

Short Paper

First serologic survey of Q fever in free-range yaks in China

Yin, M. Y.^{1,2}; Tan, Q. D.¹; Qin, S. Y.¹; Hu, L. Y.¹; Liu, G. H.³;
Zhou, D. H.^{3,4} and Zhu, X. Q.^{3,4*}

¹BSc in Veterinary Science, State Key Laboratory of Veterinary Etiological Biology, Lanzhou Veterinary Research Institute, Chinese Academy of Agricultural Sciences, Lanzhou, Gansu Province, China; ²Department of Parasitology, College of Veterinary Medicine, Hunan Agricultural University, Changsha, Hunan Province, China; ³State Key Laboratory of Veterinary Etiological Biology, Lanzhou Veterinary Research Institute, Chinese Academy of Agricultural Sciences, Lanzhou, Gansu Province, China; ⁴Jiangsu Co-innovation Center for the Prevention and Control of Important Animal Infectious Diseases and Zoonoses, College of Veterinary Medicine, Yangzhou University, Yangzhou, Jiangsu, China

*Correspondence: X. Q. Zhu, State Key Laboratory of Veterinary Etiological Biology, Lanzhou Veterinary Research Institute, Chinese Academy of Agricultural Sciences, Lanzhou, Gansu Province, China. E-mail: xingquanzhu1@hotmail.com

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Summary

The objective of the present investigation was to determine the seroprevalence of *Coxiella burnetii* infection in free-range yaks in China. A total of 552 serum samples were collected from yaks in Gansu province, northwest China between April 2013 and January 2014, and antibodies against *C. burnetii* were evaluated using enzyme-linked immunosorbent assay (ELISA). Overall, 13.59% (75/552, 95% CI: 10.73-16.45) of the examined animals were positive for *C. burnetii* antibodies. There was no significant difference in *C. burnetii* seroprevalence between female yaks (13.78%, 95% CI: 10.36-17.19) and male yaks (13.13%, 95% CI: 7.89-18.36) ($P>0.05$). *Coxiella burnetii* seroprevalence in yaks in different age groups ranged from 10.88% to 15.26%, but the difference was not statistically significant ($P>0.05$). *Coxiella burnetii* seroprevalence in yaks sampled in different seasons ranged from 12.06% (autumn) to 18.33% (summer), but the difference was not statistically significant ($P>0.05$). This is the first report of *C. burnetii* seroprevalence in free-range yaks in China, indicating the need for measures to be taken to control *C. burnetii* infection in free-range yaks in China.

Key words: China, *Coxiella burnetii*, Seroprevalence, Yaks

Introduction

Coxiella burnetii, a small gram-negative bacterium, is the causative agent of Query (Q) fever (Parker *et al.*, 2006; Tutusaus *et al.*, 2014). It was first reported by Derrick in 1935 in Queensland, Australia, during an outbreak of a febrile illness among workers in slaughterhouses. Subsequently, many similar cases have been reported from around the world (Kaplan and Bertagna, 1955). Humans are infected by inhalation of *C. burnetii* in aerosols, and it can present itself as a flu-like illness, as pneumonia, or as hepatitis (Dupont *et al.*, 1971; Tissot Dupont *et al.*, 1992). The infection in humans is often symptomless, therefore it can be diagnosed as other flu-like illnesses by mistake (Cutler *et al.*, 2007). Although acute disease is usually self-limiting, a few cases progress to the most serious form of *C. burnetii* infection (i.e., chronic stage) characterized mainly by endocarditis or vascular infection (Brouqui *et al.*, 1993).

Coxiella burnetii can also infect many animal hosts, such as cattle, pigs, horse, yak, sheep and goat (Rodolakis, 2009). *Coxiella burnetii* most notably presents as a major pathogen of abortion, leading to reproductive failure in affected cattle, sheep and goat. It is estimated that the rate of abortion ranges from 3% to 80% (Arricau-Bouvery and Rodolakis, 2005). Further-

more, it can be associated with abortion, premature delivery, stillbirth and weak offspring complex. This organism is highly infectious and is highly prevalent in almost every flock, resulting in significant economic losses worldwide (van Asseldonk *et al.*, 2013).

Zeitenok and Pille (1956) reported that 3 out of 8 yaks had Q fever antibodies in the sera (Zeitenok and Pille, 1956), and this is the first and also the only reliable report documenting the seroprevalence of *C. burnetii* infection in yaks around the world (Zeitenok and Pille, 1956). Although *C. burnetii* is considered an emerging pathogen causing major economic losses in animals and humans in China (El-Mahallawy *et al.*, 2015), it is still unknown whether yaks in the People's Republic of China (PRC) were infected with *C. burnetii*. China is the largest producer of yaks in the world.

The objective of the present investigation was to estimate the seroprevalence of *C. burnetii* infection in yaks in China. The results should provide a foundation for the execution of control strategies against *C. burnetii* infection in yaks in China.

Materials and Methods

Samples

Between April 2013 and January 2014, a total of 552 serum samples (208 in spring, 60 in summer, 199 in

autumn, and 85 in winter) were collected from free-range yaks in Gannan Tibetan Autonomous Prefecture, Gansu Province, which is one of the major yak producers in China. Blood samples were stored at 37°C for 24 h and then centrifuged for 10 min at 3000 rpm. Sera were separated from each blood sample and then kept at -20°C. Information regarding yaks including gender and age were recorded during the sampling process.

ELISA

Serum samples were tested for Q fever antibodies using the Q fever LSI ELISA kit (LSI, Lissieu, France) according to the manufacturer's instructions. Each test included the positive and the negative control. Those samples with suspicious results were retested.

