Available online at www.sciencedirect.com
SCIENCE DIRECT
ELSEVIER
International Journal of Medical Microbiology 296 (2006) S1, 144–148
IJMM
www.elsevier.de/ijmm

Detection of spotted fever group (SFG) rickettsiae in *Dermacentor reticulatus* (Acari: Ixodidae) in Poland

Joanna Stańczak*

Department of Tropical Parasitology, Institute of Maritime and Tropical Medicine, Medical University of Gdańsk, 9B Powstania Styczniowego str., PL-81519 Gdynia, Poland

Abstract

Dermacentor reticulatus ticks from Poland were investigated by molecular methods for the presence of rickettsiae. During 2003/2004, a total of 285 adult ticks was assayed using primers RpCS.877 and RpCS.1258 derived from the citrate synthase (*gltA*) gene, and 116 samples (40.7%) were positive for rickettsial DNA. Ten out of these positive samples were further assayed using SLO1F and SLO1R primers derived form the *rOmpA*-encoding gene to confirm that detected rickettsiae belong to the spotted fever group (SFG). The obtained sequence of a fragment of the *gltA* gene of *Rickettsia* sp. isolated from Polish *D. reticulatus* demonstrated 96–98% similarities to *Rickettsia slovaca, Rickettsia sibirica*, *Rickettsia honei,* and other SFG rickettsiae. The nucleotide sequences of the amplified fragments of the *ompA* gene were 98% homologous to RpA4 *Rickettsia* sp. reported from ticks collected in territories of the former Soviet Union.

Keywords: Spotted fever group rickettsiae; *Rickettsia* spp.; *Dermacentor reticulatus*; PCR; Poland

Introduction

The spotted fever group (SFG) rickettsiae are small, gram-negative, obligately intracellular bacteria. They are mainly associated with hard ticks (Ixodidae) and are known to be transmitted transstadially and transovarially in these arthropods, which serve both as vectors and reservoirs of these pathogens (Řeháček, 1984). Vertebrates, including humans, mostly serve as accidental hosts and acquire infection by a tick bite. From about 30 species described so far, at least 13 are known to be pathogenic for humans. The rest of them have been isolated only from arthropods and are often considered to be non-pathogenic (La Scola and Raoult, 1997). However, *Rickettsia helvetica,* which was isolated from *Ixodes ricinus* ticks in several European countries (Burgdorfer et al., 1979; Parola et al., 1998; Bacellar, 1999; Nilsson et al., 1999a; Beninati et al., 2002) and was originally not linked to human disease, has recently been identified as an agent of human rickettiosis. It was detected in patients with perimyocarditis (Nilsson et al., 1999b), sarcoidosis (Nilsson et al., 2002), and flu-like, febrile illness (Fournier et al., 2000, 2004). Thus, all SFG rickettsiae should be treated as organisms that may pose a threat for people exposed to tick bites.

To date, no clinical cases due to infection with SFG rickettsiae have been reported from Poland, although the presence of these bacteria has recently been detected both in *I. ricinus* (Stańczak, 2004) and *Dermacentor reticulatus* ticks (Stańczak et al., 2004). However, in those studies only small groups of ticks were investigated and information about the rickettsial species infecting ticks, tick infection rates, and the distribution

*Tel.: +48 58 69 98 551; fax: +48 58 622 33 54.
E-mail address: astan@amg.gda.pl (J. Stańczak).

1438-4221/$ - see front matter © 2006 Elsevier GmbH. All rights reserved.
doi:10.1016/j.ijmm.2006.01.014

of SFG rickettsiae in our country is still lacking. Thus, the purpose of present investigation was to detect *Rickettsia* spp. in Polish ixodids. We focused on *D. reticulatus*, a potential vector of *Rickettsia slovaca* in our country. Although in Europe *D. marginatus* is considered a main vector of this species, studies in Hungary (Řeháček et al., 1979), Russia, and Kazakhstan (Shpynov et al., 2001) suggest that also *D. reticulatus* plays a role in its perpetuation in nature.

Material and methods

Collection of ticks

Adult *D. reticulatus* were collected by flagging grassy areas in five different locations in north-eastern Poland in 2003/2004 (Fig. 1). In the laboratory, ticks were separated by sex, killed, and then preserved in 70% ethanol at room temperature until analysis.

