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Lledó, L. et al. (2003) 'A sero-epidemiological study of Rickettsia typhi infection in dogs from Soria province, central Spain', *Annals of tropical medicine and parasitology*, 97(8), pp. 861–864.

Available at <https://doi.org/10.1179/000349803225002679>

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A sero-epidemiological study of *Rickettsia typhi* infection in dogs from Soria province, central Spain

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Received 15 August 2003, Accepted 4 September 2003

Data relating to *Rickettsia typhi* infection in Spain are scarce. The seroprevalence of canine infection with this species has now been investigated, in dogs from the central province of Soria. The results of indirect immunofluorescence assays indicated that nine (12.3%) of the 73 dogs checked were carrying antibodies against *R. typhi*. The gender, age and breed of the dog, and whether it was used for hunting, shepherding, guarding or simply as a pet, apparently had no significant effect on the probability of it being seropositive. Being infested with fleas or having a history of such infestation was, however, significantly associated with seropositivity. The present results confirm that dogs may be infected with *R. typhi* and indicate their potential usefulness as sentinels in epidemiological studies of the pathogen. The results of wide-scale, serological studies of dogs may allow the geographical distribution of *R. typhi* to be mapped relatively simply.

In humans, murine typhus is usually a mild febrile disease (Azad, 1990). Most cases seem to result from the transmission of the causative agent, *Rickettsia typhi*, from rickettsaemic rats (often *Rattus rattus* or *Ra. norvegicus*) to humans, by the oriental rat flea, *Xenopsylla cheopis*. The results of epidemiological studies over the last decade have indicated, however, that mammals other than rats and humans can carry the pathogen and that rat fleas are not the only vectors (Sorvillo *et al.*, 1993; Azad *et al.*, 1997). For many years there has been a suspicion that cats or dogs may not only bring infective fleas into domestic environments but also serve as adjunct reservoir hosts (Adams *et al.*, 1970). Cats are certainly susceptible to subclinical infection with *Ri. typhi*, and of producing antibodies against the pathogen (Traub *et al.*, 1978; Sorvillo

et al., 1993; Matthewman *et al.*, 1997). Canine infection with *Ri. typhi* has not been investigated in detail but dogs are sometimes infested with cat fleas (*Ctenocephalides felis*) and these fleas can act as vectors of *Ri. typhi* (Farhang-Azad *et al.*, 1984; Dryden and Rust, 1994). Dogs are clearly important in the epidemiology of the rickettsioses of the spotted-fever group (Rehacek, 1993) but their role in the ecology of other human rickettsial infections, including murine typhus, remains obscure. The main aim of the present study was to determine the seroprevalence of *Ri. typhi* among dogs from the province of Soria in central Spain.

ANIMALS AND METHODS

Dogs

The canine sera investigated had been collected in 1993–1994 from 73 dogs that had been taken to veterinarian clinics in Soria province (Fig.). The 43 male and 30



FIG. 1. A sketch of Spain, showing the provincial boundaries and the location of Soria province and the city of Soria.

female dogs, all of which were privately owned by people living in the province, were aged 1–14 years — 22, 36 and 15 were < 4, 4–7 and > 7 years of age, respectively. Most were used for hunting (35) or as pets (29) but six were watch dogs and three sheep dogs. As numerous breeds were represented the dogs were simply classified, for the present study, as belonging to large (18), medium-sized (46) or small (nine) breeds. Six of the dogs were wire-haired, the rest having long (11), medium-length, (18) or short (28) fur. Many of the dogs lived in close contact with other animals — 32 with others dogs, two with cats, three with sheep, one with rabbits and three with doves and hens. The owners of 25 of the dogs reported that their dogs were or had been infested with fleas.

Blood was obtained, from the anaesthetized dogs, by cephalic or jugular venipuncture, and allowed to clot. Serum was isolated by centrifugation and stored at -20°C until it could be checked for anti-*Ri. typhi* antibodies by IFAT (see below).

Permission to study the local dogs was obtained from the regional government, in compliance with the relevant regulations, and the serological survey was carried out

according to the ethical guidelines of the Alcalá de Henares University's Committee on Animal Experimentation.

