

Original article

Rickettsia raoultii, the predominant *Rickettsia* found in Mongolian *Dermacentor nuttalli*[☆]

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ABSTRACT

Since the year 2005, clinical patterns resembling tick-borne rickettsioses have been noticed in Mongolia. Epidemiological data regarding species of the aetiological agent, tick vector, prevalence, and distribution as well as incidence of human cases throughout Mongolia are still sparse to date. In order to identify *Rickettsia* species occurring in Mongolia, we investigated *Dermacentor nuttalli* ($n = 179$) and *Ixodes persulcatus* ($n = 374$) collected in 4 selected provinces. *Rickettsia raoultii* was the predominant *Rickettsia* (82% prevalence) found in *D. nuttalli* and was also detected in *I. persulcatus* (0.8%). The *Rickettsia* prevalence in *D. nuttalli* from different provinces varied between 70% and 97%. In addition, *R. sibirica* was identified in approximately 4% of *D. nuttalli*, but solely from Arkhanghai province. The results of this study extend the common knowledge about the geographic distribution of *R. raoultii* and its high prevalence in *D. nuttalli*. Although the pathogenicity of this *Rickettsia* is still unclear, it should be considered in Mongolian patients suspected of having tick-borne rickettsiosis.

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Introduction

In Mongolia, human cases of tick-borne rickettsioses have been increasing in recent years. From 2005 to 2011, 240 of these cases were reported to the National Centre for Infectious Diseases with Natural Foci (NCIDNF), Ulan Bator, Mongolia. These cases were

diagnosed by clinical symptoms, and in some cases confirmed by serology or a commercially available genus-specific PCR assay (VeTek™ RICK Detection Kit, iNTRON Biotechnology, Korea). Culture has not been performed on human samples from patients with tick-borne rickettsioses so far. With regard to the literature available, data about *Rickettsia* species circulating in Mongolia are scarce, but of great importance for disease epidemiology and improvement of clinical and routine laboratory diagnostic tools. In adjacent areas close to the Mongolian border of Russia and the PR China, *Rickettsia sibirica*, *R. raoultii* (RpA4, DnS14, DnS28), and *R. heilongjiangensis* were found in *Dermacentor* spp. (Shypnov et al., 2006), whereas *Ixodes persulcatus* was shown to harbour 'Candidatus *R. tarasevichiae*' (Shypnov et al., 2006). In addition, *Haemaphysalis concinna* was described as a vector for *R. heilongjiangensis*, *R. sibirica*, and *R. raoultii* (Shypnov et al., 2006). In a collaborative study, we aimed to determine the prevalence and species of *Rickettsia* in *I. persulcatus* and *Dermacentor nuttalli* ticks

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Table 1
Rickettsia spp. found in *Dermacentor nuttalli* from different collection sites.

Province (aimag)	Collection sites	<i>Dermacentor</i> ticks positive/total number of <i>Dermacentor</i> ticks (%)					
		<i>Rickettsia raoultii</i>			<i>Rickettsia sibirica</i>		
		Female ticks	Male ticks	Total	Female ticks	Male ticks	Total
Khentii	Binder/Bayan-Adarga, Batshireet	14/15 (93%)	44/45 (98%)	58/60 (97%)	0/15 (0%)	0/45 (0%)	0/60 (0%)
Arkhangai	Ikhtamir	28/34 (82%)	33/48 (69%)	61/82 (74%)	3/34 (na)	4/48 (8%)	7/82 (8.5%)
Dornod	Data not available	7/7 (na)	NT	7/7 (na)	0/7 (0%)	NT	0/7 (0%)
Selenge	Bugant, Huder	15/21 (71%)	6/9 (na)	21/30 (70%)	0/21 (0%)	0/9 (0%)	0/30 (0%)
Total		64/77 (83%)	83/102 (81%)	147/179 (82%)	3/77 (4%)	4/102 (4%)	7/179 (4%)

NT, ticks not available.

na, prevalence in % not applicable due to low tick numbers (<10 ticks).

from 4 selected provinces of northern, north-eastern, and central Mongolia.

