Epidemiology of canine leishmaniasis by cross-sectional study in the French focus of Cévennes

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SUMMARY

A cross-sectional epidemiological survey was carried out on the distribution of canine leishmaniasis in the French focus of Cévennes. Animals were sampled in two vegetation zones: Holm and White Oak zone (WO/HO) and Holm Oak zone (HO) in which villages were chosen for collecting blood from dogs as exhaustively as possible. Sera from 259 dogs were collected and subjected to the indirect immunofluorescence antibodies test (IFAT) and counter immuno-electrophoresis (CIE). Seroprevalence was 17.9% in the WO/HO level and 9.6% in the HO level. Serological positivity to Leishmania sp. was significantly associated with a life in the WO/HO level, and sex, dog’s activity and age were found to be additional factors. Basic reproductive number \( R_0 \) was 1.21 in the WO/HO level and 1.11 in the HO level. Prevalence has moderately increased during the last ten years, especially in the peri-urban HO zone.

KEY-WORDS : leishmaniasis - dog - epidemiology - France.

RÉSUMÉ

Etude épidémiologique de la leishmaniose canine en région cévenole.
Par N. KECK ET J. DEREURE

Une étude transversale évaluant la prévalence sérologique de la leishmaniose a été conduite dans le foyer des Cévennes. Les animaux ont été prélevés dans deux strates phyto-écologiques (chênaie mixte et chênaie d’yeuse), à l’intérieur desquelles ont été choisis des villages où le recrutement s’est fait de manière la plus exhaustive possible. Des échantillons de 259 chiens ont été prélevés, puis analysés par immunofluorescence indirecte et électrosynérèse. La séroprévalence obtenue était de 17,9 % dans l’étage de la chênaie mixte et de 9,6 % dans l’étage de la chênaie d’Yeuse. Les animaux vivant en chênaie mixte ont un risque plus élevé d’être séropositif qu’en chênaie d’Yeuse (OR = 3,97, IC95% 1,33-12,71). Le taux de reproduction de base est de 1,21 (chênaie mixte) et de 1,11 (chênaie d’Yeuse). La prévalence dans ces deux strates a modérément augmenté durant les dix dernières années, principalement dans les zones péri-urbaines.

MOTS-CLÉS : leishmaniose - chien - épidémiologie - France.

Introduction

Visceral leishmaniasis is a widespread disease, especially in Mediterranean European countries, Africa, Middle East Asia and South America. In south-western Europe, it is a zoonotic disease due to Leishmania infantum.

The French focus of Cévennes is well known to be enzootic for leishmaniasis. The principal vector is Phlebotomus ariasi which is anthropo-zoophilic in its behaviour [31]. Cross-sectional serological surveys have already been carried out in this region [5, 16, 23]. They show an increase of the prevalence of canine leishmaniasis during the last ten years and a wide range of values between different parts of the focus.

Hence, this disease has become a significant veterinary problem in this focus, which may be complicated by the emergence of resistant strains to pentavalent antimonial compounds which have led to a search for alternative drugs [28]. Canine leishmaniasis is also met in non-endemic areas, concerning dogs which were exposed to the disease during travels in other endemic areas. Limited outbreaks are sometimes reported by veterinary practitioners but not always documented by epidemiological or entomological studies [27]. Yet, the potential impact of warming on the establishment of new enzootic areas has to be evaluated [14]. Furthermore, dogs are considered as the main reservoir host [10] even if other animals such as the European fox (Vulpes vulpes) or the black rat (Rattus rattus) have also been identified as potential hosts of Leishmania sp. [30, 24].

Human leishmaniasis usually occurs in young populations. However, immunodepressed adults (consequently to HIV infection or drugs used in organ transplants) are also greatly
exposed to the infection and very susceptible to the disease which was shown in the focus of Cévennes [6]. This places therefore leishmaniasis as an emerging human health problem.