Serology

Sera were tested in IFAT (Phillip *et al.*, 1976) in which Vero E6 cells (ATCC1586) infected with the Wilmington strain of *Ri. typhi* served as the antigens. Serial, two-fold dilutions of each serum were added to the antigen spots and incubated in a humid chamber for 30 min at 37°C . After washing, fluorescein-conjugated, rabbit, anti-dog immunoglobulins (Sigma) were added to each spot. After another 30-min incubation and washing, the spots were examined under a fluorescence microscope at $\times 400$. Positive and negative control sera were included with each test. Based on the results of previous studies (Suksawat *et al.*, 2001), each serum giving an IFAT titre of at least 1:64 was considered positive for antibodies against *Ri. typhi*.

Data Analysis

The prevalences (%) recorded were compared in two-way tables, using χ^2 or Fisher's exact tests.

RESULTS

Nine (12.3%) of the 73 dogs investigated were found seropositive for *Ri. typhi*: five, two, one and one at titres of 1:64, 1:128, 1:256 and 1:512, respectively. Seroprevalence among the 40 urban dogs (12.5%) was similar to that among the 33 rural dogs (12.1%; $P > 0.05$). Six (17.1%) of the hunting dogs, three (10.3%) of the pet dogs but none of the sheep or watch dogs were seropositive ($P > 0.05$). The positive dogs were six (21.4%) of those with short fur, one (5.5%) of those with medium-length fur, one (9.0%) of those with long fur, and one (16.6%) of the wire-haired dogs ($P > 0.05$). Although seven (16.2%) of the male dogs investigated but only two (6.6%) of the bitches were seropositive, this difference was not statistically significant ($P > 0.05$). The seroprevalence recorded among the older dogs (aged > 7 years), 20.0%, was higher but not significantly higher ($P > 0.05$) than that seen among the dogs aged < 4 years (4.5%) or 4–7 years (13.8%). The medium-sized dogs were more likely to be found seropositive (15.2%) than the larger (5.5%) or smaller (11.1%) and the seroprevalence among the dogs that lived with other animals (12.1%) was lower than the overall seroprevalence, but again these differences were not statistically significant ($P > 0.05$).

The only statistically significant association observed was that between seropositivity and flea infestation, the seroprevalence among the 25 dogs reported as having been infested with fleas being markedly higher than that among the other 48 dogs (28.0% *v.* 4.1%; $P < 0.05$).

DISCUSSION

Although based on a relatively small sample of dogs, the seroprevalence of *Ri. typhi* observed in the present, Spanish study (12.3%) was markedly lower than those recorded in dogs from Portugal (26.9%;

Bacellar *et al.*, 1995) or Malaysia (35%; Huxsoll *et al.*, 1977) but higher than the 0.4% recorded in Egypt (Soliman *et al.*, 1989).

A progressive increase in seroprevalence with age might be expected but neither in Spain (present study) nor in Thailand (Suksawat *et al.*, 2001) was seropositivity associated with the age, gender or size of the dog. In both Spain (present study) and Malaysia (Huxsoll *et al.*, 1977), dogs from rural environments were no more and no less likely to be seropositive than urban dogs.

Although statistically insignificant because of the small sample size, the observation that hunting dogs were more likely to be seropositive than sheep dogs, watch dogs or pet dogs may reflect differences in exposure to fleas. As hunting dogs cover large distances while working, and are often carried to many different localities by their owners, they are presumably more likely to come into contact with a flea carrying *Ri. typhi* than dogs that are more confined. Sheep dogs may be as exposed to infected fleas as hunting dogs; only three sheep dogs were investigated in the present study. As pet dogs spend much of their time in houses and close contact with humans, the observation that $> 10\%$ of the pet dogs investigated were seropositive is perhaps the most significant result in terms of the epidemiology of murine typhus. As *Ri. typhi* has flea vectors, the observed association between a history of flea infestation and seropositivity was to be expected.

In summary, the present results confirm the presence of canine infection with *Ri. typhi* in Spain. Dogs, their fleas and perhaps other peridomestic animals may play an important role in the survival of typhus-group rickettsiae and their transmission to humans. As collection of sera from dogs is, in general, considerably easier than collecting sera from rodents, dogs could play important roles, as sentinel animals, in determining the areas where there is a risk of murine typhus.

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