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SHORT COMMUNICATION

Seroepidemiology of *Mycoplasma bovis* Infection in Yaks (*Bos grunniens*) in Tibet and Hongyuan of Sichuan, China

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ABSTRACT

Mycoplasma bovis is a multi-disease producing pathogen in domestic animals causing mastitis, arthritis, and pneumonia. Seroprevalence of antibodies to *M. bovis* in yaks from Tibet and Hongyuan area of Sichuan Province in China were investigated by a commercial enzyme-linked immunosorbent assay kits. Results showed that in 2012, 44.3 and 50.8% serum samples were found positive to *M. bovis* in Tibet and Hongyuan area; and 44.5 and 55.3% positive serum samples in 2013, respectively. The investigation indicates an increasing trend in *M. bovis* infection in Chinese yaks in Tibet and Hongyuan area of Sichuan Province. These observations raise a serious public concern considering a significant loss due to such infection in yaks.

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INTRODUCTION

Mycoplasmas, member of class Mollicutes lacking the cell wall are the simplest self-replicating prokaryotic microorganisms and widespread in animals and humans (Giovannini *et al*., 2013; Gautier-Bouchardon *et al*., 2014). *Mycoplasma bovis* (*M. bovis*), one of the Mycoplasmas, was first isolated from the milk of mastitic cow in 1961 and considered as an important bovine pathogen (Fu *et al*., 2014). Apart from causing pneumonia, mastitis, otitis, conjunctivitis, arthritis and tenosynovitis in bovines, *M. bovis* infection also imparts various economic losses in terms of treatment, laboratory diagnosis and cattle production (Fu *et al*., 2014; Gautier-Bouchardon *et al*., 2014; Wawegama *et al*., 2014).

The yaks (*Bos grunniens*) are inhabitants of about 3000 m high altitude (cold plateaus) in China, Mongolia, Bhutan, Nepal, India, Russia, and other countries (Gao *et al*., 2013a; Li *et al*., 2014). About 50% of the yak population of the world lives in Tibet and Sichuan provinces of China. As an economically important domestic animal to native herdsmen, any disease threat to the yaks in such areas results into the significant economic loss in milk and meat production (Li *et al*., 2014). *M. bovis* was found first time in Hubei province of China in 2008; and since then it's out breaks have been reported in more than 10 provinces (Fu *et al*., 2014). However, to date, a little information about *M. bovis* infections is available in domestic yaks at such high altitude and from the remote areas. The aim of this study was to determine the seroprevalence of *M. bovis* infection for the first time in yaks in Tibetan and Sichuan areas of China.

MATERIALS AND METHODS

Serum samples: Blood samples were collected randomly from the jugular vein by local veterinary practitioner from 521 yaks (Tibet 273, Sichuan 248) in five regions of Tibet and Hongyuan area of Sichuan province, China, in 2012; and 675 blood samples (Tibet 418, Sichuan 257) were obtained from four regions of Tibet and Hongyuan, Sichuan of China during 2013 (Table 1). Samples were centrifuged at 1,000×g for 10 min, and sera was obtained and stored at -20°C until further analysis.

The samples were tested by means of a commercial enzyme linked immunosorbent assay (ELISA) (Bovine Mycoplasma Ab ELISA Kit, Rapidbio, RB) according to the manufacturer's instructions. The Calculate Critical (CUT OFF) value was calculated based on the optical density (OD) values according to the formula: CUT OFF = the average OD 450 of negative controls+0.15. To ensure validity, the average OD 450 of positive and negative controls were kept ≥1.00 and ≤0.10 respectively. The results were interpreted as positive when OD 450 of sample value was ≥ CUT OFF.

Table 1: Seroprevalence of Mycoplasma in yaks by ELISA in Tibet and Hongyuan, Sichuan of China in 2012 and 2013

Area studies		2012[a]		2013[b]	
Province	County	Positive/ total samples	Sero-prevalence (%)	Positive/ total samples	Sero-prevalence (%)
Tibet	Nagqu	8/33	24.2		
	Nyingchi	36/94	38.3	60/198	30.3
	Lhasa	35/78	44.9	45/73	61.6[c]
	Xigaze	18/30	60.0	71/100	71.0
	Qamdom	24/38	63.2	10/47	21.3[d]
	Total	121/273	44.3	186/418	44.5
Sichuan	Hongyuan	126/248	50.8	142/257	55.3
	Total	247/521	47.4	328/675	48.6

Differences among the regions were found statistically significant in 2012[a] ($P<0.05$, $\chi^2=17.270$) and 2013[b] ($P<0.01$, $\chi^2=70.194$). Differences between 2012 and 2013 were found statistically significant in Lhasa[c] ($P<0.05$, $\chi^2=4.258$) and Qamdom[d] ($P<0.01$, $\chi^2=15.356$).

