

Seroprevalence of *Ehrlichia canis* and of Canine Granulocytic Ehrlichia Infection in Dogs in Switzerland

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Serum samples from 996 dogs in Switzerland were examined for antibodies to *Ehrlichia canis* and to the agent causing canine granulocytic ehrlichiosis (CGE). Ehrlichiosis, borreliosis, and systemic illness not associated with ticks were suspected in 75, 122, and 157 of these dogs, respectively. The remainder of the serum samples were obtained from clinically healthy dogs which resided north ($n = 235$) or south ($n = 407$) of the Alps. The serum samples were tested by an indirect immunofluorescence technique for antibodies to the two agents incriminated, *E. canis* and *Ehrlichia phagocytophila*, a surrogate marker of the agent of CGE. Twenty-two of 996 (2.2%) serum samples had antibodies to *E. canis* and were distributed as follows: 20 of 75 (26.7%) samples from dogs suspected of having ehrlichiosis, 1 of 122 (0.8%) from dogs suspected of having borreliosis, and 1 of 407 (0.2%) from healthy dogs which resided south of the Alps. Of the 75 (7.5%) serum samples that had antibodies to *E. phagocytophila*, significantly more samples were from ill dogs than from healthy dogs. Among the sera from healthy dogs, antibodies to *E. phagocytophila* were significantly more prevalent in the north. Because seropositive dogs had a history of travel outside Switzerland and because *Rhipicephalus sanguineus* is found exclusively south of the Alps, it was presumed that, in contrast to the agent of CGE, *E. canis* is not indigenous to Switzerland.

Ehrlichia spp. are obligate intracellular microorganisms that multiply in eukaryotic cells and are believed to be transmitted by ticks (13). A number of different species of *Ehrlichia* can infect dogs, and their affinity for hematopoietic cells may result in leukopenia and thrombocytopenia. Worldwide, *Ehrlichia canis* is the most important species of *Ehrlichia* in dogs; it is transmitted by *Rhipicephalus sanguineus* and infects predominantly mononuclear cells. *Ehrlichia platys*, which is also believed to be transmitted by *R. sanguineus*, infects platelets and leads to cyclic thrombocytopenia. This species has been reported in the United States and in southern Europe. *Ehrlichia ewingii* and *Ehrlichia equi* both occur in the United States and infect predominantly neutrophils, but they cause different symptoms (17).

In addition to the disease caused by *E. canis*, canine granulocytic ehrlichiosis (CGE) has received sporadic attention in reports from Europe. Molecular comparison of isolates from Sweden and Switzerland has shown that the causative agent of CGE is an *Ehrlichia* species that is closely related to the causative agent of human granulocytic ehrlichiosis and that the nucleotide sequences of their 16S rRNA genes are 100% homologous (9, 10). The causative agent of CGE cannot be differentiated serologically from *Ehrlichia phagocytophila* and *E. equi* (6). Because of the marked cross-reactivity among members of this gene group, *E. phagocytophila* antigen or *E. equi* antigen can be used for serological detection of CGE. In Switzerland, cases of canine mononuclear and granulocytic ehrlichiosis have been described (8, 10, 16). Their respective vectors, *Ixodes ricinus* and *R. sanguineus*, are indigenous to all of Switzerland and to regions south of the Alps (e.g., the canton

of Ticino), respectively (1–3). To our knowledge, there are no epidemiological data regarding the prevalence of these diseases in Switzerland. Thus, the goal of this study was to determine the prevalence of *E. canis* and the agent of CGE in relation to the health status and geographical origin of infected dogs.

MATERIALS AND METHODS

Between March 1991 and March 1998, serum samples from 996 (642 healthy and 354 sick) dogs were collected from veterinary practices in various regions of Switzerland. Information regarding the age, sex, geographical origin, health status, and history of travel outside the country for the dogs was obtained from the participating veterinarians by use of a questionnaire. The dogs were divided into five groups based on health status and/or geographical origin. Group 1 consisted of 75 dogs that were suspected of having ehrlichiosis; clinical signs included fever, enlarged lymph nodes, and thrombocytopenia. Group 2 was composed of 122 dogs that were suspected of having borreliosis; their clinical signs included arthritis, lameness, and dermatological or renal disease of unknown etiology. Group 3 consisted of 157 dogs with generalized diseases that were not associated with ticks. In group 4, there were 235 healthy dogs that lived north of the Alps, and group 5 consisted of 407 healthy dogs that lived south of the Alps. All groups were homogeneous with regard to age and sex distribution; the mean age was 5.7 years, and 47% of the dogs were female and 53% were male. For 116 (12%) dogs, the history of travel outside the country could not be established.

Serum samples were examined for antibodies to *Ehrlichia* via an indirect immunofluorescence technique. The serological detection of antibodies to *E. canis* was performed according to the methods of Ristic et al. (14). *E. phagocytophila* antigen was used for the detection of antibodies to CGE, as described previously (11, 12). The conjugate was fluorescein isothiocyanate-conjugated rabbit anti-dog immunoglobulin G (Jackson ImmunoResearch Lab. 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 (16). Statistical analysis of the prevalence of titers was performed using the chi-square test, and a P value of ≤ 0.05 was considered significant.

