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Detection of *Ehrlichia phagocytophila* DNA in *Ixodes ricinus* Ticks from Areas in Switzerland Where Tick-Borne Fever Is Endemic

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A total of 1,523 adult *Ixodes ricinus* ticks were collected from regions where bovine ehrlichiosis is endemic and were examined for *Ehrlichia phagocytophila* via PCR. Of the ticks from cattle with ehrlichiosis, the ticks from healthy cattle, and the free-living ticks, 26.5% (18 of 68), 4.4% (35 of 802), and 0.8% (5 of 653), respectively, were positive.

Bovine ehrlichiosis is a highly febrile, systemic disease caused by *Ehrlichia phagocytophila* and transmitted by *Ixodes ricinus* (7). In Switzerland, areas in which bovine ehrlichiosis is endemic are usually found in subalpine regions that provide an ideal biotope for ticks (11, 16). Tick activity can be determined quantitatively by assessing the tick infestation of cattle and is usually moderate in May and June, low in July and August, and high in September. The seroprevalence for *E. phagocytophila* within a cattle herd has a course parallel to that of tick activity (12, 14). Methods to identify rickettsias in tick cells include indirect immunofluorescence, staining according to the method of Giménez, the hemolymph test, and electron microscopy (4, 6, 18). In addition, PCR has recently been used as a more sensitive means of identifying *Ehrlichia* DNA in ticks (1–3, 10). The purpose of this study was to determine, via nested PCR, the prevalence of *E. phagocytophila* in adult *I. ricinus* ticks from regions where bovine ehrlichiosis is endemic.

Tick collection. A total of 1,523 morphologically adult ticks of the species *I. ricinus* were collected in three regions of Switzerland where bovine ehrlichiosis is endemic (Schinberg, Obwalden; Tobelwald, St. Gallen; and Santa Maria, Tessin) during the pasture season of 1997. Of these, 68 were female ticks from six cows with bovine ehrlichiosis. The diagnosis was based on clinical signs and on the detection of *Ehrlichia* organisms in buffy coat smears and via nested PCR of leukocytes. Eight hundred two female ticks were removed from healthy calves, heifers, and cows. Animals were determined to be clinically healthy when the rectal temperature, general attitude and behavior, appetite, and milk production (in lactating cows) were normal. The ticks were divided into two groups according to the degree of engorgement: group 1 consisted of nonfed ticks and group 2 consisted of fed ticks (Table 1). In addition, 653 free-living ticks were collected. This was achieved with an umbrella that was covered with a terry towel and repeatedly pushed through the fern-rich vegetation during inspections of the pastures.

Processing of tick specimens. The ticks were examined morphologically and then frozen at −20°C until DNA extraction was performed. Each individual tick was placed in 200 μl of buffered phosphate solution in an Eppendorf tube and mechanically homogenized. The DNA extraction was performed with a QIAamp Tissue Kit (Qiagen, Basel, Switzerland) according to the manufacturer's instructions.

Nested PCR. The methods used for nested PCR for identification of the members of the *E. phagocytophila* group have been described previously (2, 13). The sensitivity of the PCR method was assessed by dilution of an *Escherichia coli*-cloned 16S rRNA gene segment from *E. phagocytophila* of approximately 1,200 bp. The linearized plasmid DNA was diluted with purified DNA from noninfected, nonfed, and fed adult ticks. The sensitivity of the PCR under these conditions was 10 copies. In contrast, a single copy of the linearized double-stranded DNA could be identified in purified herring sperm DNA. The inhibition effects were not apparent when 10% of the original tick DNA amount was used and when the DNA was heated to 95°C for 5 min before performance of the PCR (data not shown). Negative controls included DNA from 50 noninfected adult ticks of the *I. ricinus* species that were bred at the Institute of Zoology in Neuchâtel (Switzerland).

DNA sequencing. The nucleotide sequences of three isolated PCR products each from ticks from diseased and healthy cattle and from free-living ticks were determined by use of an ABI 377 DNA sequencer (Microsynth, Balgach, Switzerland) and compared to the 16S rRNA gene sequence of *E. phagocytophila* (GenBank accession no. M73220).

