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# Canine seroprevalence of *Rickettsia conorii* infection (Mediterranean spotted fever) in Castilla y León (northwest Spain)

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**Abstract.** A seroepidemiological study was conducted in 308 dogs to determine the presence of antibodies to *Rickettsia conorii*, using an indirect immunofluorescence assay (IFA). Seven of the provinces of the Castilla y León region (Burgos, León, Palencia, Salamanca, Soria, Valladolid, and Zamora) were covered by the study. Of the 308 dogs analysed, 72 (23.4%) showed significant titers by IFA (1/40 or higher). Seroprevalences were significantly differents between provinces of origin of the animals. These were below 30% in almost all the provinces studied, except for Salamanca province, where the percentage of seropositive dogs was much greater (93.3%). Potential risk factors (presence of ticks on the animals, age, sex, use, habitat, and season) relating to the presence of Mediterranean spotted fever, or Boutonneuse fever, were evaluated. Animals used for guard or pastor activities and those living in rural areas (these factors are closely linked), together with those suffering from tick infestation, had significantly higher seroprevalence than the remainder. The frequency of seropositive dogs increased during the summer months, and these coincide with the period of greatest activity by the vector. Sex and age variables were not identified as risk factors.

**Key words:** Dog, Epidemiology, Mediterranean spotted fever, Serological surveys, Spain, *Rickettsia conorii*

**Abbreviations:** IFA = Indirect immunofluorescence assay; MSF = Mediterranean spotted fever

## Introduction

Mediterranean spotted fever (MSF), or Boutonneuse fever, is a tick-borne rickettsiosis caused by *Rickettsia conorii*. This is an endemic disease in warm zones, such as the South of Europe [1], Africa [2, 3], and Asia [4, 5]. In Spain, cases have been reported from 1982 onwards in northern areas, the Mediterranean coast, and central regions [6, 7].

During the last ten years there has been an increase in the incidence of the disease, which has been linked with greater contacts between humans and infected ticks, coming mostly from dogs [8].

The biological cycle of *R. conorii* in Mediterranean areas includes the infection of the brown dog tick, *Rhipicephalus sanguineus*, and the transmission of it to dogs and humans [7, 9, 10]. Infection in dogs is subclinical or asymptomatic [11], but its epidemiological role is important, since contact with dogs is confirmed in a high percentage of human patients showing the disease [12, 13]. Dogs can be considered occasionally as a reservoir of rickettsias and a source of infection for ticks, although this had not been proved with *R. conorii* until now [14]. But their main role is as a vehicle, since their function is to carry rickettsia-infected ticks to humans [15]. Thus, seroprevelance of *R. conorii* antibodies in dogs is considered to be a good marker of the epidemiologic status of MSF in a particular area. Some authors even suggest that dogs may be used as a sentinel to assess the geographic distribution of this zoonosis [16].

Accordingly, the aim of this study was to determine the seroprevalence of *R. conorii* in dogs in Castilla y León region in the northwest of Spain, as well as to evaluate the influence of several possible risk factors in the presence of the infection.

## Material and methods

*Serum collection.* A total of 308 serum samples from dogs from various provinces in Castilla y León (northwest part of Spain), collected over the period January 1993 to May 1994, were studied. The selection method used was a probability sampling based on the approximate data for the dog population in the region, given in official datas from the Public Health Service. Samples were taken from randomly chosen dogs from the different areas. Venous blood samples

were taken in 5 millilitre vacuum tubes and allowed to coagulate at room temperature. The serum obtained was separated by centrifugation and stored at –20 °C until required. A questionnaire was completed for each dog sampled. The information obtained included data on the place of origin, age, sex, use, habitat, and the season when the blood was taken (between Winter 1993 and Spring 1994). In addition, the dog's owner indicated in each case whether the animal had suffered from ticks at any time.

*Serum analysis.* All the sera collected were analysed by indirect immunoflourescence assay (IFA) for a *R. conorii* antigen, using a commercial kit (*Rickettsia conori*-Spot IF. BioMérieux, Marcy-l'Etoile, France). As conjugate, an anti-dog IgG labelled with fluorescein isothiocyanate (Nordic, Tilburg, The Netherlands), at a dilution of 1/100 in phosphate buffer (pH = 7.2) was used. Titers of 1/40 or above were considered positive [17]. Positive and negative controls were used in each sample series analysed.

*Data analysis.* Seroprevalence was calculated for each variable studied as the number of dogs with titers of 1/40 or above divided by the total number of dogs analysed. A chi-square test was used for statistical analysis of the risk factors taken into account. The difference was considered significant when $p < 0.05$. Epidemiological analysis was carried out using the EPI-INFO computer package, version 5 [18].

