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Research paper

Molecular prevalence of spotted fever group rickettsiae in ticks from Qinghai Province, northwestern China

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ABSTRACT

Tick-borne rickettsioses is one of the oldest known vector-borne diseases and has been viewed as emerging or re-emerging disease in China. The causative agents have been increasingly recognized and exhibited a high degree of genetic diversity and widespread distribution. This study was conducted to determine the prevalence of spotted fever group rickettsiae in ticks from Qinghai Province, northwestern China. In total, 860 questing adult ticks representing six species were collected. The SFG rickettsiae were detected in *Haemaphysalis qinghaiensis* (19.6%, 79/404), *Dermacentor abaensis* (73.7%, 157/213), *Dermacentor silvarum* (50.0%, 47/94), *Dermacentor nuttalli* (67.4%, 97/144), and *Ixodes crenulatus* (100%, 3/3), with an overall infection rate of 44.5%. The infection rates of SFG rickettsiae were significantly higher in *Dermacentor* spp. than in *Haemaphysalis* spp. ($p < 0.05$). Sequence analyses of the *gltA* and *ompA* genes revealed that five SFG rickettsiae are present in ticks in Qinghai, including *R. sibirica* subspecies *sibirica*, *R. raoultii*, "*Candidatus Rickettsia* tibetani", and "*Candidatus Rickettsia* gannanii" Y27 and F107. Moreover, a potential novel *Rickettsia* species (*Rickettsia* sp. 10CYF) was identified in *D. nuttalli* and *I. crenulatus*. These findings extend our knowledge of the potential vector spectrum and distribution of rickettsiae, and provided valuable information for assessing the potential risk for public health.

1. Introduction

Rickettsiae are obligate intracellular Gram-negative bacteria associated with arthropods (e.g., ticks, fleas, mites, and lice) (Raoult and Roux, 1997; Merhej et al., 2014). They are widely distributed throughout the world, and some of which infect humans and other vertebrate or invertebrate hosts (Fournier and Raoult, 2009). This genus encompasses at least 27 *Rickettsia* species with validated and published names, and a number of putative novel *Rickettsia* species that have not been fully characterized, were continually isolated from or detected in ticks (Parola et al., 2013; Merhej et al., 2014). The members in the genus *Rickettsia* have been classified into four different groups, including the well-defined spotted fever group (SFG) and typhus group (TG), the *Rickettsia bellii* group, and the *Rickettsia canadensis* group (Merhej et al., 2014).

Ticks are important vectors that can transmit various pathogenic organisms to both humans and animals, such as *Anaplasma* spp., *Rickettsia* spp., and *Ehrlichia* spp. (Fang et al., 2015). Tick-borne rickettsioses are caused by SFG rickettsiae, and have been recognized as emerging or re-emerging diseases with worldwide distribution (Parola et al., 2005; Parola et al., 2013). To date, six validated species of SFG rickettsiae have been reported in China, including *Rickettsia heilongjiangensis*, *Rickettsia sibirica*, *Rickettsia raoultii*, *Rickettsia slovaca*, *Rickettsia aeschlimannii* and *Rickettsia massiliae*, and several potential novel *Rickettsia* spp. have also been reported based on phylogenetic analyses of several gene loci, such as "*Candidatus Rickettsia* hebeiii", "*Candidatus Rickettsia* tarasevichiae", "*Candidatus Rickettsia* tibetani", "*Candidatus Rickettsia* gannanii" and *Rickettsia* sp. XY99 (Zou et al., 2011; Jia et al., 2013b; Fang et al., 2015; Wei et al., 2015; Li et al., 2016; Yang et al., 2016). Five species of these SFG rickettsiae (*R. heilongjiangensis*, *R. raoultii*, *R. sibirica*, "*Candidatus* R. tarasevichiae", and *Rickettsia* sp. XY99) have been identified as human pathogens in China (Fang et al., 2015). Currently, several studies have revealed the extensive diversity of SFG rickettsiae in different tick species and geographic locations (Merhej et al., 2014). Qinghai is located on the Qinghai-Tibetan Plateau with unique and vigorous natural ecosystem,

