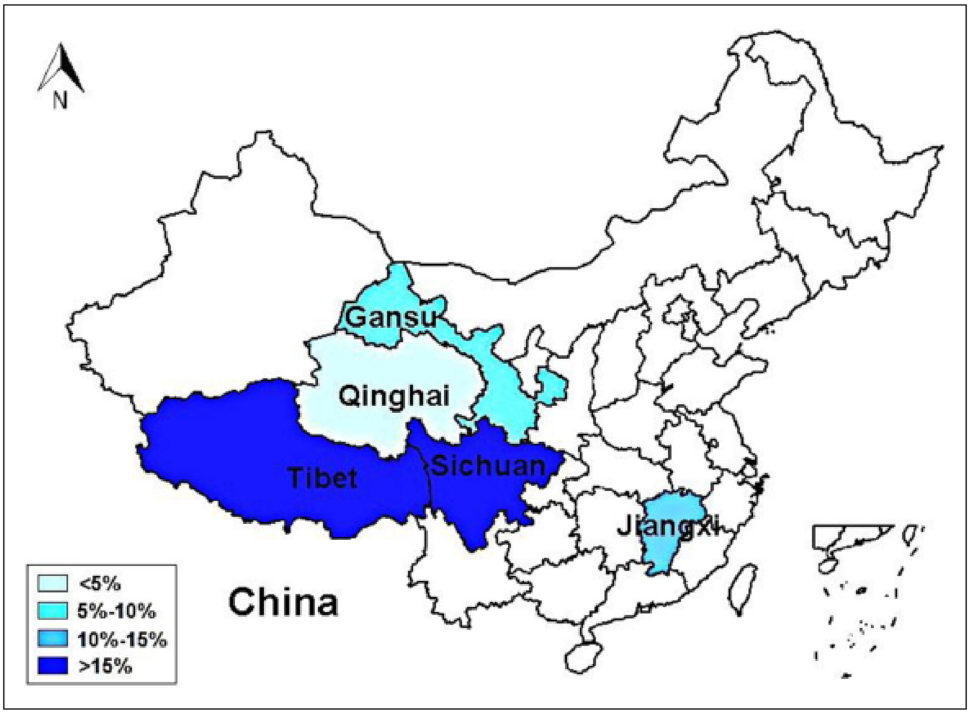

Figure 1. Geographic distribution of the yaks and cattle included in the study.

Table 1. Prevalence and Risk Factors of *PPRV* infection in yaks on the Qinghai Tibetan plateau by ELISA

Variable	Category	No. tested	No. positives	% (95% CI)	*P*-value	OR (95% CI)
Region	Qinghai	225	9	4.0 (1.8-7.5)	Reference	
	Tibet	317	49	15.5 (11.7-19.9)	<0.001	4.388 (2.108-9.133)
	Sichuan	209	33	15.8 (11.1-21.5)	<0.001	4.500 (2.097-9.655)
	Gansu	451	44	9.8 (7.2-12.9)	0.009	2.595 (1.243-5.415)
Gender	Female	661	61	9.2 (7.1-11.7)	Reference	
	Male	541	74	13.7 (10.9-16.9)	0.015	1.559 (1.088-2.234)
Age	0<year≤1	88	3	3.4 (0.7-9.6)	Reference	
	1<year≤2	63	8	12.7 (5.6-23.5)	0.030	4.121 (1.048-16.211)
	2<year≤4	457	37	8.1 (5.8-11.0)	0.123	2.496 (0.752-8.282)
	4<year	594	87	14.7 (11.9-17.7)	0.004	4.862 (1.504-15.722)
Total		1202	135	11.2 (9.5-13.2)		

Examination of PPR in yaks and cattle

Serum samples from yaks and cattle were processed by employing a commercial enzyme linked immunosorbent assay (ELISA) kit (Bovine *Peste des petits ruminants* ELISA Kit, Beijing green source dade biotechnology Co., Ltd, Beijing, China) according to the manufacturer's instructions.

The test value was based on the optical density (OD) values of OD 450. To ensure validity, the average OD 450 of positive control wells was ≥ 1.00; the average OD 450 of negative control wells ≤ 0.15. Calculate Critical (CUT OFF) = the average OD 450 of negative control wells + 0.15. The results were interpreted as: positive when the OD 450 ≥ CUT OFF; negative when the OD 450 < CUT OFF.

Statistical analysis

Multivariable logistic regression model was used to find the variables, potentially associated with the exposure to *PPR* infection in yaks and cattle. Statistically significant levels within factors and interactions was recognized, when probability (P) value found <0.05. Odds-ratios (OR) was kept with 95% confidence intervals (CI), the statistical analyses were performed through the IBM SPSS Statistics 20.0 (SPSS Somers, NY).

