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

Silvia Delgado & Pedro Cármenes
Deparamento de Sanidad Animal (Enfermedades Infecciosas y Epidemiologia), Facultad de Veterinaria, Universidad de León, Campus de Vegazana, León, 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 different 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 epidemiologic 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, seroprevalence 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 data 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 immunofluorescence 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 ($\chi^2 = 55.78; p < 10^{-10}$).

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 ($\chi^2 = 22.04; p < 10^{-4}$).

The season when samples were taken (Figure 4) also had a significant influence on the seroprevalence noted ($\chi^2 = 33.19; p < 10^{-6}$), 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