Extraction of DNA

Individual specimens were crushed and DNA was extracted by lysis in ammonium hydroxide (NH_4OH) according to Rijpkema et al. (1996). These extracts were stored at –20°C and then used as templates in a regular PCR assay.

Polymerase chain reaction

DNA amplification was assayed using a primer pair RpCS.877p (5' – GGG GGC CTG CTC ACG GCG G – 3') and RpCS.1258n (5' – AAT GCA AAA AGT ACA GTG AAC A – 3') derived from the citrate synthetase gene *gltA*, which has conserved regions shared by all known *Rickettsia* species (Regnery et al., 1991).

PCR reactions used 5 µl of genomic DNA as a template in a total volume of 50 µl reaction mixture that contained: 1 U (1 µl) *Taq* RUN polymerase, 5 µl of 10 × PCR reaction buffer containing 20 mM $MgSO_4$ (final concentration 2 mM) (A&A Biotechnology, Poland), 5 µl of 2.5 mM dNTPs mixture (final concentration 0.25 mM) (MBI Fermentas, Lithuania), 1 µl of each 10 µM primer (final concentration 0.2 µM), and 32 sterile DDW. DNA of *R. slovaca* kindly supplied by Prof. Didier Raoult from Unité des Rickettsies, Faculté de Médecine, Université de la Méditerranée, Marseille, France, was used as a positive control and DDW in place of a template as negative controls. The conditions of PCR were as already described (Regnery et al., 1991).

Ten templates chosen from samples with RpCS positive results were subjected to further PCR analysis using a combination of SLO1F (5′ – CAC CAC CTC AAC CGC AG – 3′) and SLO1R (5′ – GCC GGG GCT GCA GAT TG – 3′) oligonucleotides amplifying a part of the outer membrane protein rOmpA encoding gene (*ompA*) (Raoult et al., 2002). PCR amplification was accomplished in 50-µl volumes, using the same protocol as above. Samples were incubated for 4 min in 94 °C and then thermally cycled 30 times at 94 °C for 30 s (denaturation), 56 °C for 30 s (annealing), and 72 °C for 1 min (extension). Final extension at 72 °C lasted 5 min.

All PCR reactions were carried out in Perkin Elmer GeneAmp PCR System 2400 and 9700 thermal cyclers. Amplification products were analysed after electrophoresis in a 2% agarose gel stained with ethidium bromide.

DNA bands of 381 base pairs (bp) for RpCS primers (Fig. 2) and 459 bp for SLO primers were considered to be positive results.

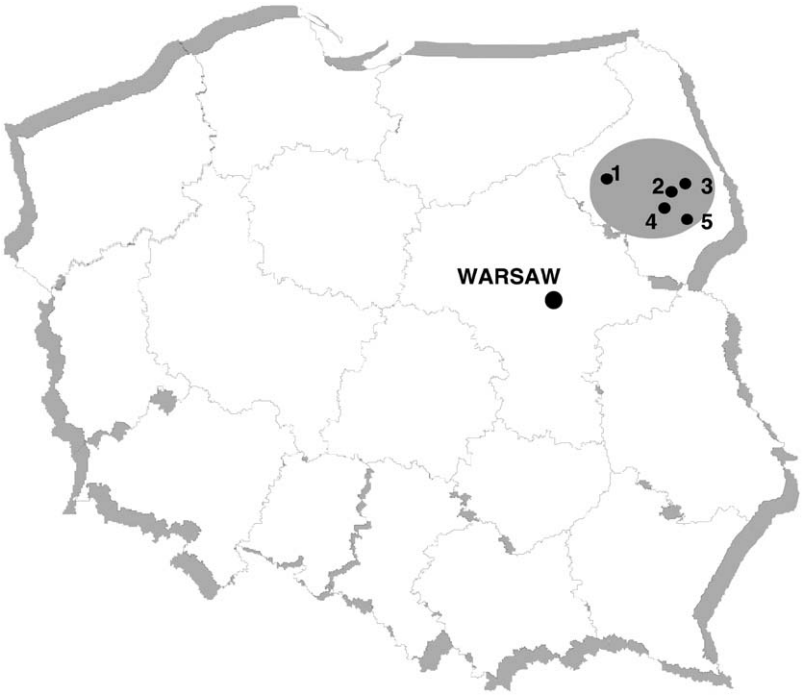

Fig. 1. Collection sites of *Dermacentor reticulatus* in north-eastern Poland.