Statistical analysis was performed by chi-square test with SPSS (Statistical Analysis System, Version 17.0.). The differences were considered statistically significant when $P<0.05$.

RESULTS AND DISCUSSION

In 2012, a total of 247 out of 521 serum samples in Tibet and Hongyuan area of Sichuan Province were interpreted as positive for antibodies to *M. bovis*. The prevalence of *M. bovis* infection in each region ranged from 24.2% to 63.2%, and differences among the regions were found statistically significant ($P<0.05$, $\chi^2=17.270$) (Table 1). In 2013, a total of 186 out of 418 serum samples at this altitude were investigated as positive for antibodies against *M. bovis* and the prevalence was ranged from 21.3% to 71.0%, and differences among the regions were statistically significant ($P<0.01$, $\chi^2=70.194$) (Table 2). A statistical significant in seroprevalence was also found during the year 2012 and 2013 in the counties of Lhasa ($P<0.05$, $\chi^2=4.258$) and Qamdom ($P<0.01$, $\chi^2=15.356$).

Since the first isolation of *M. bovis* from a cow with severe mastitis in the United States in 1961 (Fu *et al*., 2014), many countries and areas had reported this infection. A study conducted in 2011 in subtropical southern China in Guangxi Zhuang Autonomous Region (GZAR) lying near to Tibet region have shown the presence of *M. bovis* infection in dairy cows (Fu *et al*., 2011). In this study, an increasing trend (47.4 - 48.6%) in the seroprevalence of *M. bovis* infection was found during the years 2012 and 2013 respectively which is much higher than the overall seroprevalence in nearby subtropical GZAR region (7.69%). The samples collecting areas were different in geography, climate and environment, which may affect the differences in *M. bovis* infection. The yaks usually remain in small to medium herds (50-200) and they are generally reared under transhumance system by the nomadic yak herdsman. During summer, they freely graze in pastures in the plateau and are kept in farms during winter (Gao *et al*., 2103b). In our study, the sampling was done randomly during summer covering most of the area as the climate becomes too harsh to collect the samples during winter at such high altitude.

M. bovis is not ubiquitous but widely spread within the bovine population in enzootically infected areas. The infection might usually be introduced to *M. bovis*-free herds by clinically healthy calves or young cattle shedding the mycoplasma and once established on multi-age sites, it becomes very difficult to eradicate (Nicholas *et al*., 2003). In Tibetan region, more than 100 species of wildlife live in one region sharing a common source of grazing pasture, land and water with yaks which may provide an easy opportunity for the transmission of the pathogen from one yak to another (Gao *et al*., 2103b). It may enhance the chances of acquiring *M. bovis*, and increases the difficulty for veterinary surgeons too to enable the prevention and control of this infection in yaks. On the other hand, we consider that the main reason for the high seroprevalence found in this investigation may be that the groups of yaks we investigated under extensive management, have no effective immunization programs, and live in a complex environment.

In many parts of the world the significance of respiratory tract infection with *M. bovis* has become increasingly apparent in recent years because of greater recognition of its role in pneumonia, the increasing resistance of this bacterium to antimicrobial drugs. An alternative strategy to control *M. bovis* infection in cattle may be applied by separating the infected cattle from uninfected ones (Wawegama *et al*., 2014). Also, *M. bovis* can be a primary pathogen; however it may act as a predisposing factor that weakens the host immune system and facilitates the invasion of other microorganisms (Giovannini *et al*., 2013; Fu *et al*., 2014).

The study carried out first time about the seroprevalence of *M. bovis* infection in yaks in Tibet and Hongyuan area of Sichuan province, China with high prevalence which should elucidate a concern to take action.

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Author's contribution: KL and JKL participated in the design of study. KL, MS and ZQH performed the experiments and K Li and M Shahzad analyzed the data. K Li wrote the manuscript. All authors read and approved the final manuscript.

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