Evidence of the Human Granulocytic Ehrlichiosis Agent in *Ixodes ricinus* Ticks in Switzerland

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A total of 1,667 *Ixodes ricinus* ticks were collected from five regions in Switzerland where there have been sporadic occurrences of granulocytic ehrlichiosis in dogs and horses. The ticks were examined for rickettsiae of the *Ehrlichia phagocytophila* group via nested PCR. Twenty-one ticks (1.3%) were positive; 3 (0.5%) were nymphs, 6 (1.3%) were adult males, and 12 (1.9%) were adult females. The number of positive ticks varied with the stage of development and with the geographical origin. Nucleotide sequencing of the isolated PCR products identified these products as part of the 16S rRNA gene of *Ehrlichia*. In addition, these products had 100% homology with the agent of human granulocytic ehrlichiosis. The occurrence of this agent in *I. ricinus* in Switzerland presents a potential danger of transmission of granulocytic ehrlichiosis to dogs, horses, and humans.

Ehrlichiae are obligate intracellular organisms which have been divided into the following three groups based on the nucleotide sequence of the 16S rRNA gene: *Ehrlichia phagocytophila*, *E. canis*, and *E. sennetsu* (22). Members of the *E. phagocytophila* group are transmitted by ticks of the *Ixodes* genus and insect vectors. Granulocytic ehrlichiosis is a generalized disease characterized by nonspecific clinical signs such as fever, leukopenia, anaemia, and thrombocytopenia (22). In Switzerland, *E. phagocytophila* has been identified in cattle (16), and an agent with 100% homology in the 16S rRNA gene to the agent of human granulocytic ehrlichiosis (*HGE*) has been detected in dogs (17) and horses (18). Recently, Petrovec et al. (15) reported on the molecular diagnosis of the first case of *HGE* in Europe. Serodiagnostic studies indicate that *HGE* also occurs in Switzerland and poses a potential threat to humans (6, 19, 25).

Methods of identifying rickettsiae in ticks include indirect immunofluorescence, staining as described by Giménez, the hemolymph test, and electron microscopy (7, 11, 26). PCR is the most sensitive and specific technique to date for detection of *Ehrlichia* DNA in ticks (2–4, 14). This method has been used to identify agents of the *E. phagocytophila* group in *Ixodes scapularis* (13) and *I. pacificus* (4) in the United States and in *I. ricinus* in Italy (9) and Sweden (24). The purpose of this study was to investigate the distribution of rickettsiae of the *E. phagocytophila* group in *I. ricinus* in Switzerland. For that purpose, 1,667 ticks from the cantons of Zurich and Schaffhausen were collected for nested PCR and nucleotide sequencing.

MATERIALS AND METHODS

Tick collection. A total of 1,667 *I. ricinus* ticks were collected; 575 were nymphs, 448 were adult males, and 644 were adult females. The ticks were collected from April to June 1998 by using an umbrella that was covered on the outside with a Terry towel. The umbrella was pushed through grass and small bushes approximately 20 to 40 cm above the ground in forests and along the edges of forests. Ticks attached to the Terry towel were removed and placed singly in collection tubes. Ticks were collected in the regions of Uster, Wangen, Thur, and Rheinau in the canton of Zurich and in the region of Rüdlingen in the canton of Schaffhausen (Table 1). These regions were selected because there have been sporadic occurrences of granulocytic ehrlichiosis in dogs and horses.

Processing of ticks and nested PCR. The ticks were examined morphologically and then frozen at −20°C until DNA extraction was performed. Each individual tick was placed in 100 μl of buffered phosphate solution in an Eppendorf tube and mechanically homogenized by using sterile scissors. The DNA extraction was performed with a QIAamp tissue kit (Qiagen, Basel, Switzerland) according to the manufacturer’s instructions.

The components and conditions of the nested PCR for detecting *E. phagocytophila* genogroup rickettsiae (*E. phagocytophila*, *E. equi*, and the HGE agent) have been described previously (3, 20). In the first PCR, denaturation at 94°C for 5 min was followed by amplification for 35 cycles (94°C for 1 min and 72°C for 2 min) and a final extension at 72°C for 5 min. For nested PCR, 1 μl of the product from the first reaction was used as a DNA template. After denaturation, amplification was performed for 35 cycles (1 min each at 94, 60, and 72°C), followed by a final extension at 72°C for 5 min. To prevent possible inhibition of PCR by tick products, the DNA was heated to 95°C for 5 min before each PCR (20). Negative controls included DNA from 50 noninfected adult ticks of the *I. ricinus* species, which were bred at the Institute of Zoology in Neuchâtel, Switzerland.

