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

NICOLA PUSTERLA,1,4 JEANNINE BERGER PUSTERLA,2 PETER DEPLAZES,3 CELESTINE WOLFENSBERGER,1 WERNER MÜLLER,4 ANGELIKA HORAUF,4 CLAUDIA REUSCH,1 AND HANS LUTZ1

Department of Veterinary Internal Medicine1 and Institute of Parasitology, 3 University of Zurich, Zurich, and Bessy’s Kleintierklinik, Watt,2 Switzerland, and Analytisches Labor Alomed, Radolfzell, Germany4

Received 8 June 1998/Returned for modification 27 July 1998/Accepted 24 August 1998

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%) 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.

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

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

* IFA titer of 20.
* IFA titer of 40.

**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 indicates 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 seropositive 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).

**ACKNOWLEDGMENTS**

This study was supported by the Kommission zur Förderung des akademischen Nachwuchses. We acknowledge Protatek International Inc., St. Paul, Minn., for providing us with the E. canis slides at reduced costs.
We thank J. Nicolet for supplying serum samples and G. Konermann for expert technical assistance.

REFERENCES