Epidemiological survey of canine bartonellosis to *Bartonella vinsonii* subs. *berkhoffii* and canine monocytic ehrlichiosis in dogs on the Island of Reunion

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SUMMARY

Reunion Island is a French overseas department, located in Indian Ocean and with a tropical climate favourable to the development of arthropod transmitted diseases. Therefore, epidemiology of Canine monocytic ehrlichiosis and bartonellosis were studied by serology (indirect immunofluorescence) and bacterial culture (for *bartonella*) in Reunion Island. Canine bartonellosis due to *Bartonella vinsonii berkhoffii* appears to be an emerging disease. Stray dogs and medicalised dogs were followed-up during one year. Reunion is an endemic area for canine ehrlichiosis, with a total prevalence close to 31%, that includes significant differences between medicalised dogs (prevalence lower than 3%) and stray dogs (prevalence over 75%). About canine bartonellosis, seroprevalence approaches 10% and there are differences between the two groups of dogs studied. 5% of dogs are co-infected which suggests a common vector.


1- Objectives

Canine monocytic ehrlichiosis (CME) to *Ehrlichia canis* is a cosmopolitan disease transmitted by *Rhipicephalus sanguineus* (the brown dog tick). The disease varies from asymptomatic forms to its classical expression that associates fever, anaemia, thrombocytopenia and haemorrhages. Early diagnosis is crucial as prognosis relies on rapid treatment initiation. CME is particularly frequent in tropical areas where its vector abounds [2, 5, 10].

*Bartonella* bacteria infect erythrocytes and endothelial cells. Canine infections are mainly due to *B. vinsonii* subs. *berkhoffii* whose arthropod vector is not formally identified yet. They were described in several countries (USA, France) [6, 7, 9, 11]. Infection could lead to endocarditis and lymphadenopathy, even if asymptomatic infections seem to be frequent [3, 4, 8].

This study consisted in an epidemiological approach of both infections, each transmitted through arthropod vectors, probably *Rhipicephalus*. Investigations were conducted over whole Reunion Island territory from two groups of dogs: medicalised dogs and stray dogs.

Little information is currently available concerning these diseases prevalence on this Island. There are no published articles on the prevalence of *Bartonella* infection in dogs on this island, whereas CME prevalence is known to be very high, about 22% in medicalised dogs and 60% in stray dogs [2].

According to local veterinary services, out of 300 000 or 400 000 dogs on the island, some 40% have an owner, 47% are «community» animals with limited health care, and 13% are stray dogs. Domestic animal health status on the island is equivalent to metropolitan France situation, whereas stray dogs are important reservoir for pathogenic agents for their congeners and possibly for humans.

2- Materials and methods

In 2001, a total of 165 dogs were sampled. 120 were from veterinary clinics (Le Tampon and La Possession). These animals had neither clinical suspicion of CME nor infection symptom related to *Bartonella*. These samples were collected during two campaigns, the first one in February 2001, and the last one in November-December 2001 (Table 1).

<table>
<thead>
<tr>
<th>Ehrlichiosis</th>
<th>Bartonellosis</th>
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<tbody>
<tr>
<td>February</td>
<td>November/December</td>
</tr>
<tr>
<td>Medicalised dogs</td>
<td>15.38 % (8/52)</td>
</tr>
<tr>
<td>Stray dogs</td>
<td>75.55 % (34/45)</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td>15.38 % (8/52)</td>
</tr>
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Table 1: Seroprevalence of *Ehrlichia canis* and *Bartonella vinsonii* subsp. *berkhoffii* infection in 2001.

45 samples were collected from stray dogs in two kennels (Refuges of SPA Saint Denis and Saint-Anne). All of these stray dogs were shepherd type dogs, with light brown hair.

Blood samples were collected in both dry and EDTA tubes. EDTA tubes were frozen at -20°C on site. Dry tubes were kept at 4°C for 12 to 24 hours, and then the sera were frozen at -20°C.

Each animal was inspected in order to detect ectoparasites, mainly ticks. Name or number, breed, birth date, residence place, and ectoparasite presence were noted. Ticks were collected from dogs that participated in this study in order to be identified.

Anti-*Ehrlichia canis* antibody detection was performed at the Laboratoire Départemental Vétérinaire in Lyon using the reference indirect immunofluorescence technique as described by RISTIC in 1972 [14].

This serology is based on the use of infected neutrophil cells (10⁶ cells/mL diluted in Phosphate Buffer Saline (PBS) buffer without Calcium, Magnesium and added with 0.5% Bovine Serum Albumine).

10 µl are fixed on glasses, dried by air and fixed in acetone (15 min at - 20°C). The fixed glasses could be stored at -20°C during 6 months.

Dog sera to be tested were diluted at 1/12.5 in PBS buffer and then re-diluted from 1/50 to 1/1600.

The titer was considered as negative when inferior to 1/50. The result was slightly positive at 1/50 and 1/100, moderate positive between 1/200 and 1/800 (included) and strongly positive with titers superior to 1/800.