Results. The prevalence of PCR-positive ticks from the different sources and the sex of the ticks are shown in Table 2. Of 68 ticks from diseased cattle, 18 were positive for *E. phagocytophila* via nested PCR. Of the 802 ticks from healthy cattle and

TABLE 1. Distribution of 1,523 adult *I. ricinus* ticks according to origin, sex, and engorgement status

Origin or type	Sex	No. of ticks for engorgement status:	
		Nonfed	Fed
Cattle with ehrlichiosis	Female	8	60
Healthy cattle	Female	172	630
Free-living ticks	Female	260	
	Male	393	

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TABLE 2. Results of nested PCR for the identification of *E. phagocytophila* in 1,523 adult *I. ricinus* ticks

Origin or type	Sex	No. of PCR-positive ticks/total (%)
Cattle with ehrlichiosis	Female	18/68 (26.5)
Healthy cattle	Female	35/802 (4.4)
Free-living ticks	Female	2/260 (0.8)
	Male	3/393 (0.8)

of the 653 free-living ticks, 35 and 5, respectively, were positive. The PCR was negative for the 50 control ticks. The distribution of the PCR-positive ticks from diseased and healthy cattle by engorgement status is shown in Table 3. It was apparent that the percentages of positive ticks from diseased cattle were similar in the two groups, whereas in ticks from healthy cattle, there was a slight but not significant (Fisher's exact test, $P = 0.15$) increase in the percentage of positive engorged ticks.

The nucleotide sequences of the nine PCR products isolated from ticks from diseased and healthy cattle and from free-living ticks were all identified as part of the 16S rRNA gene of *E. phagocytophila* (data not shown).

Discussion. The prevalence of PCR-positive ticks varied with the origin of the ticks. The lowest prevalence (0.8%) occurred in free-living, adult *I. ricinus* ticks. In a study by Barlough et al. (3), nested PCR of 1,112 adult *Ixodes pacificus* ticks from seven different regions of California revealed an identical prevalence of members of the *E. phagocytophila* group. Cinco et al. (5) found a prevalence of 24.4% in nymphs examined for *E. phagocytophila*. Possible reasons for this discrepancy could be geographic, seasonal, or tick-stadium-associated differences between different tick populations. Interestingly, in this study the prevalence was 5.5 times higher in ticks from clinically healthy cattle than in free-living ticks. This could possibly be explained by a multiplication of *E. phagocytophila* organisms in response to the ingestion of blood. In support of this possibility, Smith et al. (15) and Lewis et al. (8) reported that a partial blood meal in *Rhipicephalus sanguineus* nymphs, infected with *Ehrlichia canis* in the larval stage, was necessary to cause ehrlichiosis in dogs. This has also been suggested for *Ixodes* spp. infected with agents of the *E. phagocytophila* genogroup (9, 17). In this study, there was a trend toward an increase in prevalence with an increase in engorgement status of the ticks from clinically healthy cattle; this may have been due to more efficient detection of *E. phagocytophila* organisms after a period of reactivation. In this population of ticks, the possible uptake of *E. phagocytophila*-infected blood prior to removal from the host did not likely play a major role in determining the prevalence. In contrast, the uptake of infected blood appeared to be the principal cause of the high prevalence of PCR-positive ticks among those collected from cattle with bovine ehrlichiosis; the prevalence in this population was six times higher than that in ticks from clinically healthy cattle. Although the number of ticks collected from cattle with bovine ehrlichiosis was small, there was no apparent difference in prevalence among ticks of differing engorgement status. Further studies are required to determine whether factors such as regional or seasonal variability of *E. phagocytophila*-infected ticks or in the *Ehrlichia* life cycle affect the prevalence of *E. phagocytophila* in ticks.

TABLE 3. Distribution of PCR-positive *I. ricinus* ticks from cattle with bovine ehrlichiosis and from healthy cattle according to engorgement status

Origin	No. of PCR-positive ticks/total (%) by engorgement status	
	Nonfed	Fed
Cattle with ehrlichiosis	2/8 (25.0)	16/60 (26.6)
Healthy cattle	4/172 (2.3)	31/630 (4.9)

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