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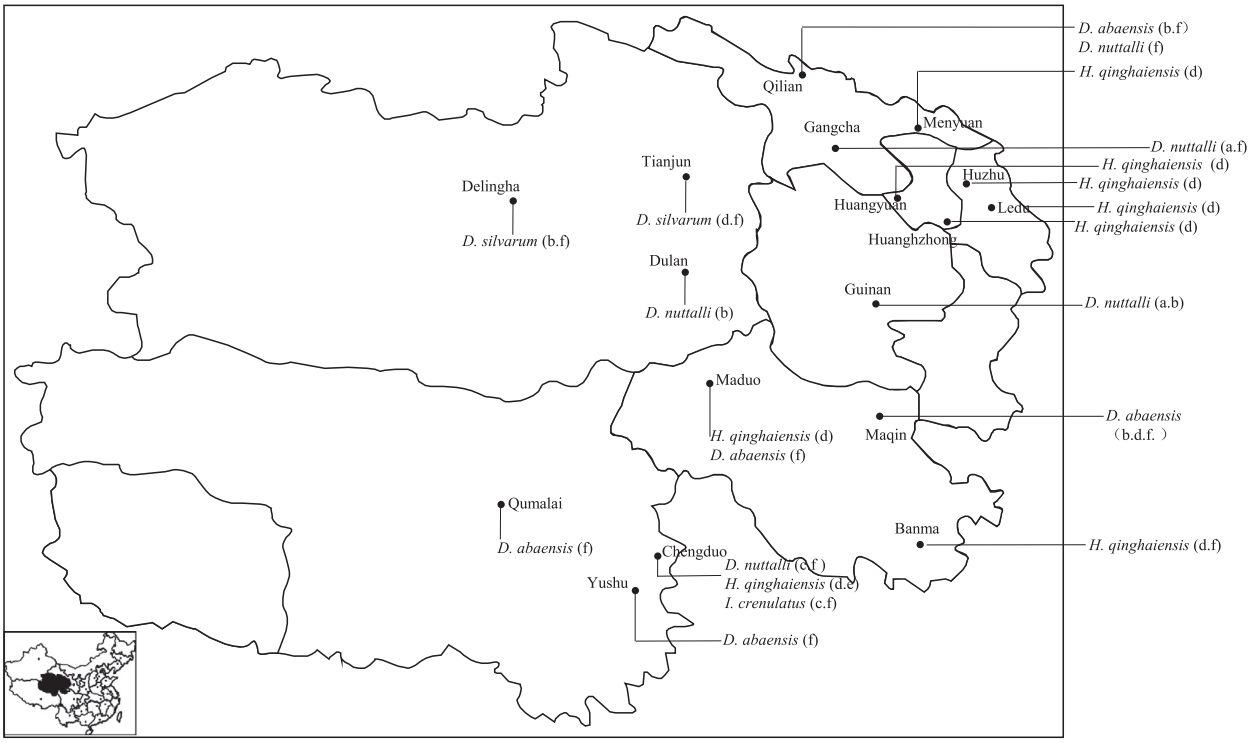

a, *R. sibirica* subspecies *sibirica* BJ-90; b, *R.raoultii*; c, *Rickettsia* sp.10CYF; d, *Candidatus* R. gannanii Y27; e, *Candidatus* R. gannanii F107; f, *Candidatus* R. tibetani

Fig. 1. Sample collection sites and distribution of rickettsiae in Qinghai, northwestern China.

which is well known for the largest and highest plateau in the world. The objective of this study was to determine the presence and molecular characterization of rickettsial species in questing ticks collected from 17 regions in Qinghai Province.

2. Materials and methods

2.1. Study sites

Qinghai Province, with an area of 720,000 km^2, is located on the northeastern of Qinghai-Tibetan plateau, which is known as the “Roof of the World”. The average elevation is 3000 m and the mean annual temperature is approximately −5 to 8 °C. Three great rivers (the Yangtze River, Yellow River and Lantsang River) originate in here, where is one of the five major pastoral areas in China, accounting for 15% of the country's total. Due to the high altitude, cold climate and oxygen deficiency, Qinghai has the unique and vigorous natural ecosystem. Geologically, this province is separated by the Riyue Mountain into two parts, the east agricultural region, and the west pastoral region, where the majority of the livestock is raised (Fig. 1). The livestock includes sheep, goats, yaks, cattle-yaks, horse and camels, and the domestic yaks ranked on the top in the country in number and accounted for one-third of the world's total.

2.2. Tick collection

Questing ticks were collected on the vegetation with the flagging methods from 17 counties in Qinghai between January and April in 2015 and 2016 (Fig. 1), including: Huangyuan (101°17′E, 36°43′N), Ledu (102°23′E, 36°29′N), Huangzhong (101°34′E, 36°29′N), Mengyuan (101°37′E, 37°22′N) and Huzhu (101°57′E, 36°50′N), situated in the east agricultural region, with an altitude of 1800 to 2800 m above sea level (a.s.l.); Qilian (100°13′E, 38°12′N), Gangcha (100°10′E, 37°19′N), Tianjun (99°02′E, 37°17′N), Dulan (98°08′E, 36°18′N), Guinan (100°45′E, 35°34′N), Maduo (98°16′E, 34°55′N), Maqin (100°16′E, 34°29′N), Qumalai (95°30′E, 34°31′N), Yushu (96°58′E, 33°02′N), Delingha (97°23′E, 37°22′N), Chengduo (97°07′E, 33°21′N), and Banma (100°44′E, 32°55′N), located in the west pastoral region, with an altitude of 2800 to 4300 m a.s.l. Based on microscopic examination, the ticks were morphologically identified to the species level according to the taxonomic key (Deng and Jiang, 1991).

2.3. DNA extraction

All tick samples were soaked in 70% ethanol and subsequently washed three times in double distilled water. They were separated by species, location and sex (Deng and Jiang, 1991). Detailed information regarding the tick samples used in this study is shown in Table 1. Ticks were homogenized individually with a tissue grinding pestle under liquid nitrogen. DNA was extracted from tick homogenates by using the QIAamp DNA Mini Kit (Qiagen, Beijing, China) according to the manufacture's protocols. DNA was stored at −20 °C until use.

2.4. PCR reactions

The extracted DNA was subject to PCR for the amplification of the citrate synthase gene (*gltA*) gene with primers RpCS.409d and RpCS.1258n as previous described (Roux et al., 1997). All the positive samples were further screened for the presence of SFG rickettsiae by PCR with Rr190.70 and Rr190.701 primers targeted on the outer surface membrane protein A gene (*ompA*) gene (Roux et al., 1996). The reaction was performed in an automatic thermocycler (Bio-Rad, Hercules, USA) with a total volume of 25 μL as previous described (Yang et al., 2016). Genomic DNA extracted from tick positive for SFG rickettsiae (GenBank accession no. KT921893) was used as the positive control, and sterile water was used as the blank control for each assay. PCR products were visualized by UV transillumination in a 1.0% agarose gel following electrophoresis and staining with ethidium bromide.