RESULTS

For yaks, the overall seroprevalence of *PPR* was 11.2% (95% CI: 9.5-13.2). In different regions, the seroprevalence was ranged from 4.0% (95% CI: 1.8-7.5) to 15.8% (95% CI: 11.1-21.5). In male and female yaks, the prevalence was 13.7% (95% CI: 10.9-16.9) and 9.2% (95% CI: 7.1-11.7), respectively. In different ages, the seroprevalence were ranged from 3.4% (95% CI: 0.7-9.6) to 14.7% (95 CI: 11.9-17.7) (Table 1). According to conditional stepwise logistic regression, region, age and gender were found to be the more influencing risk factors. In different regions, yaks in Tibet (15.5%) had 4 times (OR = 4.388; 95% CI = 2.108-9.133; $\rho < 0.001$) higher risk of infection compared to yaks in Qinghai (4.0%). Similarly, yaks in Sichuan (15.8%) had four times (OR=4.500; 95% CI= 2.097-9.655; $\rho < 0.001$) higher risk of infection than yaks in Qinghai; while yaks in Gansu (9.8%) had a double higher risk of infection when compared with yaks in Sichuan (Table 1). In different ages, yaks in 1< year ≤ 2 (12.7%) had four times (OR = 4.121; 95% CI = 1.048-16.211; $\rho = 0.030$) higher risk of being positive compared to yaks in 0 < year ≤ 1 (3.4%); yaks in year > 4 (14.7) had ad four times (OR = 4.862; 95%

CI = 1.504-15.722; ρ = 0.004) higher risk of being positive compared to yaks in 0 < year ≤ 1. Whereas, no significant difference was found in 2 < year ≤ 4 (8.1%) (ρ = 0.123) when compared to yaks in 0 < year ≤ 1 (Table 1). In different genders, male yaks had 1.5 times (OR = 1.559; 95% CI = 1.088-2.234; ρ = 0.015) higher risk of being positive compared to female yaks (Table 1). The seroprevalence of *PPR* in male yaks in Tibet was significant higher than that in female yak (ρ < 0.001; $\chi2$ = 12.350) (Fig. 2). The prevalence of *PPR* in male yaks in year > 4 was found significant higher than that in female yak (ρ < 0.01; $\chi2$ = 10.506) (Fig. 3).

For cattle, a total 66 out of 560 (11.8%; 95% CI: 9.2-14.7) were examined and found to be positive for *PPR* with the range of 0 to 23.3% (95% CI: 13.4-36.0) in the different regions of Jiangxi province. In male and female cattle, the seroprevalence was 11.9% (95% CI: 8.2-16.4) and 11.7% (95% CI: 8.3-15.9), respectively (Table 2). In the present results of conditional stepwise logistic regression, region and age were demonstrated to be the risk factors influencing seroprevalence significantly. In different regions, cattle in Yichun (20.0%) had three times (OR = 3.500; 95% CI = 1.257-9.742; ρ = 0.012) higher risk of infection comparing to cattle in Ji'an (6.70%). Similarly, cattle in Gaoan (23.3%) had four times (OR = 4.261; 95% CI = 1.674-10.843; ρ = 0.001) higher risk of infection comparing to cattle in Ji'an, while no significant difference was found in cattle in other areas (ρ > 0.05) when compared to cattle in Ji'an (Table 2). In different years, cattle in year > 4 (15.6%) had three times (OR = 3.264; 95% CI = 0.978-10.890; ρ = 0.043) higher risk of infection comparing to cattle in 0 < year ≤ 1 (5.4%), while no significant difference was found in cattle 1 < year ≤ 2 (6.5%) (ρ = 0.786) and 2 < year ≤ 4 (9.1%) (ρ = 0.388) when compared to cattle in 0 < year ≤ 1, respectively (Table 2). Gender was not a significant risk factor according to conditional stepwise logistic regression (ρ = 0.950) (Table 2). Whereas, the prevalence of *PPR* infection in female cattle in Yudu was found to be significantly higher than that in male cattle (ρ < 0.01; $\chi2$ = 7.680) (Fig. 4) and the seroprevalence of *PPR* infection in male cattle in 2 < year ≤ 4 was found to be significantly higher than that in female cattle (ρ = 0.006; $\chi2$ = 7.639) (Fig. 5).