The amplified DNA was extracted from the gel by using a gel band purification kit (Pharmacia Biotech, Dübendorf, Switzerland). Cloning was done with a TOPO TA cloning kit by using the pCR 2.1-TOPO vector system (Invitrogen, NV Leek, The Netherlands) and the *Escherichia coli* TOPO10 strain. Purification of the plasmid DNA was carried out by using a commercial plasmid kit (Qiagen). For bidirectional DNA sequencing of the insert, the following primers were used: for the pCR 2.1-TOPO vector, M13 forward primer (5'-CACGAAACAGCTATGACC-3') and M13 reverse primer (5'-GTCGAACGGATTATTCTTTATAGCTTGC-3'); for the plus strand, EE-3 (5'-GTGGAACCGATTATCTTCTTATGCGTTC-3') and EP-751 (5'-GATACTCGTGAATGTCAC-3'); and for the minus strand, EE-4 (5'-CCCTTCCTGTAAGAAGGATCTAATCG-3') and Nic-1 (5'-GGCTCAGTAAATCGGAT-3'). The nucleotide sequence was detected with a fluorescence-based automated sequencing system (ABI 377A DNA sequencer) by Microsynth, Balgach, Switzerland.

Nucleotide sequence accession number. The sequence of the 16S rRNA gene of the isolated PCR products from ticks has been deposited in GenBank under accession no. AFO84907.

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RESULTS

Of 1,667 ticks examined, 21 (1.3%) were positive in nested PCR. They consisted of 3 nymphs (0.5%), 6 adult males
(1.3%) and 12 adult females (1.9%). The prevalence of infected ticks varied slightly with the stage of development and the geographical origin (Table 2). The highest prevalence was in the Thur region (2.1%) and the lowest was in Uster (0.4%). There were no positive nymphs or adult male ticks in Uster and Rüdlingen and no positive adult female ticks in the Thur region. The highest prevalences of infected nymphs and adult male ticks occurred in the Thur region, and the highest prevalence of infected adult female ticks occurred in Wangen. The PCR was negative for the 50 control ticks.

The nucleotide sequences of all 21 isolated PCR products from ticks were identified as part of the 16S rRNA gene of *Ehrlichia*. The nucleotide sequences of the 16S rRNA genes of all of the ticks were identical and differed from the gene sequences of *E. phagocytophila* (GenBank accession no. M73220) and *E. equi* (M73223) in two and three positions, respectively. In addition, there was 100% sequence homology to the agent of HGE from the United States (U02521) and to the agent of canine and equine granulocytic ehrlichiosis from Switzerland (AF057707).

**DISCUSSION**

The goal of the present study was to investigate the distribution of rickettsiae of the *E. phagocytophila* group in various regions of the Swiss cantons Zurich and Schaffhausen. This group consists of closely related species of *Ehrlichia*, which include *E. phagocytophila*, the cause of tick-borne fever in sheep, goats, and cattle; *E. equi*, the cause of equine ehrlichiosis; and the HGE agent, a recently discovered species that infects a different host species, has a different geographical distribution, and may cause different clinical signs. However, research indicates that they may be variants of the same species (1, 10). In Europe, besides *E. phagocytophila*, there is another granulocytic species of *Ehrlichia*, which has 100% homology in the 16S rRNA gene with the agent causing HGE (12, 15, 17, 18). This agent causes granulocytic ehrlichiosis in humans, horses, and dogs. Although this disease occurs sporadically, it is more common in some regions than in others. Similar heterogeneous distributions were also observed in epidemiological studies of granulocytic ehrlichiosis in dogs (21), horses (5), and humans (19) in Switzerland and are probably due to differences in tick prevalences or to variations in the prevalences of *Ehrlichia* within tick populations. For this reason, regions with large tick populations and with known occurrences of granulocytic ehrlichiosis in dogs or horses were chosen for the present study.

The prevalence of positive ticks in this study (1.3%) was similar to that of *E. phagocytophila* in adult ticks (0.8%) from an area in Switzerland where tick-borne fever is endemic (20). Barlough et al. (4) reported a similar prevalence (0.8%) for species of the *E. phagocytophila* group in 1,112 adult *I. pacificus* ticks from seven regions of California. In contrast, our results differed from those of investigations of the prevalence of species of the *E. phagocytophila* group in ticks of the *Ixodes* genus in the United States (13, 14, 23), Italy (9), and Sweden (24). At present, we cannot explain these differences; they may be attributable to differences among tick species or among developmental stages of ticks, to geographical variations of infected ticks or intermediate host species, to seasonal variations in biological characteristics of *Ehrlichia* or of *Ehrlichia*-infected ticks, or to differences in the diagnostic methods used.

Our tick isolate was identified by means of nucleotide sequencing of the cloned PCR products. Sequencing revealed 100% homology in the 16S rRNA genes to the agent of HGE, described by Chen et al. (8) in the United States, and to the granulocytic *Ehrlichia* species in dogs and horses described by Johansson et al. (12) in Sweden and by Pusterla et al. (17, 18) in Switzerland. Despite the relatively low prevalence, the occurrence of this *Ehrlichia* agent in *I. ricinus* in certain regions of Switzerland should alert us to the possibility of *Ehrlichia* infections in humans and animals.

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