SPOTTED FEVER GROUP RICKETTSIAE IN IXODES RICINUS AND HAEMAPHYSALIS PUNCTATA Ticks in Italy

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In this study Ixodes ricinus and Haemaphysalis punctata ticks are examined. For the first time we detected Rickettsia conorii in I. ricinus and H. punctata, and Rickettsia sibirica in I. ricinus. Our results raise the question of whether other spotted fever group rickettsiae, in addition to R. conorii subsp. conorii and R. conorii subsp. israelensis, are involved in bacterial diseases in Italy and whether I. ricinus and H. punctata can act as new vectors for these rickettsiae.

Over fifteen pathogenic species of spotted fever group (SFG) rickettsiae have been recognized worldwide, nine since 1991. In Italy, until 2002, Rickettsia conorii was thought to be the only SFG rickettsia present, yet five other tick-transmitted rickettsiae that are pathogenic for humans have since been described: Rickettsia helvetica in Ixodes ricinus ticks (1); the Israeli spotted fever agent (R. conorii subsp. israelensis) in Rhipicephalus sanguineus ticks (2); Rickettsia slovaca in Dermacentor marginatus and Haemaphysalis punctata ticks (3); and Rickettsia africae and Rickettsia aeschlimannii in Hyalomma marginatum ticks (3). Moreover, in 2004, three people who developed a mild, non-eruptive disease, showed serological evidence of R. helvetica infection (4). More recently, Giammanco et al. (5) used molecular-sequence-based identification techniques to retrospectively study clinical isolates obtained from 24 Mediterranean spotted fever (MSF) cases occurring in Western Sicily from 1987 to 2001. Sequence analysis of the ompA gene identified 5 clinical isolates as R. conorii subsp. israelensis and demonstrated the occurrence of Israeli spotted fever in Sicily. The remaining 19 of the 24 isolates were R. conorii subsp. conorii.

MATERIALS AND METHODS

From June to July 2001, we conducted a tick-spirochete and tick-TBE survey in 41 sites in the region of Alto Adige (northeast Italy). A total of 330 adult ticks and 140 nymphs of the Ixodidae family were collected by flagging over vegetation; 308 ticks were identified as I. ricinus and 162 as H. punctata, according to standard taxonomic keys (6-7). Ticks were pooled according to species and the area and date of collection (40 pools of I. ricinus and 9 pools of H. punctata). They were rinsed in sterile distilled water and homogenised with sterile glass pestles in 2 ml of Hank’s medium containing 7.5% BSA. As a side experiment, a sample of each extract was tested by PCR for SFG rickettsiae after DNA extraction with a QIAmp tissue kit (QIAGEN, Hilden, Germany), following the manufacturer’s instructions. PCR amplification was performed as previously described, using a rickettsial citrate synthase gene (gltA) primer pair (Rp.CS.877p and Rp.CS.1258n) and an SFG rickettsial

Key words: spotted fever group rickettsiae, Ixodes ricinus, Haemaphysalis punctata, Italy

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Table 1. Identification of Rickettsia spp. in tick samples from June to July 2001, in the region of Alto Adige (Northeast Italy).

<table>
<thead>
<tr>
<th>Tick species identified (n° examined /n° pools)</th>
<th>Pool infection rate (%)</th>
<th>N° Rickettsia spp. identified (% identity with gltA /% identity with rompA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ixodes ricinus (308/40)</td>
<td>3/40 (7.5)</td>
<td>2 Rickettsia sibirica (100/100)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 Rickettsia conorii (100/100)</td>
</tr>
<tr>
<td>Haemaphysalis punctata (162/9)</td>
<td>2/9 (22.2)</td>
<td>2 Rickettsia conorii (100/100)</td>
</tr>
</tbody>
</table>

Fig. 1. PCR experiments with primers Rp.CS.877p/Rp.CS.1258n and Rr.190.70p/Rr.190.602n for gltA and rompA genes respectively. Lanes 1, 2 and 3 amplicons of I. ricinus samples. Lanes 4 and 5 amplicons of H. punctata samples.

190-kDa surface antigen gene (rompA) primer pair (Rr.190.70p and Rr.190.602n) (8-9). Each positive PCR product was purified using a QIA Quick Gel extraction kit (QIAGEN), following the manufacturer’s instructions, and cloned in the pGEM-T easy vector (Promega, Madison, Wis.). Sequencing was performed with a commercial T7 sequencing kit (Amersham Biosciences, Uppsala, Sweden) with M13 forward and reverse primers, and the results were analysed on a Pharmacia Biotech ALFExpress automated DNA sequencer. The sequences obtained were compared to others using the BLAST search tool (http://www.ncbi.nlm.nih.gov/BLAST/).