DISCUSSION

Nowadays, cattle production is the 3rd largest agricultural commodity in China, with 1.08 billion head cattle in 2015 (National Bureau of Statistics of China. http://data.stats.gov.cn/easyquery.htm?cn=C01) (Li *et al.*, 2016b; Li *et al.*, 2017). So, it is of great importance to perform serological examination as the

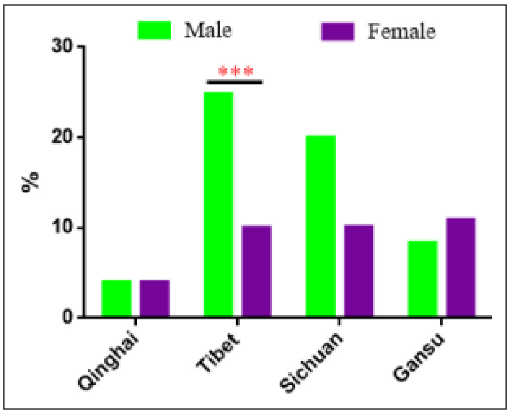

Figure 2. Prevalence of *PPRV* infection in male and female yaks on the Qinghai Tibetan plateau by ELISA.

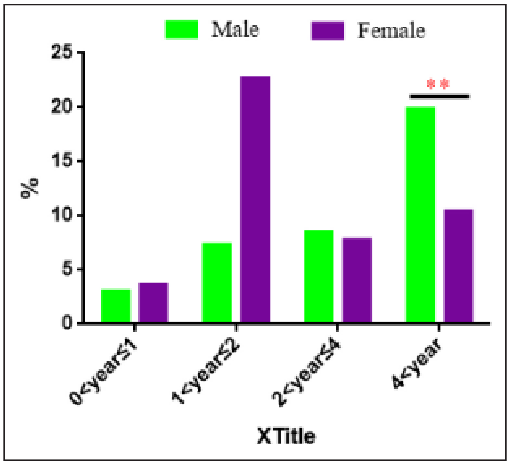

Figure 3. Prevalence of *PPRV* infection in male and female yaks in different years by ELISA.

Table 2. Prevalence and Risk Factors of *PPRV* infection in cattle in Jiangxi province by ELISA

Variable	Category	No. tested	No. positives	% (95% CI)	*P*-value	OR (95% CI)
Region	Pingxiang	15	0	0		
	Jingdezhen	35	0	0		
	Ji'an	120	8	6.7 (2.9-12.7)	Reference	
	Yingtan	15	1	6.7 (0.2-31.9)	1.000	1.000 (0.116-8.600)
	Xinyu	35	5	14.3 (4.8-30.3)	0.152	2.333 (0.711-7.653)
	Yichun	45	9	20.0 (9.6-34.6)	0.012	3.500 (1.257-9.742)
	Nanchang	80	9	11.3 (5.3-20.3)	0.255	1.775 (0.654-4.813)
	Shangrao	35	5	14.3 (4.8-30.3)	0.152	2.333 (0.711-7.653)
	Ganzhou	35	3	8.6 (1.8-23.1)	0.699	1.313 (0.329-5.237)
	Fuzhou	25	2	8.0 (1.0-26.0)	0.811	1.217 (0.243-6.110)
	Gaoan	60	14	23.3 (13.4-36.0)	0.001	4.261 (1.674-10.843)
	Yudu	60	10	16.7 (8.3-28.5)		
Gender	Female	299	35	11.7 (8.3-15.9)	Reference	
	Male	261	31	11.9 (8.2-16.4)	0.950	1.017 (0.608-1.701)
Age	0<year≤1	56	3	5.4 (1.1-14.9)	Reference	
	1<year≤2	77	5	6.5 (2.1-14.5)	0.786	1.277 (0.281-5.361)
	2<year≤4	132	12	9.1 (4.8-15.3)	0.388	1.767 (0.479-6.520)
	4<year	295	46	15.6 (11.6-20.2)	0.043	3.264 (0.978-10.890)
Total		560	66	11.8% (9.2-14.7)		