## Results

Seventy-two out of 308 (23.4%) dogs studied showed titers of 1/40 or greater to *R. conorii*. The number of seropositive animals were heterogeneously distributed over the different provinces. The greatest seroprevalence was for Salamanca province with 93.3%. The remaining provinces covered had a seroprevalence below 30%, being the lowest percentage for Soria province with only 8% (Figure 1). A highly significant difference in seropositive dogs was found between the provinces sample ($\chi^2 = 55.78$; $p < 10^{-n}$).

No significant differences were found for age or sex (Figure 2).

With regard to functional use (Figure 3), guard and pastor dogs showed a larger percentage of seropositives (34.1%) compared to hunting dogs (17.6%) and animals kept as pets (14.4%). Similarly, dogs living in rural areas had a higher seroprevalence (39.1%) than those from urban areas (16.6%) or breeding kennels (7.1%). The chi-square test showed important differences by use ($\chi^2 = 11.03$; $p = 0.004$) and by habitat ($\chi^2 = 22.04$; $p < 10^{-n}$).

The season when samples were taken (Figure 4) also had a significant influence on the seroprevalence

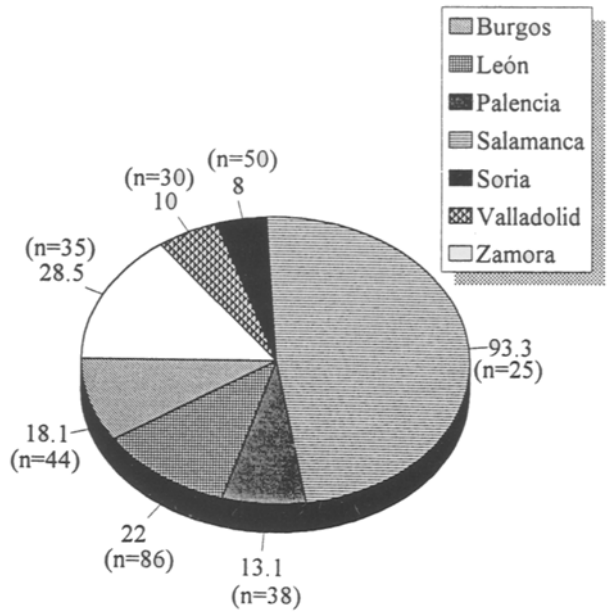


**Figure 1.** Seroprevalence to *Rickettsia conorii* in dogs in the provinces of Castilla y León, Spain.

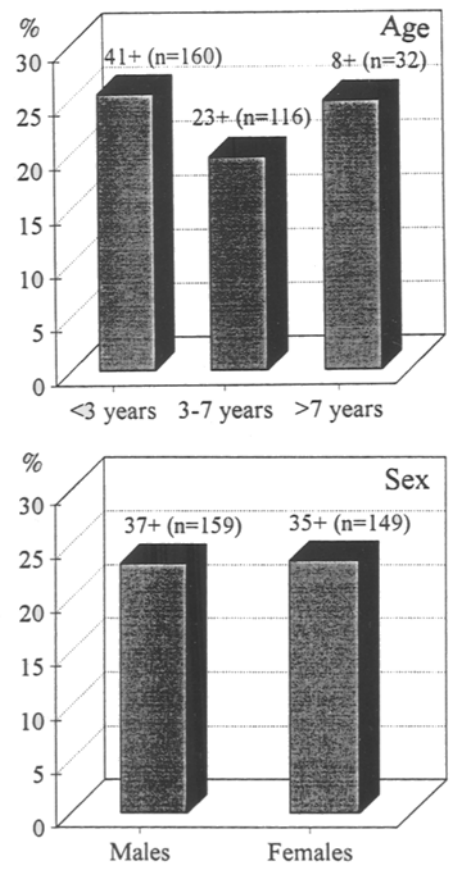


**Figure 2.** Canine seroprevalence to *Rickettsia conorii* according to the age and sex of the dogs.

noted ($\chi^2 = 33.19$; $p < 10^{-n}$), being this highest in summer (66.6%), followed by spring and winter, and lowest in autumn (13.6%).

