



Short communication

A fatal case of tick-borne rickettsiosis caused by mixed *Rickettsia sibirica* subsp. *sibirica* and “Candidatus *Rickettsia tarasevichiae*” infection in Russia

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ABSTRACT

North Asian tick-typhus (NATT), also known as Siberian tick typhus, is the main tick-borne rickettsiosis in Siberia, Russia. Recently, a fatal infection in a four-year-old girl with typical tick-borne rickettsiosis symptoms (fever, rash, eschar at the site of the tick bite, myalgia) and meningeal syndrome was registered. In order to identify the etiology of this infection, blood and brain samples from the patient were examined for the presence of a wide range of tick-transmitted agents and enteric viruses by polymerase chain reaction with subsequent sequencing. *Rickettsia sibirica* subsp. *sibirica* and “*Candidatus Rickettsia tarasevichiae*” DNA was identified in both blood and brain samples. *Rickettsia sibirica* was characterized by the *gltA*, *ompA* and *ompB* genes, and “*Candidatus R. tarasevichiae*” was characterized by the *gltA* and *ompB* genes. We report the first case of mixed *R. sibirica* and “*Candidatus R. tarasevichiae*” human infection with a fatal outcome in Russia.

1. Introduction

Several tick-borne rickettsioses have been identified in Russia, of which North Asian tick-typhus (NATT), also known as Siberian tick typhus, is the most widespread, being identified in 17 administrative territories of Russia. Clinical manifestations of NATT include fever, eschar at the site of the tick bite, rash, headache, and myalgia (Lyskovtsev, 1968; Rudakov and Obert, 2001). The causative agent of NATT is *Rickettsia sibirica* subsp. *sibirica*, which was confirmed by the isolation of strains from ticks and from patients (Shpynov et al., 2006a). In addition to *R. sibirica*, several agents of tick-borne rickettsioses were identified in ixodid ticks in Siberia, namely *Rickettsia heilongjiangensis*, *Rickettsia helvetica*, *Rickettsia raoultii*, and ‘*Candidatus Rickettsia tarasevichiae*’ (Igolkina et al., 2015; Rar et al., 2017; Shpynov et al., 2006b). DNA of *R. heilongjiangensis*, *R. raoultii*, and *R. helvetica* was detected in the blood samples from febrile patients from Western Siberia and the Pre-Ural region (Granitov et al., 2015; Igolkina et al.,

2018; Nefedova et al., 2008). To date, clinical cases caused by “*Candidatus R. tarasevichiae*” have not been described in Russia.

Usually, rickettsioses in Russia have mild clinical manifestations (Rudakov and Obert, 2001). However, a fatal infection in a four-year-old girl with typical tick-borne rickettsiosis symptoms (fever, rash, eschar at the site of the tick bite, myalgia) and meningeal syndrome was registered in Krasnoyarsk province, Siberia. The purpose of this study was to confirm its etiology and characterize the etiological agent(s) using molecular methods.

2. Case presentation

In May 2017, a four-year-old girl was admitted to the Idrinsky District Hospital in Krasnoyarsk province, Russian Siberia, four days after a tick bite behind the left ear. The patient presented with symptoms including a fever reaching 39.5 °C, headache and rash. She lived in a village in Kuraginsky district situated in the southern part of

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Krasnoyarsk province; the tick bite was recorded in Idrinsky district. This tick was removed by the child's parents and was not examined for the presence of infectious agents. Upon admission, there was fever (38.6 °C), enlargement of the posterior lymph nodes, eschar at the site of the tick bite, and a maculopapular rash. No underlying conditions prior to this illness that complicated treatment were documented. The rash appeared on the upper extremities on the fourth day after the tick bite and spread throughout the body the next day. Examination of the heart, lungs and abdominal organs revealed no pathology. The preliminary diagnosis in this case was a severe form of tick-borne rickettsiosis. The prescribed treatment included cefotaxime (2 × 650 mg). Laboratory tests indicated leucopenia (2.7×10^9 cells/L) and lymphopenia (9%); the remaining blood counts were normal. On the sixth day of the disease, weakness and lethargy were recorded and treatment was corrected with this: cefotaxime was replaced by tetracycline (3×0.05 g, *per os*) and benzylpenicillin ($4 \times 500,000$ units). Then, the child's condition worsened sharply, and an infectious toxic shock with tonic-clonic convulsions and bradycardia developed. The patient was transferred to the intensive care department of the Kuraginsky Central District Hospital. The girl died a few hours later despite the ongoing intensive therapy.

