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Serologic Evidence for *Babesia bigemina* Infection in Wild Yak (*Bos mutus*) in Qinghai Province, China

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ABSTRACT: Piroplasmosis, caused by tick-borne protozoan parasites of the genera *Theileria* and *Babesia*, is one of the most economically important infections of domestic ruminants in China. We evaluated the prevalence of antibodies to *Babesia bigemina* in wild yaks (*Bos mutus*) from Qinghai Province, China. Of 91 samples, 24% had detectable *B. bigemina* antibodies, and significant differences in prevalence were observed between those older than 5 yr and those younger than 5 yr. We collected 1,679 ticks belonging to two Ixodid genera from the infested wild yaks. The most prevalent tick species was *Dermacentor nuttalli* (48.1%), followed by *Haemaphysalis qinghaiensis* (33.3%) and *Dermacentor silvarum* (18.6%). These results indicated that *B. bigemina* is most likely endemic in the study area, and active surveillance programs for piroplasmosis are needed for monitoring animal health status.

Key words: *Babesia bigemina*, serology, ticks, wild yaks.

Piroplasmosis, caused by tick-borne protozoans in the genera *Theileria* and *Babesia*, is the most economically important parasitic infection of domestic ruminants in various areas worldwide (Uilenberg 2006; Schnittger et al. 2012). Clinical signs usually include anemia, fever, and anorexia and, in severe cases, can lead to widespread organ failure and death (Uilenberg 1995; Schnittger et al. 2012; Tian et al. 2013; Gebrekidan et al. 2014). Cattle (*Bos taurus*) that recover from the acute disease and infections can become chronic piroplasm carriers and act as a reservoir for ticks, maintaining the life cycle of the parasite (Gomes et al. 2013; Tian et al. 2013). At least five species of *Babesia*—*B. bigemina*, *B. bovis*, *B. major*, *B. orientalis*, and *B. ovata*—have been described in cattle in China. Among them, *B. bigemina* and *B. bovis* are the most common and are major causative agents of bovine babesiosis and often result in coinfection because they share the same vectors, *Rhipicephalus* (*Boophilus*) *microplus*, *Rhipicephalus annulatus*, and *Rhipicephalus geigyi* (Yin et al. 1997; Caeiro 1999; Liu et al. 2008, 2014; Silva et al. 2009; He et al. 2012). The clinical manifestations of these two parasite infections in cattle are similar, but disease caused by *B. bovis* is more serious than that caused by *B. bigemina* (Liu et al. 2014).

The semidomesticated yak (*Bos grunniens*), is an iconic symbol of the Qinghai-Tibet Plateau and occurs across Qinghai, Tibetan, Sichuan, Gansu, Xinjiang, and Yunnna provinces, where it contributes significantly to the highland economy and nutritional security of the tribal population living in the difficult terrain of Qinghai-Tibet Plateau (Saravanan et al. 2013). There is exhaustive literature describing *Babesia* species infecting several livestock and a variety of wildlife species. The incidence of babesiosis in domesticated yaks has also been described (Bai et al. 2002; Yin et al. 2002, 2004; Liu et al. 2010; Schnittger et al. 2012; Saravanan et al. 2013). Until now, however, no information on *Babesia* species infecting wild yaks (*Bos mutus*) has been reported.

We investigated the prevalence of antibody to *B. bigemina* in wild yaks in

Qinghai Province, China, June–December 2011. The wild yaks selected for this study were aged according to the number of incisors and divided into those younger than 5 yr and those older than 5 yr. We collected 91 blood samples from apparently healthy wild yaks in rural areas of Haixi Mongolian and Tibetan Autonomous Prefecture, Qinghai Province. Sera were separated and stored at −20 C until analyzed. Simultaneously, the body surface of each yak was thoroughly examined, and all ticks attached to the skin were collected and brought to the laboratory for morphologic identification according to standard identification keys (Chen et al. 2010; Kumsa et al. 2014).

We used a commercial enzyme-linked immunosorbent assay (ELISA) (Lanzhou Veterinary Research Institute, Lanzhou City, Gansu Province, China) to measure the prevalence of *B. bigemina* infection (as indicated by antibodies) in wild yaks. The ELISA was performed according to the manufacturer's instructions. The sensitivity and specificity were 94.2% and 96.5%, respectively, and the highest rate of intrareproducibility and interreproducibility were 1.92% and 1.76%. There was no cross-reactivity between *B. bigemina* and other *Babesia* and *Theileria* species, and the ELISA also distinguished infection with *B. bovis* and *B. bigemina*. The mean optical density (OD) was measured at 630 nm using a Multiskan MK3 (Thermo Fisher Scientific China, Changhai, China). The critical value was the mean OD of negative controls plus 0.15. A mean OD of test samples greater than or equal to the critical value was considered positive; values below that value were negative.

Pearson's χ^2 test was used to determine statistical significance of the differences in prevalence of *B. bigemina* infection between age groups (Gomes et al. 2013). Differences were considered statistically significant at $P<0.05$. Statistical calculations were performed using SPSS, version 16.0 (SPSS, Chicago, Illinois, USA).

Of 91 serum samples, 22 (24%) were positive for *B. bigemina* antibody. This was higher than reported by Saravanan et al. (2013) who found *B. bigemina* infection in 5.32% of yaks in India. The differences between China and India in prevalence of *B. bigemina* infection may be due to different diagnostic methods, the vector ticks' distribution, climate variation, and time of sampling (Swai et al. 2007; Saravanan et al. 2013). The prevalence of *B. bigemina* infection in yaks younger than 5 yr was 28% (18 of 64) and in yaks older than 5 yr was 15% (4 of 27); the difference was statistically significant ($P< 0.05$). Prevalence decreased with increasing age, which differs from the results of Swai et al. (2007), who found the opposite. This difference may be due to the type of animal population, the species of tick vectors, climatic variation, or the stratification of animals into different age classes (Swai et al. 2007).

We collected 1,679 ticks from the sampled yaks in Qinghai Province during June and December 2011. Three species, *Haemaphysalis qinghaiensis* (559, 33.3%), *Dermacentor nuttalli* (808, 48.1%), and *Dermacentor silvarum* (312, 18.6%), were identified based on morphologic characters. There was a significant difference in collection frequency between the two *Dermacentor* species ($P<0.05$). Li et al. (2013) reported only *D. nuttalli* in Haixi and Qinghai provinces. This difference may be attributed to the different type of animal population, ecologic environment of study sites, or migration of wild yaks (Swai et al. 2007).

To our knowledge, ticks of the family Ixodidae are the main vectors of piroplasms, and their geographic distribution influences the epidemiology of piroplasmosis (Ibrahim et al. 2013). Although we detected antibodies to *B. bigemina* in yak sera, we did not collect the vector ticks of *B. bigemina* (*Rhipicephalus* [*Boophilus*] *microplus*, *R. annulatus*, or *R. geigyi*), suggesting that *H. qinghaiensis*, *D. nuttalli*, or *D. silvarum* may be the vectors

that maintain endemicity of *B. bigemina* in Qinghai Province, but that will need to be experimentally validated (Li et al. 2013).

Our work adds to the knowledge of the epidemiologic features of *Babesia* spp. infections in Qinghai Province and indicates that *B. bigemina* may be endemic in wild yaks in our study region. Our data on the prevalence of *B. bigemina* infection in wild yaks in Qinghai Province can inform management and control programs for piroplasmosis in wild yaks in Qinghai Province.

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