Thus, it is important to estimate the prevalence of canine leishmaniasis in order to evaluate the risk for human and animal health. The aim of this work was to estimate the prevalence of leishmaniasis with a cross-sectional study in two areas of the French focus of Cévennes and to define epidemiological patterns and risk factors.

Material and methods

The Cévennes focus can be divided into five vegetation zones with different prevalences of leishmaniasis [16]. The main difference between the stages is vegetation, which is correlated with the frequency of Phlebotomus ariasi and thus the disease prevalence.

The data come from a cross-sectional serological survey of the dog population carried out during the months of May and June 1999. We used Lanotte’s assertion [16] for our work but only carried out the survey in two zones:

- the holm and white oak (Quercus ilex and Q. pubescens) zone (WO/HO zone) which is a rural stage where altitude is generally high (500-700 m) and prevalence is expected to be high.
- the holm oak (Q. ilex) zone (HO zone) which is more peri-urban, where prevalence is expected to be lower.

In this area, transmission by Phlebotomus ariasi occurs from late May to early September, but is usually concentrated in July [7].

According to the results obtained by DERERUE [5] who found that prevalence was 16.51 % in HO/WO zone and 5.51 % in HO zone, the number of dogs needed to be sampled was 143 in each zone in order to compare prevalence using statistical analysis with at least 80 % power.

In each zone, villages were chosen for collecting blood from all native dogs as exhaustively as possible. Dogs younger than six months were excluded in order to sample dogs which were exposed at least to one transmission season. Six villages of the HO/WO zone and four villages of the HO zone were studied.

Dog owners were informed of a free survey by announcements in the local press or in public places and by veterinary surgeons working in the villages. Each dog owner provided the age, breed, sex, activity, origin, nature of movements of the dog. Activity was classified as pet dogs, guard dogs, sheep-dogs and hunting dogs. Origin allowed to know whether the dog was recently living in the place or if it has been there for a long time. Nature of movements was useful to know if the dog had been in other countries where leishmaniasis is also enzootic.

SEROLOGICAL TESTS

Blood samples were collected by venepuncture with the agreement of the dog owner. Each sample was subjected to an immunofluorescent antibody test (IFAT) and counter immunoelectrophoresis (CIE) as it has already been done in other studies realized in the Cévennes focus [5, 16, 23]. Dogs with titers ≥ 1/80 and positive CIE were considered positive. Furthermore, in order to compare our results to previous studies in the focus, the prevalence rate was also estimated by considering dogs with titers ≥ 1/80 as positive (with or without positive CIE).

STATISTICAL ANALYSIS

The Woolf method was used for stratified analysis. Odds Ratios (OR) for each risk factor, Khi²-test, Khi²-woolf for interaction tests was calculated with Epi-Info®. The level of significance was set at 5 %.

The model proposed by HASIBEDER et al. [12] in the case of homogeneous transmission was used to calculate basic case reproduction number R0. This considers two kinds of dogs, type A and type B. After infection, type A dogs enter a latent period during which they are serologically positive but asymptomatic and not infectious to sandflies. Afterwards, they become symptomatic and infectious. Type B dogs are also serologically positive but are never infectious to sandflies and can self-cure. This model was simplified by DYÉ et al. [8] who considered that type B dogs were negligible [18] and that the proportion of infected sandflies was very low [19]. The R0 value gives an idea of the number of new infections occurring after the introduction of one infected animal into a safe host population.

Results

During the study, 259 sera were collected : 134 in the HO/WO zone and 125 in the HO zone. A few dogs had been imported to the Cévennes focus but at less than three months of age.

The characteristics of dogs in each vegetation zone were quite similar (table I) : sex ratio was approximately 50 % ; cross-breed dogs and young dogs (less than three years) were more numerous than other categories of breed and age. Hunting dogs were more frequent in HO/WO zone which is more rural and pet dogs were more frequent in the HO zone. As they were few, sheepdogs were grouped with hunting dogs for statistical analysis.