Materials and methods

Tick sampling

D. nuttalli and *I. persulcatus* were collected by flagging the vegetation and were stored at -80°C until further investigation. Ticks originated from 4 provinces (aimags) in northern (Selenge), north-eastern (Khentii, Dornod), and central Mongolia (Arkhangai). Selenge aimag ($49^{\circ}45'\text{N}$ $106^{\circ}30'\text{E}$) is located adjacent to Siberia and is characterised by coniferous forests and open steppe vegetation. The aimag is situated at an altitude of 1400–2200 m above sea level (asl). More than 40% of the area is covered by forest, and the remaining parts constitute of pasture and agricultural land. Animal husbandry is practiced widely. The average annual precipitation has been reported as 250–300 mm (www.infomongolia.com). Khentii aimag ($47^{\circ}19'\text{N}$ $110^{\circ}39'\text{E}$) also borders Russia to the North and comprises forested taiga, forested steppe, and arid fields, the latter mainly covered by gramineous plants. Nearly 20% of its northern part is elevated >1800 to 2400 m asl. The annual precipitation is 200–300 mm (www.infomongolia.com). Dornod aimag ($48^{\circ}04'\text{N}$ $114^{\circ}30'\text{E}$; 560–1300 m asl) offers areas of maritime climate in summer and Siberian cold temperatures during winter. Average precipitation is reported as 150–300 mm/year, but locally achieves 400–500 mm. Plain steppe and meadows dominate most parts of this aimag (www.infomongolia.com). Arkhangai aimag ($47^{\circ}23'\text{N}$, $101^{\circ}30'\text{E}$) is situated on the northern slopes of the Khangai Mountain at an altitude of 1300–2400 m asl. The area

consists of high mountains, forest, and steppe with an annual precipitation of >300 mm. More than 70% of the territory is represented by pasture. Animal husbandry is the main field of economy (www.infomongolia.com).

Sample preparation

Dermacentor and *Ixodes* ticks were disinfected in 70% ethanol for 10 min, washed $2\times$ in PBS (pH 7.4), dried on sterile filter paper, and transferred to lysing matrix A tubes (MP Biochemicals) containing 1 ml Minimum Essential Medium (MEM) + Gibco® GlutaMAX™ supplemented with MEM non-essential amino acids solution ($1\times$) (Life Technologies). All ticks were processed individually using the Bio 101/Thermo Savant FastPrep® FP120 (Qbiogene). DNA was extracted from tick homogenates applying the MagNA Pure LC Total Nucleic Acid Isolation Kit (Roche) and the MagNA Pure LC 2.0 system (Roche) according to the manufacturer's instructions.

PCR and rickettsial species identification

The presence of rickettsial DNA was determined by a real-time PCR assay targeting the citrate synthase gene (*gltA*) (Wölfel et al., 2008) with minor modifications. PCR was performed using LightCycler® FastStart DNA Master HybProbe chemistry (Roche) on a Stratagene Mx3000P real-time cyclor. Uracil-DNA-glycosylase (UDG) was added to eliminate carry-over contamination according to Schex et al. (2011). Preceding investigation of 20 *D. nuttalli* revealed a *R. raoultii* prevalence of >90%. Therefore, *gltA* PCR-positive samples were subjected to a *R. raoultii*-specific real-time PCR assay targeting the outer membrane protein B gene (*ompB*)

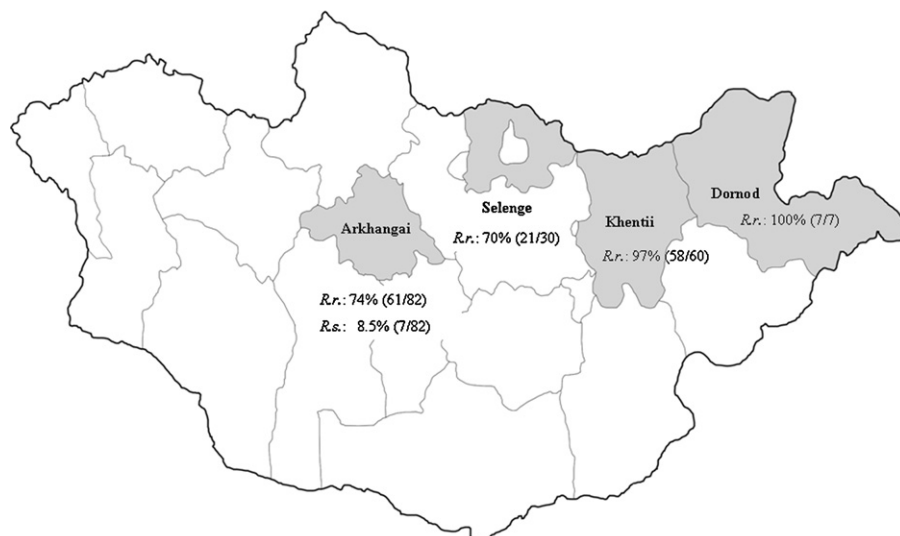


Fig. 1. Map of Mongolia. Aimags of tick origin are shaded in grey. Numbers given indicate *Rickettsia raoultii* (R.r.) and *Rickettsia sibirica* (R.s.) prevalence in *Dermacentor nuttalli*.

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