RESULTS

A total of 22 (2.2%) and 75 (7.5%) serum samples had antibodies to *E. canis* and *E. phagocytophila*, respectively (Ta-

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TABLE 1. Serological examination of 996 dog sera for *E. canis* and *E. phagocytophila* with indirect immunofluorescence

Dog group (no. of samples)	<i>E. canis</i>		<i>E. phagocytophila</i>	
	No. of positive samples ^a (%)	IFA titers (no. of samples)	No. of positive samples ^b (%)	IFA titers (no. of samples)
1 (75)	20 (26.7)	20 (7), 40 (3), 80 (1), 160 (2), 640 (2), 1,280 (2), 10,240 (3)	13 (17.3)	40 (4), 80 (2), 160 (1), 320 (3), 640 (3)
2 (122)	1 (0.8)	320 (1)	19 (15.6)	40 (9), 80 (4), 160 (3), 320 (3)
3 (157)	0		21 (13.4)	40 (11), 80 (4), 160 (2), 320 (2), 640 (1), 1,280 (1)
4 (235)	0		17 (7.2)	40 (6), 80 (5), 160 (3), 320 (1), 640 (2)
5 (407)	1 (0.2)	640 (1)	5 (1.2)	40 (2), 80 (2), 160 (1)
Total (996)	22 (2.2)		75 (7.5)	

^a IFA titer of 20.

^b IFA titer of 40.

ble 1). Dogs suspected of having ehrlichiosis had the highest prevalence of antibodies to *E. canis*. The levels of seroprevalence were significantly different from those in healthy dogs ($P < 0.001$). Two dogs with an *E. canis* antibody titer of 20 and all dogs with a titer equal to or greater than 80 had a history of travel to a country where *E. canis* is endemic (e.g., Italy, France, or Spain). There was no established history of travel outside the country for 5 dogs with *E. canis* antibody titers of 20 and for 3 dogs with titers of 40. In group 1, positive *E. canis* titers were significantly related to the history of travel outside the country ($P < 0.01$). One dog in group 2 and 1 in group 5 were seropositive for *E. canis*. The 2 dogs with titers of 320 and 640 had traveled in Italy and Spain, respectively. The seroprevalence of *E. phagocytophila* varied with the health status and geographical origin of the dogs; there was a significant difference in seroprevalence between diseased and healthy dogs from north ($P < 0.05$) and south ($P < 0.01$) of the Alps. In contrast, there were no significant differences ($P > 0.05$) among groups 1, 2, and 3. Healthy dogs from north of the Alps had a significantly higher seroprevalence ($P < 0.05$) of *E. phagocytophila* than healthy dogs from south of the Alps. All of the 75 dogs that were seropositive for *E. phagocytophila* resided in Switzerland and had never traveled outside the country. Seven sera positive for *E. canis* cross-reacted with *E. phagocytophila*, but the titers were two to seven times lower for the latter. In contrast, 11 sera positive for *E. phagocytophila* cross-reacted with *E. canis* at dilutions that were two to five times lower.

DISCUSSION

The prevalence of *E. canis* is largely dependent on the distribution of the vector, *R. sanguineus*, which occurs mainly in tropical and subtropical regions. This tick, which is indigenous to southern Europe (Italy, Spain, Portugal, and France), is occasionally introduced by dogs into Switzerland, where it may overwinter in dog kennels and other buildings. However, for climatic reasons, this tick can survive only south of the Alps, where its sporadic occurrence was first described in the 1980s (2). It appears that in recent years this tick has become an established resident of areas south of the Alps; adult- and juvenile-stage ticks have been found in the canton of Ticino on dogs, cats, and people who have never traveled outside this area (3). The extremely low prevalence of *E. canis* in healthy dogs indicated that this *Ehrlichia* species is not yet indigenous to that region. The infection in the seropositive dog in group 5 was presumably contracted during travel to a country where *E. canis* is endemic. This was probably also true for the seropos-

itive dog of group 2, because for biological reasons, *E. canis* infection in dogs north of the Alps is unlikely.

The high percentage of dogs seropositive for *E. canis* in group 1 was in agreement with the occurrence of specific clinical signs in these dogs, in contrast to healthy dogs. Also, the occurrence of *E. canis* antibodies in this group was significantly related to a history of travel outside the country. In Switzerland, cases of canine mononuclear ehrlichiosis were often associated with a travel history in southern Europe or Asia (16). An indirect immunofluorescence technique was used to detect specific antibodies to *E. canis*, because detection of the agent itself in peripheral blood is difficult. The low titer of 20 is considered positive for *E. canis*. However, low false-positive titers (up to 80) may occur in samples contaminated with bacteria (17). Thus, to enhance the diagnostic sensitivity of the immunofluorescent-antibody (IFA) test, *E. canis* infection should be suspected in dogs from Switzerland that have characteristic symptoms and titers equal to or greater than 80 after traveling in southern Europe, especially when a serological follow-up is not available. Otherwise, rising titers or a persistently positive IFA titer is considered indicative of active *E. canis* infection (17).

In the United States, the agent of CGE is *E. equi*; however, in Europe, the agent is a related *Ehrlichia* species that is transmitted by *I. ricinus* (15) and whose 16S rRNA gene has 100% homology to that of the causative agent of human granulocytic ehrlichiosis (9, 10). CGE can be acute or subclinical and is usually characterized by mild fever, depression, and lethargy (7). Seropositive dogs have been diagnosed throughout Switzerland. The seroprevalence of *E. phagocytophila* differed among the five groups, depending on health status and geographical origin of the dogs. Based on the significantly higher seroprevalence of *E. phagocytophila* in dogs with generalized illnesses (group 3) than in healthy dogs from the same geographical area (group 4), it appears that in the past CGE may have been overlooked as a clinical entity. This is supported by reports of CGE in a number of European countries (5, 7, 10). The differences between the two groups of healthy dogs (groups 4 and 5) may have been due to a different distribution of the CGE agent between ticks north and south of the Alps. A comparable distribution (a higher seroprevalence north of the Alps) has been reported in healthy horses that were examined for antibodies to equine granulocytic ehrlichiosis (4).

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