The prevalence obtained in the HO/WO stage was 17.2 % and 9.6 % for HO when we considered dogs as positive if IFAT was ≥ 1/80 (table II). It was lower if we considered dogs as positive when IFAT was ≥ 1/80 and CIE positive (14.2 % for HO/WO and 4 % for HO). All sera with positive CIE had a titre of 1/80 or more. The frequency distribution of antibody titre (figure 1) was unimodal. Low titres (1/20, CIE had a titre of 1/80 or more. The frequency distribution of antibody titre (figure 1) was unimodal. Low titres (1/20, 1/40) were more frequent in HO/WO zone than in HO.

The odds ratio measuring the risk of seropositivity (IFAT ≥ 1/80, CIE+) in the HO/WO zone compared to HO zone was 3.97 (IC95% = 1.33 - 12.71). This indicates a strong association between seropositivity and the place where the dog lives.

In each zone, results were different between the villages (table III). We obtained a wide range of prevalence rate
values, especially in HO/WO zone (for example 33.3 % in Monoblet and 0 % in Montoulieu). There was no statistical difference between the villages of the HO/WO (Khi² = 1.14, p = 0.76) which was not the case between those of the HO (Khi² = 9.95, p = 0.08).

Stratified analysis calculating the OR for each class of sex, activity and age shows that they are interacting factors (table IV). Odds ratios are much higher for male dogs (7.61) and watch dogs (10.29). In the population studied, the seroprevalence leishmaniasis is higher for watch dogs (20.5 %), than for hunting dogs (9.3 %) or pet dogs (5.3 %). It is also higher for males (12 %) than for females (6.5 %). Furthermore, there was no association between the breed and seropositivity (Khi² = 0.76, p = 0.68).

Assuming the informations presented earlier, the basic reproductive number if biting rates are homogeneous would be 1.21 for the HO/WO zone and 1.11 for the HO zone.

Discussion

We have used in this study the same sampling strategies and serological diagnosis than in previous studies already done in the Cévennes. DEREURE (1993) found seroprevalence rates of 5.51 % in HO and 16.52 % in HO/WO. According to our results, canine leishmaniasis prevalence seems to be stable, at least in HO/WO zone. This is illustrated by the low value of R₀ (near 1) which is characteristic of an endemic disease. Nevertheless, seroprevalence has increased in the HO zone (9.6 %) which is relevant to other observations showing that canine leishmaniasis may become a more peri-urban disease. As human populations are more and more present in peri-urban areas, this could represent a public health problem. Prevalence values are different between the two studied zones but we also find great disparities within

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TABLE I. — Categories of dogs sampled. Characteristics of dogs in each vegetation zone concerning sex, race, age and activity.

<table>
<thead>
<tr>
<th>Vegetation zone</th>
<th>Number of dogs</th>
<th>Seroprevalence (%)</th>
<th>Seroprevalence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>IFAT≥1/80</td>
<td>IFAT≥1/80, CIE +</td>
</tr>
<tr>
<td>HO/WO</td>
<td>134</td>
<td>17.2 (10.8-23.6)</td>
<td>14.2 (8.3-20.1)</td>
</tr>
<tr>
<td>HO</td>
<td>125</td>
<td>9.6 (4.4-14.8)</td>
<td>4.0 (0.6-7)</td>
</tr>
</tbody>
</table>

WO : White Oak zone
HO/WO : Holm Oak and White Oak zone
IFAT : immunofluorescent antibody test
CIE : counter immunoelectrophoresis

TABLE II. — Prevalence of canine leishmaniasis in two vegetation zones. Prevalence rate was calculated when dogs were considered as positive when IFAT was ≥ 1/80 and when it was ≥ 1/80 with a positive CIE.
those zones, especially in the HO/WO. This may be explained by the possible existence of micro foci where distinctive local climatic conditions favour the development of Phlebotomus ariasi. In fact, if the geographical classification presented by LANOTTE [16] is confirmed, it is likely that some differences, which are linked to the altitude or exposure to the sun also exist within the vegetation zones. Thus, this sandfly is more frequently found on the slopes of the hillsides at altitude of 300-500 m, which is its preferred habitat and also the place where villages are implanted [32].