Anti-*Bartonella vinsonii* subsp. *berkhoffii* antibody detection was performed in the microbiology department at the Ecole Nationale Vétérinaire in Alfort, using Regnery's technique described in 1992 and modified Chomel’s technique in 1995 [11]. Cells were of feline origin, FCWF (*Felis catus* whole foetus) or simian origin (*Vero*). Source of antigens was ATCC strain 51672 of *Bartonella vinsonii* subsp. *berkhoffii*. Dog sera were diluted to 1/50 in a PBS buffer solution. For each microscopy slide, a positive control serum (cat or coyote serum positive to 1/50) and a negative control (PBS) were prepared. A fluorescent conjugated product with total canine anti-immunoglobulin of rabbit origin (Jackson ref. 304 095 003) was diluted to 1/100 in 5mL of PBS solution with one drop of Evans Blue solution (0.001%). Specific fluorescence intensity was evaluated with a scale ranging from 1 to 4, and fluorescence equal or superior to dilution to 1/50 was considered as positive. All serums were initially tested with a 1/50 dilution. Then all positive serums were tested with successive binary dilutions. Slides’ interpretation was double-blinded.

*Bartonella* spp. cultures were performed according to method described by CHOMEL et al. in 1995 [8, 11]. Unfrozen EDTA tubes that contained dog blood were centrifuged (3000 rpm and 1800 G) for one hour. Supernatant was eliminated and centrifugel pellet extracted in 250 µL of Eagle medium modified by Dulbecco. Pellet was then sprayed on gelose (Brain Heart Infusion gelose (DIFCO) with fresh and defibrinated rabbit blood to 5%) and placed at 35°C in an atmosphere (5%) of CO₂ for 4 weeks. Petri boxes were regularly examined to observe bacteria growth.

Statistical analysis was done using a Chi² method.

3- Results and Discussion

43 serums out of 165 (26%) were positive for *Ehrlichia canis* serology (Tables 1 and 3). During sample campaign in November-December, this study clearly distinguished medicalised dogs from stray dogs: the former had a prevalence of 1.47%, whereas 19 out of 20 stray dogs were positive in Saint-Anne refuge, and 15 out 25 were positive at Saint-Denis refuge, for a total of 75.55% (34/45). The difference between these dog populations was significant (p<10⁻⁶). Global prevalence in November/December was 31%.

On the total of 43 positive dog sera, only 1 was 1/50; 3 were equal to 1/100; 35 were between 1/200 and 1/800 and 4 were equal to 1/1600. The 4 lowest titers were all obtained from medicalised dogs. The 3 highest titers were coming from stray dogs.

Table II: *Bartonella vinsonii* subsp. *berkhoffii* serology during November/December 2001 period on the 113 dogs with reference to tick infestation.
10 samples out of 113 were positive using anti-Bartonella indirect immunofluorescence assay with titres ranging from 1/50 to 1/100 (Tables 1 and 2). Seroprevalence of Bartonella vinsonii berkholffii infection in studied population was around 9%. Two animals from veterinary clinics out of 68 and eight stray dogs out of 45 were positive for B. vinsonii berkholffii. This difference was significant (p < 0.05).

Bartonella direct detection by culture in 113 blood samples was negative.

6 serum samples out of 113 were positive to both serologies, representing a prevalence of 5% for co-infection. During the November/December study, 16 dogs out of 113 (14.5%) were infested by ticks (Tables 2 and 3). All ticks except 2 were Rhipicephalus sanguineus, the 2 others belong to the Ixodes genus.

Regarding CME, this study performed on Reunion Island could not find significant differences between various dog breeds, confirming what BEUGNET et al. [2] observed earlier. There was no significant difference between males and females and age did not influence results.

Tick carriage that was noted when blood samples were taken could be positively correlated to seropositivity: tick-infested dogs were significantly more frequently affected than non-infested animals at the time of observation (p<0.05).

Ehrlichia infection seroprevalence in medicalised population was low. Hypothesis include satisfying health status and regular care to dogs that consult in veterinary clinics, particularly the prescription of acaricide products.

The difference in prevalence rates observed in February and November/December in the medicalised group was significant (p=0.004). Such variability could be explained by climate changes and also by geographic origin of animals as 33 dogs were recruited in a veterinary clinic located at an altitude of 400m whereas 20 were recruited on the coast in February.

Seroprevalence of Ehrlichia canis infection was estimated to 31% (November/December period) on Reunion Island, almost one third of the total canine population, all groups included. However, seroprevalence was clearly higher in stray dogs (75%) than in medicalised animals (less than 3%). This suggests stray dogs to be a reservoir for Ehrlichia canis and efforts to improve health status in this population should be developed.

As far as Bartonella vinsonii berkholffii is concerned, the analysis of seropositive dogs did not reveal any influence from gender, breed, and neither age.

The presence of ectoparasites, whether ticks or fleas, could not be related to the Bartonella serological status in this study. Nevertheless, hypotheses that could explain prevalence differences between stray dogs and domestic dogs for canine ehrlichiosis could apply in similar terms for canine bartonellosis.

Bartonella vinsonii berkholffii infection seroprevalence was estimated to 9%. Such rates are similar to rates reported in other studies in the United States of America [7, 11, 12], in Israel [1] and in France [9].

Co-infection global prevalence was 5%, or 6/113 dogs. This is in favour of a common vector for Ehrlichia canis and Bartonella vinsonii berkholffii. Actually, this signifies that 60% of dogs infected with Bartonella are infected with Ehrlichia too (6/10), and inversely, 17% (6/35) of dogs infected with Ehrlichia are infected with Bartonella too. This natural co-infection has been observed by other authors [12, 13, 15].

The example of the Reunion Island demonstrates that in favourable climate and with an important vector population, some vectorial diseases like ehrlichiosis can become preponderant or even dominant. This is particularly a concern when dogs move from such a territory to Europe. In addition, some of the vector-transmitted diseases like bartonellosis are potential zoonosis. Disease control implies both prevention of vector infestation and control of reservoirs. In this case, as in many tropical settings, stray dogs are the main reservoir.

**References**


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