Statistical analysis

Differences in the seroprevalence of *C. burnetii* among yaks of different genders, seasons and different age groups were analyzed with Chi-square tests using the SPSS software (SPSS Inc., IBM Corporation, Version 18, USA). It was regarded statistically significant when the p-value <0.05.

Results

Seventy-five (13.59%) of 552 examined yak serum samples were positive for *C. burnetii*. *Coxiella burnetii* seroprevalence was higher in female yaks (13.78%, 95% CI: 10.36-17.19) than in male yaks (13.13%, 95% CI: 7.89-18.36), but the difference was not statistically significant (P=0.84) (Table 1). *Coxiella burnetii* seroprevalence in yaks increased with age but the difference was not statistically significant (P=0.41) (Table 1). In terms of sampling seasons, *C. burnetii* seroprevalence in yaks was higher in summer (18.33%, 95% CI: 8.54-28.12) and winter (14.12%, 95% CI: 6.72-21.52) than in spring (13.46%, 95% CI: 8.82-18.10) and autumn (12.06%, 95% CI: 7.54-16.59), but the difference was not statistically significant (P=0.67) (Table 1).

Discussion

The present survey revealed a 13.59% seroprevalence

of *C. burnetii* infection in yaks in China for the first time. This seroprevalence was significantly higher than that detected in cattle (3.4%) in the USA (McQuiston and Childs, 2002) and beef cattle (6.7%) in northern Spain (Ruiz-Fons *et al.*, 2010). But this result is much lower than those observed in Europe. For example, ELISA testing showed a 38% *C. burnetii* seroprevalence in cattle in Hungary (Gyuranecs *et al.*, 2012). The seroprevalence was also lower compared to the results from other tropical countries. The seroprevalence in cattle was between 40% and 59.8% in Nigeria, Sudan and Zimbabwe (Guatteo *et al.*, 2011). A PCR testing showed that *C. burnetii* prevalence in vaginal swabs of cow and ewe was 0.81% and 4.4%, respectively (Cardinale *et al.*, 2014). A previous study reported a 20% *C. burnetii* seroprevalence in sheep in Turkey (Kennerman *et al.*, 2010), which is higher than the *C. burnetii* seroprevalence in free-range yaks in China. The difference in *C. burnetii* seroprevalence in yaks, cattle and beef cattle may be explained by the fact that different species of cattle had different susceptibility to *C. burnetii* infection. The aerosol route (inhalation of infected fomites) is the primary mode of *C. burnetii* transmission. When the weather gets warmer, the *C. burnetii* seroprevalence gets higher (Maurin and Raoult, 1999). All the sampled yaks were free-ranging and the animal welfare and animal husbandry practices were poor, which may contribute to the high *C. burnetii* seroprevalence in yaks. In conclusion, the reported difference in *C. burnetii* seroprevalence is likely due to differences in susceptibility, animal welfare, climates, and animal husbandry practices. Another possible reason for the difference in seroprevalence may be due to the use of different investigative methods.

The present survey showed that *C. burnetii* seroprevalence in yaks was the highest in summer, but the lowest in autumn although the difference is not statistically significant; suggesting that *C. burnetii* infection in yaks was prevalent all year round, with a peak in summer. Possible explanations include mild temperature and high humidity in summer in the region which may be suitable for the survival of *C. burnetii*, leading to increased risk of infection (Cardinale *et al.*, 2014). Ticks can transport *C. burnetii* and cause Q fever

Table 1: Prevalence of *Coxiella burnetii* antibodies in yaks by gender, age and season in China using enzyme-linked immunosorbent assay (ELISA)

| Factor | Category | Sample No. | Sample No. | Prevalence (%) | 95% CI | χ^2 | P-value |
|--------|----------|------------|------------|----------------|-------------|----------|---------|
| Gender | Male | 160 | 21 | 13.13 | 7.89-18.36 | 0.04 | 0.84 |
| | Female | 392 | 54 | 13.78 | 10.36-17.19 | | |
| Age | ≤1 | 147 | 16 | 10.88 | 5.85-15.92 | 1.77 | 0.41 |
| | 2 | 97 | 12 | 12.37 | 5.82-18.92 | | |
| | ≥3 | 308 | 47 | 15.26 | 11.24-19.28 | | |
| Season | Spring | 208 | 28 | 13.46 | 8.82-18.10 | 1.57 | 0.67 |
| | Summer | 60 | 11 | 18.33 | 8.54-28.12 | | |
| | Autumn | 199 | 24 | 12.06 | 7.54-16.59 | | |
| | Winter | 85 | 12 | 14.12 | 6.72-21.52 | | |
| Total | | 552 | 75 | 13.59 | 10.73-16.45 | | |

in many animals, including yaks (Maurin and Raoult, 1999; Yu, 2000). With the increase of temperature, especially in summer, ticks may strengthen their activity, which may contribute to the higher *C. burnetii* seroprevalence in yaks in summer.

The present survey showed that *C. burnetii* seroprevalence in female yaks was only slightly higher than that in male yaks, suggesting that both genders of yaks are similar susceptible to *C. burnetii*. Although yaks of different age categories had slightly different *C. burnetii* seroprevalence, the difference was not statistically significant, which is consistent with those of previous studies (Kennerman *et al.*, 2010; McCaughey *et al.*, 2010).

In conclusion, the present investigation provides first evidence that *C. burnetii* infection is highly prevalent in free-range yaks in China, which may cause major economic impact on these animals. Further studies are required to clarify the exact epidemiological situation of *C. burnetii* infection in different yak flocks in China, and to elucidate the negative impact of *C. burnetii* infection on yak production.

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