Identity of Ehrlichial DNA Sequences Derived from *Ixodes ricinus* Ticks with Those Obtained from Patients with Human Granulocytic Ehrlichiosis in Slovenia

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In 1996, four cases of human granulocytic ehrlichiosis (HGE) were identified near Ljubljana, Slovenia (5, 8). All four patients reported exposure to ticks, and none had traveled outside of Slovenia within the putative incubation period. The aim of this study was to collect *Ixodes ricinus* ticks from the Ljubljana area to determine if they were infected with an ehrlichial species and, if so, to assess the DNA sequence homologies between amplicons derived from ticks with those obtained from three of the patients (one patient developed antibodies to the HGE agent, but no ehrlichial DNA was recovered). *I. ricinus* is the most abundant tick in central Slovenia, and this species typically comprises more than 98% of the ticks recovered during tick sampling (9, 11). In Slovenia, as in other European countries, *I. ricinus* is the main vector of the causative agents of Lyme borreliosis and tick-borne encephalitis (9, 11). In Europe, the tick *I. ricinus* is known as a vector of the agent causing tick-borne fever (*Ehrlichia phagocytophila*) (14).

In early summer of 1996, 101 unfed adult *I. ricinus* ticks were collected by flagging vegetation. The collection site was a woodland with clearings in which the major tree species were immature birch (*Betula pendula*), oak (*Quercus cerris*), hazel (*Corylus avellana*), and pine (*Pinus silvestris*) trees, and the undergrowth was predominantly fern (*Pteridium aquilinum*) and bilberry (*Vaccinium myrtillus*) shrubs. All ticks were collected at the same location near Ljubljana, and gender and species were identified by an entomologist. DNA was extracted by first digesting individual ticks overnight in TE buffer supplemented with proteinase K (2,500 μg/ml), followed by phenol-chloroform-isoamyl alcohol extraction and ethanol precipitation. To confirm that the DNA extraction was successful, PCR assays were conducted as previously described to amplify 16S mitochondrial rRNA genes (rDNA) of tick origin (3).

To examine for ehrlichial DNA, each sample was tested with PCR assays based on the 16S rRNA gene. Three (3.2%) of 93 ticks were found to contain granulocytic ehrlichiae. Nucleotide sequences of portions of the bacterial *groESL* heat shock operon amplified from these ticks were identical or nearly (99.8%) identical to those previously determined for human patients with HGE from Slovenia, providing additional evidence that the ticks were infected with the HGE agent. This study identified *I. ricinus* as the likely vector for these ehrlichial pathogens of humans in this part of Europe.

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GroEL Heat Shock Operon

![Diagram indicating PCR primer positions and amplicon sizes. Primers HS1 and HS6 were used in primary PCRs, and amplicons produced in nested PCRs with the indicated primers were sequenced. The nucleotide sequence of the 1,256-bp region flanked by primers HS43 and HSVR was obtained from five of six samples, two of three human patients, and three *I. ricinus* ticks. Sequences of the regions flanked by primers HS43 and HSVF (442 bp) and primers HSVF and HSVR (395 bp) were obtained from the remaining human patient. The overlapping sequences from five samples were identical in size. The sequence from one of the female ticks contained three single nucleotide substitutions: A to G at position 51, G to A at position 450, and T to C at position 660. The sequences were numbered by designating the A of the *groEL* translation initiation codon nucleotide 1. The nucleotide substitutions did not alter the deduced amino acid sequence.](http://jcm.asm.org)
should be noted that we tested only adult stages of
(3.1%) reported from the eastern coast of Sweden (12). It
lower (3.2%) than the 24.4% prevalence found in ticks col-
13). The prevalence of infection of
ticks by means of electron microscopy and PCR assays (4, 7, 12,
except reference 7).
and North American strains of the HGE agent (5, 10).
sequences previously amplified from one of the Slovenian HGE
patients (GenBank accession no. AF033101) from which ehr-
licial DNA was obtained. These regions are flanked by primers
HS43 and HSVR were previously amplified from the
other two Slovenian HGE patients (5) from which ehrlichial
DNA was obtained. These regions are flanked by primers
HS43 and HS45 (442 bp) and primers HSVF and HSVR (395
bp) (Fig. 1) (5, 10). The full 1,256-bp sequence was amplified
and sequenced for one of these patients during this study and
was found to be identical to the 1,256-bp sequence from the
first patient (GenBank accession no. AF033101). We did not
have a sufficient sample to obtain the full 1,256-bp sequence
from the remaining patient. However, the sequences previ-
ously amplified from this patient were identical to the overlap-
ing regions of the 1,256-bp sequence (GenBank accession no.
AF033101) from the other patients. The groESL sequences
from the Slovenian patients and ticks were more similar to se-
quences previously obtained from European strains of E. pha-
gocytophila than to groESL sequences amplified from E. equi
and North American strains of the HGE agent (5, 10).
Researchers from Scotland, Italy, Sweden, and France have
reported detection of granulocytic Ehrlichia spp. in I. ricinus
ticks by means of electron microscopy and PCR assays (4, 7, 12,
13). The prevalence of infection of I. ricinus ticks by granu-
locytic Ehrlichia detected by our methods in Slovenia was much
lower (3.2%) than the 24.4% prevalence found in ticks col-
lected from Italy (4) but indistinguishable from the prevalence
(3.1%) reported from the eastern coast of Sweden (12). It
should be noted that we tested only adult stages of I. ricinus,
while the cited studies reported results from nymphal ticks
(except reference 7).

To our knowledge, this is the first study in which ehrlichial
DNA sequences derived from ticks and HGE patients from the
same location in Europe were compared. The identity of these
ehrlichial sequences implicate I. ricinus as a vector of E. phago-
cytophila, or closely related agents, in Slovenia. The public
health and biologic significance of variation among ehrlichial
groESL sequences amplified from ticks and patients cannot be
determined at this time. Further studies in Slovenia will assess
the prevalence of ehrlichial infection among I. ricinus ticks in
various life stages and identify the maintenance cycle in reser-
voir and vector species that may ultimately lead to human in-
fec tion.

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