The parents of the child provided written informed consent. The investigation was approved by the local ethics committee at the Omsk State Medical University (Fig. 1).

The autopsy data (hepatosplenomegaly, cerebral edema) and the results of histological examination (productive vasculitis of the brain, spinal cord and skin, polymorphocellular perivascular infiltrates in the liver and lungs, serous meningitis, myeloid hyperplasia of the spleen

and lymph nodes, interstitial lymphoid infiltration in the myocardium) were consistent with the clinical diagnosis of tick-borne rickettsiosis.

Total DNA was extracted from 100 µl of the total blood and brain samples taken *post mortem* using the Proba NK kit (DNA-Technology, Russia) according to the manufacturer's protocol. *Rickettsia* spp. DNA was detected using nested polymerase chain reaction (PCR) with primers for the *gltA* gene (Table 1), as described previously (Igolkina et al., 2018). DNA extraction and subsequent PCR assays were performed twice for both blood and brain samples. A negative control (sterile water) and a positive control (confirmed *R. helvetica* DNA) were used for the *gltA* nested PCR. To prevent cross-contamination, DNA extraction, amplification, and PCR product detection were carried out in separate rooms. Aerosol-free pipette tips were used at each stage. For species determination in the case of possible mixed infections, nested reactions were performed independently using primers specific to "*Candidatus R. tarasevichiae*" belonging to the ancestral group (Gillespie et al., 2018) and spotted fever group rickettsiae (SFGR). Species determination was confirmed by subsequent sequencing. The DNA of both *R. sibirica* subsp. *sibirica* and "*Candidatus R. tarasevichiae*" was identified in duplicated blood and brain samples.

In addition, the *ompA* gene fragment of *R. sibirica* was amplified in nested PCR using SFGR-specific primers and *ompB* gene fragments of both "*Candidatus R. tarasevichiae*" and *R. sibirica* were amplified independently using nested PCR with primers specific to "*Candidatus R. tarasevichiae*" and SFGR (Table 1).

All amplified fragments were sequenced in both directions; the obtained sequences were deposited in the GenBank database under accession numbers: MK048467–MK048475. The determined *gltA*, *ompA*