According to DYE et al. [8], the fact that antibody titre distribution is unimodal is characteristic of an endemic disease. We also obtained a unimodal distribution but think that this may also be due to the fact that more and more dogs are treated against leishmaniasis. This could lower the antibody titre of infected dogs, as treatments markedly reduce antibody titres [28]. Hence, 11 of the dogs that were serologically positive had already been treated or were receiving a treatment when the study was done. Furthermore, we observed that low IFAT antibody titres (1/20, 1/40) were more frequent in

<table>
<thead>
<tr>
<th>Vegetation zone</th>
<th>Village</th>
<th>Number of dogs</th>
<th>Seroprevalence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HO/WO</td>
<td>Montoulieu</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Monoblet</td>
<td>51</td>
<td>33.3</td>
</tr>
<tr>
<td></td>
<td>S² Martial</td>
<td>12</td>
<td>16.7</td>
</tr>
<tr>
<td></td>
<td>S² Roman de Codières</td>
<td>25</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>Colognac</td>
<td>27</td>
<td>3.7</td>
</tr>
<tr>
<td></td>
<td>S² Laurent le Minier</td>
<td>9</td>
<td>11.1</td>
</tr>
<tr>
<td>HO</td>
<td>Montarnaud</td>
<td>21</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>S² Paul et Valmale</td>
<td>47</td>
<td>10.6</td>
</tr>
<tr>
<td></td>
<td>Vailhauqués</td>
<td>36</td>
<td>11.1</td>
</tr>
<tr>
<td></td>
<td>Combaillaux</td>
<td>21</td>
<td>14.3</td>
</tr>
</tbody>
</table>

WO : White Oak level  
HO/WO : Holm Oak and White Oak level

Table III. — Serological test results in each village of the two vegetation zones. Number of dogs tested and the leishmaniasis seroprevalence results in each village of the two vegetation zones.

<table>
<thead>
<tr>
<th>Sex</th>
<th>Activity</th>
<th>Age (years)</th>
<th>OR (Confidence Interval 95%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male (N=135)</td>
<td>Petdogs (N=113)</td>
<td>&lt; 3 (N=103)</td>
<td>7.61 (1.53-51.53)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>3.1 (0.45-26)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>11.7 (0.65-210)</td>
</tr>
<tr>
<td>Female (N=123)</td>
<td>Hunting dogs/Sheepdogs (N=107)</td>
<td>3 to 7 (N=91)</td>
<td>1.6 (0.33-7.86)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>4.23 (0.5-94.57)</td>
</tr>
<tr>
<td></td>
<td>Watch dogs (N=39)</td>
<td>&gt; 7 (N=64)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1.7 (0.33-9.59)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>10.29 (1.34-98.68)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>9.33 (1-219.4)</td>
</tr>
</tbody>
</table>

Table IV. — Results of stratified analysis. OR for each class of sex, activity and age are mentioned in dark characters (IC95%).
HO/WO zone. In this zone, dogs may be more frequently in contact with the parasite, and respond to infection by producing low titres of antibodies but without becoming symptomatic. According to DYE et al. [8], it is possible that some positive animals are included among them. This underlines the question concerning the sensitivity of IFAT, although it appears to be universally recognized as the standard. LANOTTE et al. [17] evaluated IFAT sensitivity at 99 % (± 1 %) and specificity at 68 %. The limitations of serology are due to the long periods of serological latency, as antibodies are often detectable only several months after infection [11]. It has also been investigated by DYE et al. [9] during a two years cohort study showing that sensitivity and specificity are high during short period of 2-3 months (March-April) after a long incubation period. Furthermore, a proportion of seropositive dogs spontaneously convert to negative [1].

Although we considered dogs as positive when IFAT was ≥ 1/80 for comparing our results to previous studies, we also used the results of CIE to analyze the effect of risk factors. This serological technique has already been used by REZAI et al. [29] who found that this test correlated well with the results of the IFAT. We also observed by operating field diagnosis serology for veterinary practitioners that it is a good indicator of the evolution of the dog’s disease compared to IFAT, which is consistent with observations made by MANSUETO [20].

