

## Tick-borne rickettsiosis in the Altay region of Russia

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### INTRODUCTION

North Asian tick typhus (NATT) is a tick-borne zoonosis caused by *Rickettsia sibirica* subsp. *sibirica* (*R. sibirica*) in the Asiatic part of Russia [1,2]. In the Russian Far East, this disease was identified as early as 1932 [3]. Currently, active foci of this communicable disease are identified in 18 administrative territories of Siberia and the Russian Far East. More than 66 000 cases of NATT have been registered in Russia between 1936 and summer 2007. The Altay region of western Siberia (average morbidity 24.3–70.4 per 100 000 inhabitants per year) and the Altai Republic (54.2–97.3) exhibit the highest morbidity for NATT in Russia. The highest incidence was observed in 2001 in Altay when 1867 cases of NATT were registered, representing 54% of all NATT cases in Russia. Cases of NATT are registered between April and October–November, with a peak in May–June (67.4% of cases). Following a period of decrease in July and August, a second peak, though less intense than the first one, is registered in September. The morbidity of NATT is registered in all landscape-geographical areas of the Altaian region, but is distributed unevenly [4]. NATT is known to be transmitted to humans by *Dermacentor nuttalli*, *D. silvarum*, *D. marginatus*, *D. reticulatus*, *Haemaphysalis concinna* and *Ixodes persulcatus*. However, the diagnosis of NATT is mostly based on clinical and serological data, and no direct confirmation is available. As *R. raoultii* has also been detected in *D. nuttalli* ticks in the Altai Republic using PCR,

we investigated whether some patients diagnosed serologically or by culture as having NATT could have another rickettsiosis. In addition, we searched for members of the *Rickettsiales* order in ticks collected in this area, and we characterised genetically 13 rickettsial strains cultivated from ticks.

### MATERIAL AND METHODS

In spring 2005, inoculation eschar biopsies were taken from 12 patients with clinically typical NATT in the city of Barnaul, Altay. In addition, we studied 15 rickettsial strains cultivated from patients (2) and from ticks (13) from Altai, from 1965 to 1989. We also screened 115 ticks collected in the forest-steppe zone of the Altai region (Aleiskiy, Byiskiy and Shypunovskiy districts) for bacteria from the *Rickettsiales* order.

Rickettsiae were detected using partial amplification and sequencing of the 5'-end of the *ompA* gene (190–70 and 190–701 primers), and of the *gltA* gene (CS1d-CS535r and CS409d-RP1258n primers). Ehrlichial DNA was detected using the 16EHRD and 16EHRR primers which amplify a 345-bp fragment of the 16S rRNA gene of all known ehrlichiae and anaplasma. PCR reactions were carried out in a PTC-200 thermal cycler (MJ Research, Inc., Watertown, MA, USA). PCR-positive products were sequenced using the d-Rhodamine Terminator Cycle Sequencing Ready Reaction kit (Applied Biosystems, Warrington, UK) and an ABI 3100 PRISM automated sequencer (Applied Biosystems). Obtained sequences were compared with the GenBank database.

### RESULTS

Ten of the 12 skin biopsies were positive by PCR amplifying the *ompA* and *gltA* genes. Sequences obtained from PCR products were identical and were 100% similar to those of *R. sibirica* subsp. *sibirica* (*gltA* GenBank accession number: U59734; *ompA*: U43807). All 10 proven NATT cases were characterised by a mild clinical picture different from that of Far-Eastern rickettsiosis caused by *Rickettsia heilongjiangensis* in the Russian Far East.

Fourteen strains were identified as *R. sibirica*. These strains were isolated from humans (2) and

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**Table 1.** Detection and identification of bacteria described in ticks from Altay

Tick species	Number of positive ticks/ total examined (%)	Gene	Identification
<i>D. marginatus</i>	12/27 (44,4)	<i>ompA</i> , <i>gltA</i>	<i>Rickettsia raoultii</i>
<i>D. reticulatus</i>	17/41 (41,5)	<i>ompA</i> , <i>gltA</i>	<i>Rickettsia raoultii</i>
<i>I. persulcatus</i>	3/8 (37,5)	<i>gltA</i>	«Candidatus <i>Rickettsia tarasevichiae</i> »
	3/8 (37,5)	16S rRNA	Bacterium 'Montezuma'
	3/8 (37,5)	16S rRNA	<i>Ehrlichia muris</i>
	1/8 (12,5)	16S rRNA	<i>Anaplasma phagocytophilum</i>
	1/8 (12,5)	<i>ompA</i> , <i>gltA</i>	<i>Rickettsia raoultii</i>
	1/8 (12,5)	<i>ompA</i>	closely related to <i>R. tamurae</i>
<i>H. concinna</i>	6/20 (30)	<i>ompA</i> , <i>gltA</i>	<i>Rickettsia raoultii</i>
	4/20 (20)	<i>ompA</i> , <i>gltA</i>	<i>Rickettsia heilongjiangensis</i>

from six tick species: *D. nuttalli* (2), *D. silvarum* (2), *D. marginatus* (4), *D. reticulatus* (2), *H. concinna* (1) and *I. persulcatus* (1). The remaining strain, isolated from *H. concinna*, was identified as *R. heilongjiangensis* [5].

In *D. reticulatus* and *D. marginatus* ticks, we detected *R. raoultii* (Table 1). In *I. persulcatus*, we detected 'Candidatus *R. tarasevichiae*', *Ehrlichia muris*, *Anaplasma phagocytophilum*, a rickettsia closely related to *R. tamurae*, bacterium 'Montezuma' and *R. raoultii*. In *H. concinna*, we detected a co-infection with *R. heilongjiangensis* and *R. raoultii*.

## DISCUSSION

We demonstrate for the first time by direct identification the causative role of *R. sibirica* in NATT in the Altay territory of Russia. To date,

this is the only rickettsia species detected in humans in this area. However, we acknowledge the fact that our investigation was conducted on a small number of patients, and further studies will be necessary to evaluate the incidence of other rickettsioses. In particular, we herein demonstrated the presence of another pathogenic species, *R. heilongjiangensis*, in ticks collected in a NATT focus, and have previously found *R. slovaca*, *R. aeschlimannii* and *R. helvetica* in ticks in Russia. In addition, we also detected in ticks *Anaplasma phagocytophilum*, the agent of granulocytic ehrlichiosis. Our results highlight the need to pursue the molecular identification of tick-borne human infections in different regions of Russia.

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