As might be expected, the exposure to ticks was

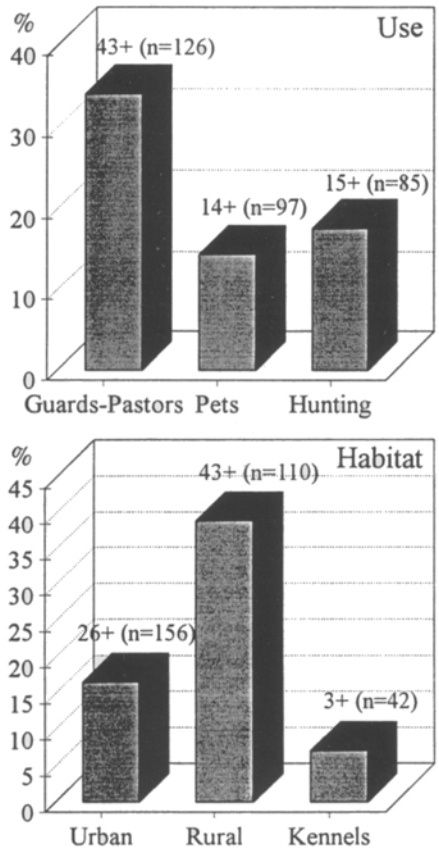


**Figure 3.** Canine seroprevalence to *Rickettsia conorii* according to the use and habitat of the dogs.

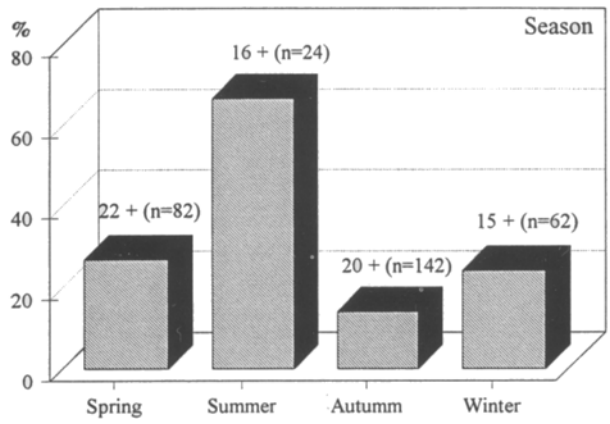


**Figure 4.** Canine seroprevalence to *Rickettsia conorii* according to the season of sampling.

a factor that also had a significant influence on prevalence. Of animals that had had ticks, 32.2% were seropositive, versus the 19.1% of seropositives among the animals not exposed to them ($\chi^2 = 5.70$; $p = 0.016$).

## Discussion

Studies carried out in Mediterranean countries have indicated seroprevalence status to *R. conorii* between 23% and 85% of analysed dogs [10, 17, 19]. Furthermore, high prevalence has been discovered in some African countries, above 80% [20].

The seroprevalence observed in dogs from Castilla y León was 23.4%, which is lower than the percentages found in other areas of Spain: 57.8% in Barcelona [13], 58.6% in the central area [10], and 75.9% in Madrid [8]. However, this seroprevalence could be related with the presence of other rickettsiae transmitted by *R. sanguineus*; until 1992, the only rickettsia known in Spain was *R. conorii*, but *R. rhipicephali* had been identified from ticks eggs in Madrid [21], and his presence in dogs may induce antibody cross-reactions with *R. conorii*.

Nevertheless, this prevalence varied a great deal from one province to another within the region. In Salamanca province 93.3% of the dogs showed antibodies, this result being almost identical to the 92.4% that was reported by Herrero-Herrero et al. [22] in a survey undertaken between 1981 and 1985. This is related to the high seroprevalence noted by these authors in the human population (73.5%) or the high rate of human disease in 1992 (10.22 per hundred thousand) [23], which means that this province must be considered as an area of high risk for MSF. In the remaining provinces studied the prevalence was below 30%, lower than the figures found in any other region of Spain. This high seroprevalence in Salamanca province could be related with the greater number of dogs infested with ticks in this province.

We noted higher seroprevalence in dogs from rural areas and those used as guard or pastor dogs, which are two factors closely related to each other; this would be explained by a greater contact between dogs and the vector in such conditions. These results correspond to what was reported by Herrero et al. [10], who pointed out that dogs dedicated to hunting activities and in contact with livestock were more frequently infested than those remaining in the house and surrounding home area.

Espejo-Arenas et al. [24], in Vallés Occidental County (Barcelona province, Spain), observed a seropositivity of 37% in a canine population which was predominantly urban. This value is much higher than what we found in urban areas of Castilla y León (18.4%).

The different prevalence by season that we observed is noteworthy, and these data are close to those reported by other authors [8, 13, 24]. There is a greater seroprevalence in summer, followed by spring, this coincides with the periods of greatest activity of *R. sanguineus*, which usually infests dogs from the beginning of spring to autumn [25]. On the other hand, this seasonal serological response could suggest a short duration of the canine immune response to *R. conorii* [13]. However, the possible presence of *R. rhipicephali* could influence the short lasting duration of the immune response [26].

Neither sex nor age is a risk factor for MSF in the

canine population studied. However, Herrero-Herrero et al. [22] noted a significantly higher prevalence in dogs aged under five years.

In summary, the authors believe that the presence of the infection in all the provinces of Castilla y León, and the high level of it in some zones should lead to an increased vigilance on the part of the authorities to ensure prevention of this zoonosis.

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