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Short communication

Distribution of *Cryptosporidium* species in Tibetan sheep and yaks in Qinghai, China

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ABSTRACT

Few data are available on the distribution of *Cryptosporidium* species in Tibetan sheep and yaks, which are free-range animals living in a cold, low oxygen, and high ultraviolet radiation habitat. In this study, 904 fecal specimens were collected from 350 Tibetan sheep and 554 yaks in six counties. *Cryptosporidium* spp. were detected and differentiated by PCR and sequence analyses. Altogether, 43 (12.3%) Tibetan sheep and 158 (28.5%) yaks were positive for *Cryptosporidium* spp. In Tibetan sheep, *Cryptosporidium xiaoi* (39/43, 90.7%) was the dominant species, with the remaining cases (4/43, 9.3%) by *Cryptosporidium ubiquitum*. All *C. ubiquitum* specimens belonged to the subtype family XIIa. In contrast, *Cryptosporidium andersoni* (72/158, 45.6%), *Cryptosporidium bovis* (47/158, 29.7%), *Cryptosporidium ryanae* cattle type (35/158, 22.2%), *C. ryanae* buffalo type (2/158, 1.3%), and *Cryptosporidium suis*-like (2/158, 1.3%) were identified in yaks. Contradictory to previous observations, *C. andersoni* was one of the dominant *Cryptosporidium* species in yaks in this study. Despite sharing habitats, Tibetan sheep and yaks are evidently infected with different *Cryptosporidium* species.

1. Introduction

Cryptosporidium spp. are common parasites in humans and domestic animals. In ruminants, cryptosporidiosis can cause diarrhea and weight loss in neonatal animals, leading to economic losses (Santín, 2013). In addition, molecular biological studies have increased the recognition of zoonotic transmission of cryptosporidiosis. Consequently, sheep and cattle are major reservoirs of *Cryptosporidium* spp. in humans and source of environmental contamination (Ryan et al., 2014).

Several *Cryptosporidium* species have been identified in sheep. The dominant species are *Cryptosporidium ubiquitum*, *Cryptosporidium xiaoi* and *Cryptosporidium parvum* (Ryan et al., 2014). To date, there are very few studies on the distribution of *Cryptosporidium* species in sheep in China. In the study reported by Wang et al. (2010), *C. ubiquitum* was identified as the major species in sheep in Henan Province. Similarly, Shen et al. (2011) detected only *C. ubiquitum* in four *Cryptosporidium*-positive pre-weaned lambs in Sichuan Province. In contrast, *C. xiaoi* was the most common species in sheep in Inner Mongolia (Ye et al., 2013). Thus, the distribution of *Cryptosporidium* species in sheep in China is not clear.

Most investigations have demonstrated that the distribution of *Cryptosporidium* species in dairy cattle is age-related. *C. parvum* is the dominant species in pre-weaned calves, *Cryptosporidium bovis* and *Cryptosporidium ryanae* predominate in post-weaned calves, whereas *Cryptosporidium andersoni* is the dominant species in juvenile and adult cattle (Santín, 2013; Santin et al., 2008). Several other *Cryptosporidium* species have been reported occasionally in cattle (Ryan et al., 2014). The distribution of *Cryptosporidium* species in free-range bovine animals is less well known.

Yaks (*Bos grunniens*) are bovine animals living on the Qinghai-Tibetan Plateau of China. They are kept outdoors and share pastures with Tibetan sheep and wild animals. In recent studies six *Cryptosporidium* species, including *C. bovis*, *C. ryanae*, *C. andersoni*, *C. parvum*, *C. xiaoi*, and *C. ubiquitum* have been identified (Feng et al., 2007; Karanis et al., 2007; Ma et al., 2014; Mi et al., 2013; Qi et al., 2015a). However, only three cases of *C. andersoni* infection have been detected, although *C. andersoni* is the dominant species in

