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New records of spotted fever group rickettsiae in Baltic region

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Abstract

Tick-borne rickettsiae are considered to be emerging, but there is still a lack of data on the occurrence and prevalence of the spotted fever group rickettsiae across Europe, especially in the Baltic countries. The aim of the present study was to investigate the presence of *Rickettsia* spp. in *Dermacentor reticulatus* and *Ixodes ricinus* ticks, as well as to determine their prevalence in various regions of Lithuania and Latvia. The prevalence of *Rickettsia* spp. in geographically distinct populations of *D. reticulatus* on transect Baltic-Adriatic regions was compared. The molecular analyses of 1859 *D. reticulatus* and 361 *I. ricinus* from 48 localities in Lithuania and Latvia revealed the presence of *Rickettsia raoultii* in *D. reticulatus* and *Rickettsia helvetica* in *I. ricinus*. The prevalence of pathogens in *D. reticulatus* (4.9%) and *I. ricinus* (17%) ranged in different locations from 0% to 36.9% and 0%–31.3%, respectively. Higher overall infection rates were detected in *D. reticulatus* collected in Poland (15.8%) and Slovakia (11.4%), with the prevalence range in different locations from 0% to 59.6%. Our study is the first demonstration of the presence of *R. raoultii* in *D. reticulatus* ticks in the Baltic countries and *R. helvetica* in questing *I. ricinus* ticks in Lithuania. © 2015 Institut Pasteur. Published by Elsevier Masson SAS. All rights reserved.

Keywords: Spotted fever group rickettsiae; Rickettsia raoultii; Rickettsia helvetica; Ticks; Baltic region

1. Introduction

Rickettsiae are Gram-negative intracellular vector-borne bacteria in the family *Rickettsiaceae* and order *Rickettsiales* that cause mild to severe diseases in humans and animals [1]. *Rickettsiae* are transmitted by various arthropod vectors: ticks, fleas, lice and mites [2]. Tick-borne rickettsioses are caused by a number of pathogenic organisms belonging to the spotted fever group (SFG) of the genus *Rickettsia* [3]. Tick-borne rickettsiae are considered to be emerging, but there is still a lack of data on the occurrence and prevalence of SFG rickettsiae across Europe, especially in the Baltic countries. Ixodid ticks serve as the main vectors and reservoirs of SFG rickettsiae. The geographical distribution of some SFG rickettsiae

most likely corresponds to the geographical distribution of these ticks [1]. *Ixodes ricinus* and *Dermacentor reticulatus* are the most important hard tick species in central Europe and are implicated in the transmission of several pathogens including species of tick-borne rickettsiae [3]. *I. ricinus* can be found in all Baltic countries. During the past two decades *D. reticulatus* has expanded its range in Europe and has been detected in new localities in Lithuania and southern Latvia in which has not been reported thus far [4]. However, the importance of *D. reticulatus* in the transmission of *Rickettsia* spp. pathogens has not been well recognized.

The first initial investigations of *Rickettsia* spp. in Lithuania were conducted about forty years ago. Studies carried out during 1976–1977 confirmed the circulation of SFG rickettsiae in the territory of Lithuania: rickettsiae and rickettsia-like organisms were detected in *I. ricinus* ticks by a haemocyte test (Gimenez staining) and antibodies against *Rickettsia sibirica*

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and *Rickettsia slovaca* were found in human and cattle serum samples [5] (Tarasevich et al., 1981). However, to the best of our knowledge, since that time no other data on rickettsial pathogens or clinical cases due to SFG rickettsiae in Lithuania have been reported. The detection and identification of *Rickettsia* have greatly improved in term of accuracy and sensitivity since the advent of molecular tools.

The aims of the present study were to investigate the presence of rickettsiae in sympatric populations of *D. reticulatus* and *I. ricinus* ticks, to characterize the detected rickettsiae using partial sequencing of the *gltA* gene, and to determine the prevalence of *Rickettsia* spp. in various regions in Lithuania and Latvia. We also compared the prevalence of *Rickettsia* spp. in *D. reticulatus* on transect Baltic-Adriatic regions.

2. Materials and methods

2.1. Tick collection

A total of 1859 questing *D. reticulatus* and 361 *I. ricinus* collected from 48 locations in Lithuania and Latvia, and 635 *D. reticulatus* from 15 locations in Poland, Slovakia, and Croatia were examined for rickettsial pathogens (Fig. 1; Table 1).

Questing ticks were collected using the flagging technique [6] in the spring (April and May) of 2013–2014. Ticks were identified to the species level, stage and sex using taxonomic keys [6].

2.2. PCR and sequencing

DNA from ticks was extracted using ammonium hydroxide solution (2.5%) [7]. The presence of rickettsiae in ticks was determined by amplification of a 381-bp fragment of *gltA* gene by PCR using primers RpCS.877p and RpCS.1258n as previously described [8]. The PCR products of *Rickettsia* positive samples were extracted from agarose gel and purified using the GenJet PCR purification kit (Thermo Fisher Scientific, Lithuania) and then sequenced using the ABI Prism 3130 genetic analyser (Applied BioSystems, USA). The obtained sequences were analysed using the Mega 6.05 software package, version 6.05 and compared with the sequence data available from GenBank using the BLAST program. A phylogenetic tree was constructed using the Neighbour joining algorithm and the Kimura 2-parameter model as implemented in the Mega 6.05.

Partial *gltA* sequences for representative samples were submitted to GenBank under the accession numbers KR816891 to KR816898.

2.3. Statistical analysis

The prevalence of *Rickettsia* spp. infection in ticks was statistically analysed by means of the Pearson's χ^2 test performed using the statistical software package STATISTICA for Windows 7.0.

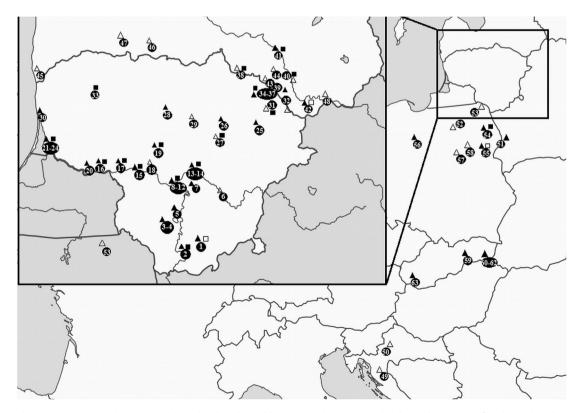


Fig. 1. Map showing *D. reticulatus* and *I. ricinus* ticks collection sites with ticks positive and negative for *Rickettsia* spp. \bigcirc – Collection site number; $\square - I$. *ricinus* negative for *Rickettsia* sp.; \blacksquare *I. ricinus* infected with *Rickettsia* sp.; $\triangle - D$. *reticulatus* negative for *Rickettsia* sp.; $\blacktriangle - D$. *reticulatus* infected with *Rickettsia* sp.; \triangle

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