To confirm the identity of the tick species in the positive rickettsia samples, the complete 18S rRNA was obtained by PCR using primers A and G as previously described (10). The PCR products were sequenced and the nucleotide sequences were matched up in the GenBank with BLAST search tool (http://www.ncbi.nlm.nih.gov/BLAST/).

RESULTS

Rickettsial DNA was detected in 5 (10.2%) of the 49 tick samples studied (Table I). The identity of the tick species in the positive rickettsia samples was confirmed with the 18S rRNA sequence analysis (10). Sequences displayed 100% identity to I. ricinus accession no. Z74479 and H. punctata accession no. Z74478.

A rickettsia exhibiting 100% gltA (GenBank accession no. U59734) and rompA (GenBank accession no. U43807) identity to Rickettsia sibirica was found in 2 samples of I. ricinus (Table I, Fig. 1). R. sibirica is the etiological agent of Siberian tick typhus which is mainly transmitted by Dermacentor sylvarum and Haemaphysalis concinna ticks.

A rickettsia exhibiting 100% gltA (GenBank accession no. U59730) and rompA (GenBank accession no. U43806) identity to R. conorii (the agent of MSF) was identified in 1 sample of I. ricinus and in 2 samples of H. punctata (Table I, Fig. 1).

DISCUSSION

The Siberian Tick TIPHUS was first recognized in 1932 and has been extensively documented in Siberia.
and western China. To the best of our knowledge, this is the first report of \textit{R. sibirica} (or a closely related rickettsia) in ticks in Italy and of its presence in \textit{I. ricinus} ticks. The first evidence of a rickettsia closely related to \textit{R. sibirica} in a European country was found in 1996 in southern France (11-12). This rickettsia (\textit{Rickettsia sibirica mongolotimonae}) belongs to the \textit{R. sibirica} species yet exhibits specific serotypic and ecological characteristics. While North Asian tick typhus is confined to Siberia and western China, \textit{R. sibirica mongolotimonae} infection has been found in \textit{Hyalomma} ticks in Inner Mongolia, Niger and Greece, in a \textit{Rhipicephalus pusillus} tick in Portugal, and in humans in France, Greece, Portugal and in northern and southern Africa. To date, only 12 human cases of \textit{Rickettsia sibirica mongolotimonae} infection have been described (13-15). However, there was 98% similarity in the nucleotide sequence of \textit{rompA} gene between the rickettsia found in the 2 \textit{I. ricinus} samples and the \textit{Rickettsia sibirica mongolotimonae} strain HA-91 (GenBank accession no. U43796). Consequently, the rickettsia we found is likely to be \textit{R. sibirica}.

Although the finding of \textit{R. conorii} in Alto Adige is not a novelty - in that cases of MSF, though infrequent, have been reported - this species has never been found in \textit{I. ricinus} or \textit{H. punctata} ticks. However, whether \textit{I. ricinus} and \textit{H. punctata} ticks can act as vectors of MSF remains to be determined. MSF is transmitted by \textit{R. sanguineus} and is prevalent in countries around the Mediterranean and Black Seas; in Sicily, it has also been detected in \textit{Rhipicephalus turanicus} ticks (3). In Italy, as in other European countries, \textit{I. ricinus} is the vector of \textit{Borrelia burgdorferi sensu lato}, tick-borne encephalitis virus, \textit{Anaplasma phagocytophilum} and possibly \textit{R. helvetica} (1). It is widespread and represents more than 90% of all the ticks removed from humans in northern Italy (16). \textit{H. punctata} has been found to be infected with \textit{R. slovaca} in Sicily (3).

The results of this study raise the question of whether other SFG rickettsiae, in addition to \textit{R. conorii} subsp. \textit{conorii} and \textit{R. conorii} subsp. \textit{israelensis}, are involved in bacterial diseases in Italy and whether \textit{I. ricinus} and \textit{H. punctata} can act as vectors for \textit{R. conorii}, and \textit{I. ricinus} as a vector for \textit{R. sibirica}. Since rickettsiae are fastidious and culturing them is very complex, serology is the most widely used microbiological means of diagnosing SFG rickettssioses, with indirect immunofluorescence assay (IFA) being the most common method. However, given that IFA cannot identify rickettsiae at the species level (because of cross-reactivity among SFG rickettsiae), microbiological confirmation could be performed using a multiple-antigen IFA and/or cross-adsorption and western blotting. PCR on eschar biopsy samples has a high probability of detecting rickettsial DNA. In any case, clinicians need to be aware of emerging tick-borne diseases in Italy, in particular, infections due to \textit{R. sibirica}.

REFERENCES


