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Serological Evidence of Infection with *Ehrlichia* spp. in Red Foxes (*Vulpes vulpes*) in Switzerland

NICOLA PUSTERLA,[1]* PETER DEPLAZES,[2] UELI BRAUN,[1] AND HANS LUTZ[1]

Department of Veterinary Internal Medicine[1] and Institute of Parasitology,[2] University of Zurich, CH-8057 Zurich, Switzerland

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Serum samples from 1,550 red foxes in Switzerland were tested for antibodies to the agents of canine granulocytic and monocytic ehrlichiosis by an indirect immunofluorescent technique. Forty-four (2.8%) of the samples were positive for *Ehrlichia phagocytophila*, which is an antigen marker for granulocytic ehrlichiosis. In contrast, none of the samples had antibodies specific to *Ehrlichia canis*, the agent of monocytic ehrlichiosis.

Several species of the genus *Ehrlichia* cause clinical and subclinical infections in dogs and wild canids (3, 15). In Europe, *Ehrlichia canis* and the agent of canine granulocytic ehrlichiosis (CGE) are the most important species of *Ehrlichia* in canids. *E. canis* infects primarily mononuclear cells (15) and is transmitted by *Rhipicephalus sanguineus*, which is indigenous to areas of Switzerland south of the Alps (2, 4). The agent of CGE and the agent of human granulocytic ehrlichiosis are closely related (8, 9), based on 100% sequence homology of the 16S rRNA gene. The agent of CGE is transmitted by ticks of the genus *Ixodes* and cannot be differentiated serologically from *Ehrlichia phagocytophila* and *Ehrlichia equi* (6). Because of the strong serological cross-reactivities within this genogroup, *E. phagocytophila* and *E. equi* can be used as antigens for the detection of antibodies to CGE. To investigate the role of foxes as a possible reservoir of CGE and canine monocytic ehrlichiosis, serum samples collected from Swiss foxes (a feral relative of the dog) were examined serologically.

Samples. This investigation involved 1,550 red foxes (*Vulpes vulpes*) that were killed during the hunting seasons from 1989 to 1998. Samples of clotted blood collected from the heart and samples of fluid collected from the pleural cavity were kept at −80°C until used. Based on the region of Switzerland where they were killed, the foxes were assigned to one of the following five groups: north (562 foxes), central (269), south (108), east (207), and west (404).

IFA. Serum samples were examined for antibodies to *Ehrlichia* by an indirect immunofluorescent assay (IFA). *E. phagocytophila* antigen was used for the detection of antibodies to CGE, as described previously (11). The serological detection of antibodies to *E. canis* was performed according to the method of Ristic et al. (12). The conjugate was fluorescein isothiocyanate-conjugated rabbit anti-dog immunoglobulin G (Jackson ImmunoResearch Laboratories Inc., West Grove, Pa.) The cutoff titers were 20 for *E. canis* and 40 for *E. phagocytophila*, according to the reference range of our laboratory (14). Statistical analysis of the prevalence of titers was performed with Fisher's exact test, and a P value of ≤ 0.05 was considered significant.

Results. A total of 44 (2.8%) of the samples had specific antibodies to *E. phagocytophila*. The frequency of titers is shown in Table 1. None of the samples had antibodies to *E. canis*. Seroprevalence for *E. phagocytophila* varied with the geographical region. The highest prevalence (3.7%) was in the northern and western regions, and the lowest (1.1%) was in central Switzerland. Seroprevalences in foxes from the northern and western regions differed significantly from those in central Switzerland ($P < 0.05$), but there were no significant differences among foxes from the northern, western, eastern, and southern regions ($P > 0.05$). Furthermore, there were no significant differences between seroprevalences in foxes from the southern and eastern regions and those from central Switzerland ($P > 0.05$).

Discussion. CGE and canine monocytic ehrlichiosis are generalized diseases that occur sporadically in specific regions of Switzerland (7, 14). Infected dogs have fever and other nonspecific clinical signs. The vector of CGE, *Ixodes ricinus*, occurs throughout Switzerland. In contrast, the vector of monocytic ehrlichiosis, *R. sanguineus*, occurs only in southern Switzerland (1, 2, 4). Thus, it would be expected that in Switzerland cases of canine monocytic ehrlichiosis would occur only in the southern regions. In a recent paper, members of our group reported that healthy dogs from areas north of the Alps had a significantly higher prevalence of antibodies to *E. phagocytophila* than healthy dogs from regions south of the Alps (11). Furthermore, in the same study, it was concluded that canine monocytic ehrlichiosis was not indigenous, because the majority of seropositive dogs had travelled to regions of endemicity outside of Switzerland. In areas where the disease occurs in domestic dogs, wild foxes and other wild canids may serve as reservoir hosts for canine ehrlichiosis (3). In our opinion, the fox is an ideal species for the study of the importance and spread of canine ehrlichiosis. The fox is closely related to the dog and thus is susceptible to most diseases specific to dogs. In addition, because of their territorial nature, foxes usually remain in a specific area.

The overall prevalence of 2.8% that was calculated in the present study is similar to that of granulocytic ehrlichiosis in dogs (3.4% [11]) and in horses (4.0% [5]). Molecular comparison of the 16S rRNA gene has demonstrated that granulocytic ehrlichiosis of canids and equids in Switzerland is caused by the same species of *Ehrlichia* and that there is 100% homology between the 16S rRNA gene of this agent and that of the agent of human granulocytic ehrlichiosis (9, 10). The highest seroprevalence occurred in foxes from the northern and western regions of Switzerland, which are the areas known to have the largest number of ticks (1). In contrast, *I. ricinus* is found less often in the eastern and particularly central regions of Switzerland, and this was reflected in the low seroprevalence of

* Corresponding author. Present address: University of California, School of Veterinary Medicine, Department of Medicine and Epidemiology, Davis, CA 95616. Phone: (530) 752-7991. Fax: (530) 752-0414. E-mail: npusterla@ucdavis.edu.

TABLE 1. Serological examination of 1,550 fox sera for *E. phagocytophila* and *E. canis* by IFA

Sample origin (region of Switzerland)	*E. phagocytophila*		No. reactive[b] for *E. canis*
	No. reactive[a]/ total (%)	IFA titers (no. of samples)	
North	21/562 (3.7)	40 (7), 80 (6), 160 (4), 320 (3), 640 (1)	0
Central	3/269 (1.1)	40 (2), 160 (1)	0
South	2/108 (1.8)	80 (1), 160 (1)	0
East	3/207 (1.4)	40 (1), 80 (1), 160 (1)	0
West	15/404 (3.7)	40 (7), 80 (8)	0
Total	44/1,550 (2.8)		0

[a] IFA titer of ≥40.
[b] IFA titer of ≥20.

CGE in foxes from these areas. The variation in geographical prevalence of CGE may be attributable to the differences in tick populations among these areas. Another possible explanation is the heterogeneous distribution of *Ehrlichia* within the tick population. This has been shown to be true for the agent of central European tick-borne meningoencephalitis, which is transmitted by *I. ricinus* (13).

The finding that none of the foxes had antibodies to *E. canis* supports the conclusions of earlier studies of dogs that *E. canis* presently is not carried by the *R. sanguineus* population in Switzerland. However, the spread of this *Ehrlichia* species into southern Switzerland is possible because this species is endemic to neighboring countries and because of the wide distribution of the vector. New areas of endemicity may develop when *Ehrlichia* agents are transmitted from infected dogs to noninfected tick populations. In the future, it should be feasible to monitor the distribution of ehrlichiosis by periodical serological examination of an indigenous population of canids, such as the fox.

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