However, the major problem is to know wether dogs found positive for leishmaniasis are also infective to phlebotomine sandflies. This information would allow to evaluate the burden of infectiousness of a dog population in an endemic area. There are proven connections between antibody titre and infectiousness [9]. DYE et al. [9] concluded that the aim of control programmes using serology should be to detect infectiousness rather than infection.

Other epidemiological studies conducted in France found prevalence rates ranging from 3.5 to 20 % [13, 21, 26, 36]. In other European countries, values are sometimes higher, especially in Spain and Italy where rates of 30 % were observed [22, 25]. Despite the fact that leishmaniasis seroprevalence seems to be stable, the absolute number of cases may be higher due to the growth of the canine population (France: 7 million dogs in 1971 and 9 millions in 2000, FACCÔ/SOFRES survey 2000 and Association Française d’Information et de Recherche sur l’Animal de Compagnie, personal communication). This increase of reservoir host numbers could contribute to the spread of leishmaniasis.

The OR measuring the risk of infection in WO/HO zone compared to HO (OR = 3.97 ; IC95% =1.33-12.71) is high. This difference seems essentially linked to an unequal distribution of sandflies in these two ecological zones. Concentration of P. ariasi is positively correlated with canine leishmaniasis seroprevalence [16]. This difference between different biotopes (altitude, rural/peri-urban) has also been demonstrated by numerous authors. For example, OZON et al. [26] found that seroprevalence varied with altitude (OR = 2.8, IC95% = 2.03-3.86) and COURTENAY et al. [4] showed that the force of infection was 17 times higher in a rural than in a periurban place. On the contrary, AMELA et al. [2] did not find such difference.

Many authors consider that dog activity is an important risk factor. It shows also an effect in our study since the risk was higher for watch dogs, contrary to MILHAU [23] who considered that hunting dogs were more exposed. In fact, hunters are nowadays well informed about leishmaniasis and know better how to prevent this disease. This may be different for watchdog owners, although the dog lives almost entirely outdoors and are bitten more frequently by the rather exophilic P. ariasi. Pet dogs may be less affected by leishmaniasis because they spend more time inside the house.

Also, we showed that the age of dogs is a modifying factor. Some authors found that prevalence was higher in old dogs [16, 33]. In fact, old dogs may be more affected by this disease since they have experienced more transmission seasons. Nevertheless, it seems that this effect is not so much clear because other authors have observed an asymptotic distribution [8] or a bimodal distribution [2].

Sex is not often considered to be a risk factor [25, 26]. According to our results, the risk for males could be higher. This has already been observed by CIARAMELLA et al. [3] and ZAFFARONI et al. [37] and could be explained by an increased out-door activity in males.

This study illustrates the difficulty to realize a representative epidemiological study. We used the voluntary recruitment of subjects, which does not permit the estimation of the number of dog owners who may come. Random selection from a clearly defined population would have been preferable, but was not possible because the French dog population is not completely registered.

Furthermore, as it was not possible to estimate the proportion of the dog population sampled, many of them may have escaped to our study. This represents a partial source of bias. Indeed, the population of dogs which were not sampled may be different to those tested. We observed that dog owners preferred to bring young dogs and leave the old or ill dogs at home. On the contrary, people who are interested by leishmaniasis are often those who have already been affected by it.

Some of the problems described may be solved by prospective epidemiological studies, which could also be useful to estimate the incidence of canine leishmaniasis. Repeated investigations on the same dog cohorts coupled with the study of the changes in vector populations could be a mean to elaborate a mathematical model of the epidemiology of leishmaniasis. It could also be important to use other diagnosis techniques as IFAT may be not enough sensitive [9]. For example, PCR has already been widely studied for the diagnosis of leishmaniasis [15, 35]. The prognosis interest of this technique for the diagnosis of canine leishmaniasis for veterinary practitioners deserve to be evaluated.

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