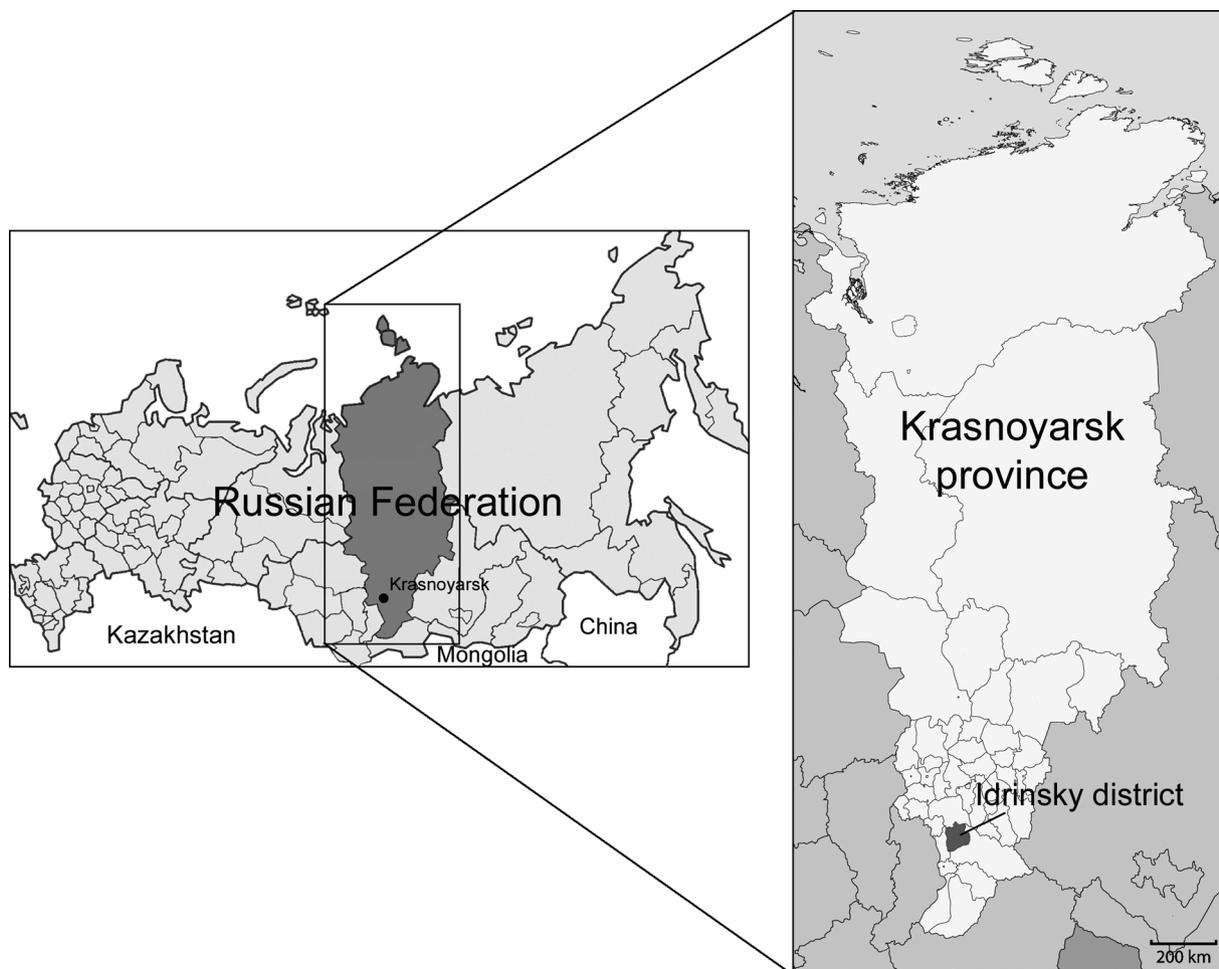


Fig. 1. The location of Idrinsk district, Krasnoyarsk province.

Table 1
Primers used for PCR.

Target genes	Reaction	Primer sequences (5'-3')	Size (bp)	Annealing temperature (°C)	Reference
<i>Rickettsia</i> spp. <i>gltA</i>	Primary reaction	<i>glt1</i> (gattgctttacttacgacc) <i>glt2</i> (tgattttttccattgtgc)	1087	52	Igolkina et al., 2015
	Nested reaction	<i>glt3</i> (tatagacgggtgataaaggaatc) <i>glt4</i> (cagaactaccgatttcttaagc)	667	53	Igolkina et al., 2015
<i>Candidatus R. tarasevichiae</i> <i>gltA</i>	Nested reaction	RT1 (tactaaaaaagctcgtgttacc) RT2 (tgttgcaaacatcatcgtaag)	300	56	Igolkina et al., 2015
SFGR <i>gltA</i>	Nested reaction	RH1 (gtcagtctactatcacctatatag) RH3 (taaaatattcttcttaagagcga)	906	54	Igolkina et al., 2018
SFGR <i>ompA</i>	Primary reaction	A1 (taacattacaagctggaggagcc) A2 (ttcagagcctgaccaccgg)	1335	58	Igolkina et al., 2015
	Nested reaction	A5 (caagtctggtgatgttacta) A6 (tagttacatttctgcacctac)	975	56	Igolkina et al., 2018
<i>Rickettsia</i> spp. <i>ompB</i>	Primary reaction	B1 (atatgaggtatcgctact) B2 (ccatataccgtaagctacat)	1270	56	Igolkina et al., 2015
	Nested reaction	B3 (gcaggtatcggtactataaac) B4 (aatttacgaaacgattactccgg)	843	56	Igolkina et al., 2015
<i>Candidatus R. tarasevichiae</i> <i>ompB</i>	Nested reaction	Btar1 (gcaggtctgttactgtaaatgac) Btar2 (gcgaaacgaaccccgcttgg)	800	59	Igolkina et al., 2018
SFGR <i>ompB</i>	Nested reaction	BSFG1 (ctaggtcttggagcagataacg) BSFG2 (ccgttaaatctagcaccacc)	842	56	This study

and *ompB* gene sequences of *R. sibirica* (827 bp, 782 bp, and 745 bp, respectively) were identical to the corresponding sequences of *R. sibirica* subsp. *sibirica* strain 246 (GenBank NZ_AABW01000001); the *gltA* and *ompB* gene sequences (575 bp and 741 bp, respectively) of “*Candidatus R. tarasevichiae*” corresponded to known sequences of “*Candidatus R. tarasevichiae*” from *Ixodes persulcatus* ticks (GenBank AF503167, KR150783).

