Ticks are recognized as the main vectors and reservoirs of spotted fever group rickettsiae. We searched for the most prevalent Rickettsia spp. in Poland and found R. slovaca and R. helvetica bacteria in ticks in southern and central Poland; R. raoulti was found in ticks in all parts of Poland.

Ticks are ectoparasites infesting many mammals, including humans and their pets. In Poland, Ixodes ricinus ticks are widely distributed throughout the country whereas Dermacentor reticulatus ticks are limited to the northeast region. Both I. ricinus and D. reticulatus ticks are known to be the main vectors and reservoirs of the spotted fever group rickettsiae (SFG) worldwide. Detection of the SFG pathogens requires a 2-step procedure: PCR and sequencing of selected genes (16S rRNA, citrate synthase [gltA], outer membrane protein A [ompA], ompB, and 17-kDa protein) (1–6). The aim of the present study was to identify and characterize rickettsial species occurring in ticks in Poland.

The Study

We collected 214 ticks in 3 regions of Poland: the Warsaw region (central Poland) (107 I. ricinus ticks), the Radomsko region (southern Poland) (47 I. ricinus ticks), and the Białowieża Primeval Forest National Park (northeastern Poland) (60 D. reticulatus ticks). The specimens were collected from April 2005 through August 2007 and identified on the basis of morphologic characteristics. All I. ricinus ticks were obtained from dogs and cats, and D. reticulatus ticks were collected from vegetation.

DNA was extracted from I. ricinus specimens by using the QIAMP DNA Tissue Kit (QIAGEN, Hilden, Germany). D. reticulatus specimens were crushed in Eppendorf (Hamburg, Germany) tubes, after which DNA extraction was performed by boiling the specimens in 200 µL of 0.7 M NH₄OH for 30 min.

Bacterial DNA was examined for the Rickettsia spp. gltA gene by using RpCS.409d and RpCS.1258n primers. Each positive specimen was amplified with paired primers RpCS.190-70 and RpCS.190-701, which were specific for the SFG rickettsiae ompA gene. In the absence of amplifiable fragments of the ompA gene, molecular identification was conducted by using PCR with paired primers RpR17.61p and RpR17.492n, which are able to anneal the specific 17-kDa outer membrane protein gene (Table 1).

The QIAquick PCR Purification Kit (QIAGEN) was used to purify PCR products for sequencing. All amplicons were sequenced with the ABI Prism 377 DNA Sequencer (Applied Biosystems, Foster City, CA, USA) in accordance with the manufacturer’s recommendations. All sequences were edited by using the AutoAssembler software (Applied Biosystems), identified with the BLAST software (http://blast.ncbi.nlm.nih.gov/Blast.cgi), and compared with sequences available in GenBank.

The DNA of Rickettsia spp. was found in 70 (32.7%) I. ricinus and D. reticulatus ticks by using PCR and primers specific for the gltA gene. All 70 tick samples that tested positive for the genus Rickettsia were subjected to amplification with primers specific for the ompA gene. Twenty-eight I. ricinus and 34 D. reticulatus ticks were positive in PCR for the specific ompA fragment. In DNA samples extracted from 8 I. ricinus specimens (7 negative for the ompA gene and 1 having a faint reaction to the gltA gene), gene fragments characteristic of the 17-kDa protein were found.

Sequences of the gltA gene fragment (688 nt) from 62 samples were identical to the R. raoultii Marne and R. raoultii Khabarovsk strain sequences (GenBank accession nos. DQ365803.1 and DQ365804.1). Results were confirmed by sequencing the ompA fragment; all 62 showed 99%–100% nucleotide similarity with the ompA gene (610 nt) of the R. raoultii Marne and the R. raoultii Khabarovsk strains (GenBank accession nos. DQ365799.1 and DQ365801.1).

Seven sequences of the gltA gene fragment amplicons were identical with the R. helvetica gltA gene (GenBank accession nos. U59723.1 and DQ31912.1). These samples also had 100% identical sequences of the 17-kDa protein gene characteristic of R. helvetica (GenBank accession nos. EF392726.1 and AJ427881.1). The sequence of 1 amplicon with primers specific for the ompA gene showed 99% similarity with the R. slovaca ompA gene (GenBank accession no. U43808.1). Sequencing of PCR products with primers specific for the gltA and 17-kDa protein gene were not conducted due to the small amount of DNA in the sample and also due to poor amplification.

R. raoultii DNA was detected in 34 (56.7%) of the 60 D. reticulatus specimens from Białowieza and in 28 (18.2%) of the 154 I. ricinus specimens tested, including 25 (23.4%) of 107 from the Warsaw region in central Poland and 3 (6.4%) of 47 I. ricinus specimens from the Radomsko region in the south. R. helvetica and R. slovaca were noted
exclusively in DNA extracted from the *I. ricinus* ticks. In 3 (2.8%) of the 107 ticks from the Warsaw area, DNA of *R. helvetica* was found. In the Radomsko area, 4 (8.5%) of 47 specimens had *R. helvetica* DNA, and 1 (2.1%) had *R. slovaca* DNA (Table 2).

### Conclusions

The DNA of *R. raoultii* was found in *Ixodes* spp. ticks in southern Poland and in *Dermacentor* spp. ticks in northeastern Poland. Until recently, *R. raoultii* had been reported only in *D. nutalli*, *Rhipicephalus* sp., and *Dermacentor* spp. ticks in Europe and Asia (i.e., Siberia and the Astrakhan area) (8–11).

SFG rickettsiae in Poland have been previously noted solely in *D. reticulatus* ticks (12). The gltA gene fragment sequence demonstrated similarities with *R. honei* and other unidentified SFG rickettsiae. The nucleotide sequences of amplified fragments of the *ompA* gene were 98% homologous to RpA4 *Rickettsia* sp.

Our findings show that *R. raoultii* occur in all regions of Poland. These bacteria were noted in 18.2% of *I. ricinus* and 56.7% of *D. reticulatus* specimens. Their occurrence in various species of ticks may suggest that they are capable of being distributed all over Europe.

Although the pathogenic role of these genotypes has not yet been established, their ability to cause infection cannot be ruled out. In fact, the RpA4 strain has been isolated from a patient with symptoms resembling those of *R. slovaca* infection (D. Raoult, unpub. data) (10). *R. slovaca*, a member of the SFG rickettsiae, is DNA, and 1 (2.1%) had *R. slovaca* DNA (Table 2).

of the severe and characteristic symptoms that occur after being bitten by these ectoparasites.

*R. helvetica*, which was detected in Switzerland in 1979, is also widespread in Europe. This species has recently been found in *I. ricinus* ticks in the northern part of Poland (14). Our study also confirms the occurrence of *R. helvetica* in central and southern Poland. These reports suggest that *R. helvetica* can infect *I. ricinus* ticks and may be extensively distributed in several European countries, including Poland. The pathogenicity of *R. helvetica* as a self-limiting illness associated with headache, myalgias, rash, or eschar has been confirmed. Also, several patients with perimyocarditis associated with *R. helvetica* have been observed in Sweden (15).

Our findings indicate that SFG rickettsiae transmitted by ticks could penetrate biotopes in various parts of Europe. Though the pathogenicity of the newly recognized species of the genus *Rickettsia* has not yet been proven definitively, it is prudent for clinicians in Poland and other European countries to be alert to possible appearances of infections caused by these pathogens.

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