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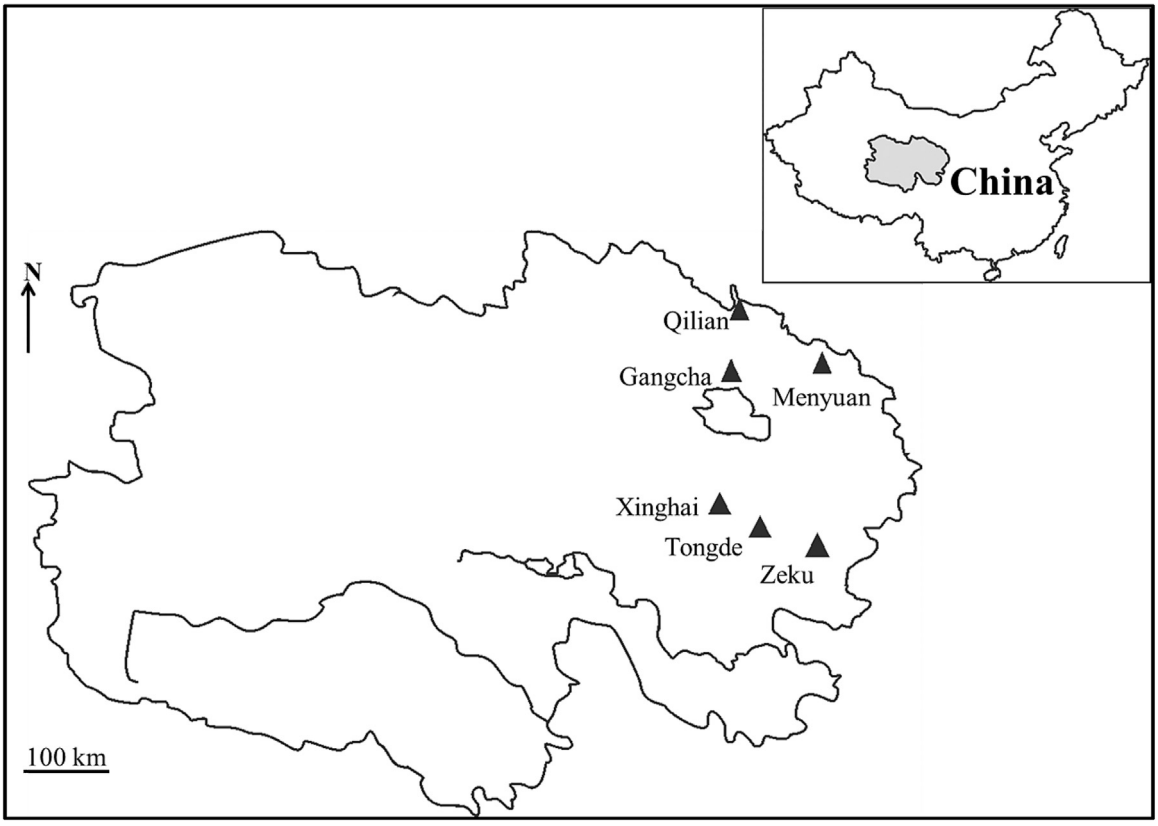

Fig. 1. Counties (▲) in Qinghai Province, northwestern China sampled in this study.

dairy cattle in several locations in China (Liu et al., 2009; Wang et al., 2011). Thus, the distribution of *Cryptosporidium* species could be different between yaks and dairy cattle in China, which may be attributed to the free-range nature (sharing pastures with Tibetan sheep) and cold habitat of yak farming (Ma et al., 2014).

To improve our knowledge of cryptosporidiosis epidemiology in China, we examined in this study the distribution *Cryptosporidium* species in Tibetan sheep and yaks from Qinghai Province.

2. Materials and methods

2.1. Specimen collection

A total of 904 fresh fecal specimens were collected between June 2013 and May 2015 from 350 Tibetan sheep and 554 yaks in Qinghai Province, northwestern China. The sheep specimens were collected from Zeku, Qilian, Xinghai, Tongde, Menyuan, Gangcha Counties, whereas the yak specimens were collected from the same counties except Gangcha (Fig. 1). Qinghai Province has a continental plateau climate, with cool and short summers and cold and long winters. The average altitude, annual rainfall, and annual temperature of the six counties range from 2800 to 4500 m, 360 to 540 mm, and −2.4 to 3.0 °C, respectively. Tibetan sheep and yaks are main domestic animals there, are raised outdoor, and share grazing lands with wild animals.

The animals were divided into three age groups (<1 year, 1–2 years and >2 years old). The first age group (<1 year) were young animals with close associations to their mothers, the second group (1–2 years) were juveniles, whereas the third group (>2 years) were breeding age animals. Only a small number of young animals were sampled because of the seasonal nature of breeding and difficulties in sampling free-range animals during cold seasons. The sampled animals were mostly healthy, with no obvious occurrence of diarrhea. Specimens were stored at 4 °C in 2.5% potassium dichromate before DNA extraction.

2.2. DNA extraction

Approximately 0.5 g of stored fecal specimens were washed three times with distilled water by centrifugation at 2000 × *g* for 10 min. DNA was extracted from the washed fecal material using the FastDNA SPIN Kit for Soil (MP Biomedicals, Santa Ana, CA) as previously described (Jiang et al., 2005), and stored at −80 °C before analysis by PCR.

2.3. Cryptosporidium *detection, genotyping, and subtyping*

To detect *Cryptosporidium* spp., a ~587-bp fragment of the small subunit (SSU) rRNA gene was amplified by nested PCR (Ryan et al., 2003). Each specimen was analyzed at least twice using 1 μl of the extracted DNA in PCR. Reagent-grade water was used as the negative control, whereas *Cryptosporidium canis* DNA was used as the positive control. To identify the *C. ubiquitum* subtype, a ~948 bp fragment of the 60 kDa glycoprotein (gp60) gene was amplified by nested PCR (Li et al., 2014).

All positive PCR products of the SSU rRNA or gp60 genes were sequenced in both directions using an ABI 3130 Genetic Analyzer (Applied Biosystems, Foster City, CA). The obtained sequences were assembled using ChromasPro 1.32 (http://technelysium.com.au/ChromasPro.html) and compared with reference sequences in the NCBI database using ClustalX (http://clustal.org/) to determine *Cryptosporidium* genotypes and *C. ubiquitum* subtypes. Representative nucleotide sequences generated in this study were submitted to GenBank database under accession numbers KU052802–KU052815.