The samples were screened by nested PCR for the presence of other tick-transmitted agents, as described previously (Rar et al., 2017). All clinical samples were negative for tick-borne encephalitis virus, Kemerovo virus, *Borrelia burgdorferi* sensu lato, *Borrelia miyamotoi*, *Anaplasma* spp., *Ehrlichia* spp., and “*Candidatus Neohrlichia mikurensis*”. In addition, the samples were screened by PCR for the presence of enteric viruses: enteroviruses, adenoviruses, parechovirus, group A and C rotaviruses, genogroup GI and GII noroviruses, human astrovirus and sapovirus according to Oberste et al. (2000) and Zhirakovskaia et al. (2016). The enteric viruses that could lead to meningeal syndrome were also not identified.

3. Discussion

Krasnoyarsk province is one of the endemic areas for NATT. The Idrinsky district, where the patient was bitten by a tick, belongs to the regions with a high incidence of NATT with 80.2 cases per 100,000 people in 2000–2016 (Kostyrykina, personal communication). Two tick species, *Haemaphysalis concinna* and *I. persulcatus* coexist in Idrinsky district, with *H. concinna* as dominant (Khazova and Yastrebov, 2001). *Haemaphysalis concinna*, along with *Dermacentor* spp., is a recognized vector of *R. sibirica* (Rudakov and Obert, 2001). Approximately 6.5% of *H. concinna* were infected with *R. sibirica* in the southern part of Krasnoyarsk province (Khazova and Yastrebov, 2001). The main vector of “*Candidatus R. tarasevichiae*” is *I. persulcatus*, and the prevalence of “*Candidatus R. tarasevichiae*” in this tick species reached 60–90% in several areas of Siberia (Igolkina et al., 2015; Rar et al., 2017). However, “*Candidatus R. tarasevichiae*” has been identified in *H. concinna* ticks (Cheng et al., 2016), and *R. sibirica* has been identified in *I. persulcatus*; approximately 2% of *I. persulcatus* ticks infected with both rickettsia species were found in the southern regions of Western Siberia (Rar et al., 2017).

Coinfection with two SFGR species is rare in humans (Nogueras et al., 2015). The case of mixed infection of patients with *R. sibirica* and “*Candidatus R. tarasevichiae*” is described for the first time. Importantly, other tick-transmitted agents or enteric viruses were not

identified in the patients’ samples. Clinical symptoms of mixed infection described in this study differed from those of typical NATT by the presence of meningeal syndrome, cerebral and spinal cord vascular inflammation and damage of liver, lungs, spleen and heart. Notably, a meningitis-like manifestation has been previously described in a fatal case of rickettsiosis caused by “*Candidatus R. tarasevichiae*” (Jia et al., 2013).

In this case, both mixed infection with two rickettsial agents and inadequate treatment could have contributed to the fatal outcome. Tetracycline antibiotics are known to be the most effective drugs for the treatment of rickettsial diseases (Parola et al., 2005). However, there are no protocols for the treatment of rickettsial diseases approved by the Ministry of Health of Russian Federation and both chloramphenicol, which is indeed a proper preparation, and cephalosporin are used in different hospitals (Sidorova et al., 2016). Moreover, the use of tetracycline antibiotics is contraindicated in children less than eight years of age, and these preparations can be used only in life-threatening cases. Recently, the safety of tetracycline antibiotics for treating Rocky Mountain spotted fever in pediatric patients has been reported (Todd et al., 2015). In Russia, rickettsiosis caused by *R. sibirica* is usually a mild disease compared to Mediterranean spotted fever or Rocky Mountain spotted fever, and patients recover from NATT even without treatment (Rudakov and Obert, 2001). In this case, the severity of the disease was underestimated and the proper treatment with tetracycline was started only on the sixth day.

The use of molecular methods is required for correct identification of the causative agents of rickettsial infection. This might help in detection of coinfection cases and clarify whether “*Candidatus R. tarasevichiae*” increases the severity of the disease caused by SFGR species.

Declaration of Competing Interest

The authors declare that they have no competing interests.

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