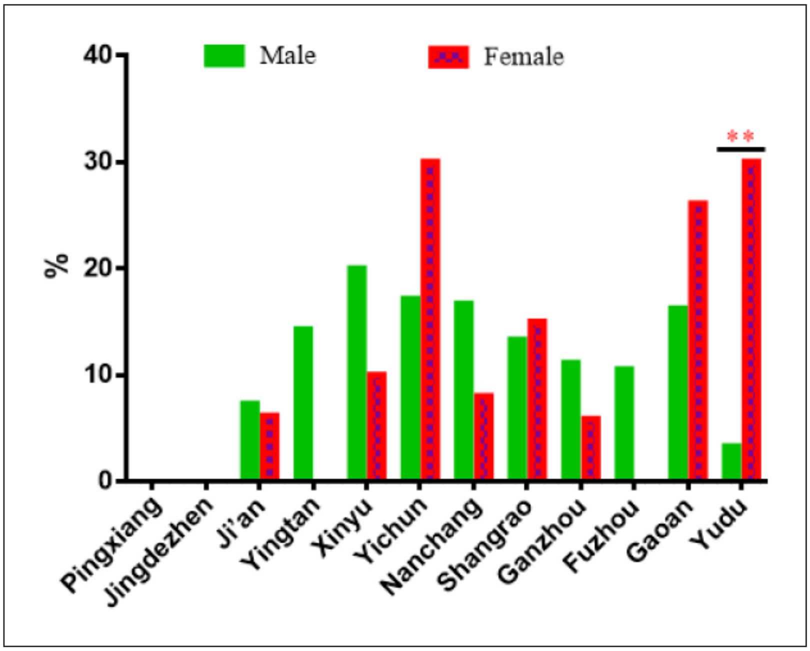

Figure 4. Prevalence of *PPRV* infection in male and female cattle in Jiangxi province by ELISA.

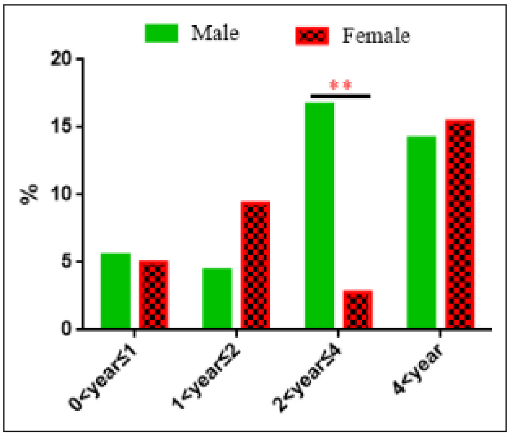

Figure 5. Prevalence of *PPRV* infection in male and female cattle in different years by ELISA.

disease in domestic animals is of key economic relevance (Li *et al.*, 2017).

In the current survey, the seroprevalence of *PPR* infection in yaks and cattle was 11.2% and 11.8%, respectively, which is significantly lower than that in cattle in Sudan (25.8%), and cattle (41.9%) and buffalo (67.4%) in Pakistan (Khan *et al.*, 2008; Intisar *et al.*, 2017). The difference may be caused by the change in seasonal, climatic and geographic conditions, as this disease is mostly endemic. However, it is in accordance with the prevalence of cattle (11.1%) and buffaloes (16.2%) in India (Balamurugan *et al.*, 2014), and cattle (10.0%) and buffaloes (14.2%) in Pakistan (Abubakar *et al.*, 2017). There is no significant difference in the prevalence of *PPR* in yaks and cattle, which is in line with previous results that showed no significant difference in cattle and buffaloes (Abubakar *et al.*, 2017). It reveals that bovine species is not a risk factor in *PPR* infections.

According to the results of conditional stepwise logistic regression, region, age and gender were demonstrated to be risk factors in yaks (Table 1), while gender was not a risk factor in cattle (Table 2). However, significant difference was found in female and male cattle in Yudu ($\rho < 0.01$) (Fig. 4), and male and female cattle in 2<year≤ 4 ($\rho < 0.01$ (Fig. 5). The possible reason may be the change in geographical location, climate and samples numbers, as the average altitude is 3000-5000 m and 300-500 m in the Qinghai Tibetan plate and Jiangxi province, respectively.

The infected yaks and cattle may transmit this economically important disease to other animals, as bovine are social animals living together with other wild and domesticated animals (Li *et al.*, 2014). Transmission is through aerosols in situations where there is close contact between infected and susceptible animals (Taylor, 2016). Infected ruminants excrete *PPRV* prior to showing signs of the disease and this results in faster spreading of disease. This has adverse effects on the livestock industry as it places limitation on the export of livestock and their products to countries that are free from this disease. These countries in order to prevent trans-boundary transmission introducing trade sanctions (Gowane *et al.*, 2016). This virus may cause an economic disaster in the region as they depend on the ruminants and their products for trade (Banyard *et al.*, 2010). Another issue is if infected animals are introduced to a naive population, morbidity and mortality can reach almost 100%, causing a major setback to the livelihoods of livestock keepers (Jones *et al.*, 2016).

In conclusion, the current study reports the seroprevalence and associated risk factors of *PPR* in yaks and cattle for the first time in China. These findings have important epidemiological significance and direct influence in yaks on the remote plateau and domestic cattle in China. It is recommended that based on these results effective action be taken to control and eradicate this disease.

Conflict of interest

The authors state that there is no competing interest.

Acknowledgements. This study was supported by the General Project of Education of Zhejiang Province, 2017 (Y201737824) and the Startup Project for Doctoral Scientific Research of Wenzhou Vocational College of Science and Technology, 2016 (No. 201604).

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