Spotted fever group Rickettsia in brown dog ticks Rhipicephalus sanguineus in southwestern Spain

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Abstract A total of 2,229 adults ticks (1,428 males and 801 females) belonging to the brown dog tick, Rhipicephalus sanguineus Latreille, 1806, collected from dogs in Seville province (Andalusia), distributed in 500 lots ranging from one to eight specimens per lot, were examined for the presence of rickettsiae by molecular techniques. Specific rickettsiae DNA were detected in 90 lots (18%) of ticks tested. Sequence analysis of amplicons revealed that R. sanguineus ticks were infected exclusively with Rickettsia massiliae (including the strain Bar-29). The results of this study extend the knowledge of the geographic distribution and prevalence of these spotted fever group (SFG) rickettsiae and indicate that at least two of them, with yet uncertain pathogenicity to humans, are present in brown dog ticks in south western Spain. Although Mediterranean spotted fever (MSF) is an endemic disease in Andalusia, Rickettsia conorii was not found, whereas R. massiliae, recently described as a pathogenic species, was highly prevalent in this area. Our data suggest that in Andalusia a number of MSF or MSF-like cases attributed to R. conorii could have been actually caused by other SFG rickettsia present in R. sanguineus, particularly, R. massiliae.

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Introduction

In the Mediterranean area, some spotted fever group (SFG) rickettsiae have been implicated in human disease and are therefore defined as pathogenic species (Parola et al. 2005; Brouqui et al. 2007). These rickettsiae include Rickettsia conorii, the causal agent of Mediterranean spotted fever (MSF), comprising a variety of genospecies of R. conorii (Zhu et al. 2005), and several other tick-borne species of SFG that are considered scarcely or not pathogenic to humans, as Rickettsia massiliae (Beati and Raoult 1993; Vitale et al. 2006).

The epidemiology of rickettsiae and rickettsial diseases in Andalusia (south Iberian Peninsula) is not well known. Seroepidemiological studies have revealed the presence of SFG rickettsiae (R. conorii, Rickettsia felis, and R. massiliae) as well as Rickettsia typhi in western Andalusia (Bernabeu-Wittel et al. 2006a, b). The purpose of this study was to investigate, identify, and characterize SFG rickett-

siae in brown dog ticks collected from dogs in Seville province. The particular feature of brown dog tick enables it to complete its entire life cycle indoors. Because of this, this tick can establish populations in colder climates and has been found in extensive areas of the world. Many tick species can be carried indoors by their animal hosts but cannot complete their entire life cycle inside. Although Rhipicephalus sanguineus feed on a wide variety of mammals, dogs are the preferred hosts in Andalusia and appear to be required to develop large infestations Millán et al. (2007). The transovarial and transtadial transmission of SFG rickettsiae within tick vectors in nature ensures rickettsial survival and determines their limited distribution to that of their tick vectors (Raoult and Roux 1997; Azad and Beard 1998; Parola et al. 2005).

Prevalence studies of infection in the tick vector can be used as an indicator of possible changes in the intensity of Rickettsia spp. transmission. However, these studies are difficult to carry out, as prevalence in vectors is usually low, and its estimation requires large number of ticks to be dissected.

Material and methods

Sampling

A total of 2,229 dog brown ticks (1,428 males and 801 females) were collected from 500 domestic dogs in urban and suburban areas from 89 different areas in Seville Province (western Andalusia, Spain) between April 1999 to March 2005. After collection, the ticks were immediately placed in vials with 70% ethanol, were properly labeled, and were later identified in the laboratory by species, gender, and stage by a professional entomologist using specific taxonomic keys (Walker et al. 2000).

Methods

Ticks were rinsed with distilled water, dried on sterile filter paper, and then crushed in sterile Eppendorf tubes. DNA was extracted from 500 pools of adult ticks (from one to eight specimens per pool, average of 4.46 ticks per pool) using the Macherey–Nagel DNA tissue Kit (Düren, Germany) according to the manufacturer's instructions. The efficiency of DNA extraction was verified in all samples by polymerase chain reaction (PCR) assay, which amplifies 12S ribosomal RNA (rRNA) of tick origin (Zahler et al. 1997; Bernasconi et al. 2002) as well as in the cases that doubt persisted in discrimination between R. sanguineus and Rhipicephalus turanicus. Discrimination was made using an amplification of a fragment of mitochondrial 12S rRNA using the oligonucleotides T1B and T2A (Beati and Keirans 2001) and amplification conditions described elsewhere (Bernasconi et al. 2002). Negative controls consisted of distilled water extracted in the same laboratory. Specific rickettsial sequences were detected by using PCR primers that amplify a portion of glta, ompA, ompB, and 16S rRNA genes, respectively Márquez et al. (1998). Subsequent direct sequencing of amplified products was performed on selected samples in order to provide an objective and precise identification, using specific PCR primers and the Genome DTCS-quick Start kit (Beckman Coulter, Fullerton, CA, USA) in a capillary DNA sequencer CEQ 2000XL (Beckman Coulter).

Results and discussion

Overall, rickettsial DNA was detected in 90 (18%) of the examined tick pools by PCR, amplifying at least two rickettsiae-specific fragments. Six of positive samples only amplified glta and ompA fragments. The alignment of ompB sequences obtained from the remaining 84 positive pools detected, in all the cases, two variants of R. massiliae (Beati and Raoult 1993). From a geographic point of view, positive samples were dispersed in Seville province and did not show a specific geographic distribution pattern (Fig. 1). Pools with infected ticks appeared in 49 of examined areas (55.06%) with values ranging from 4% to 100% of processed samples.

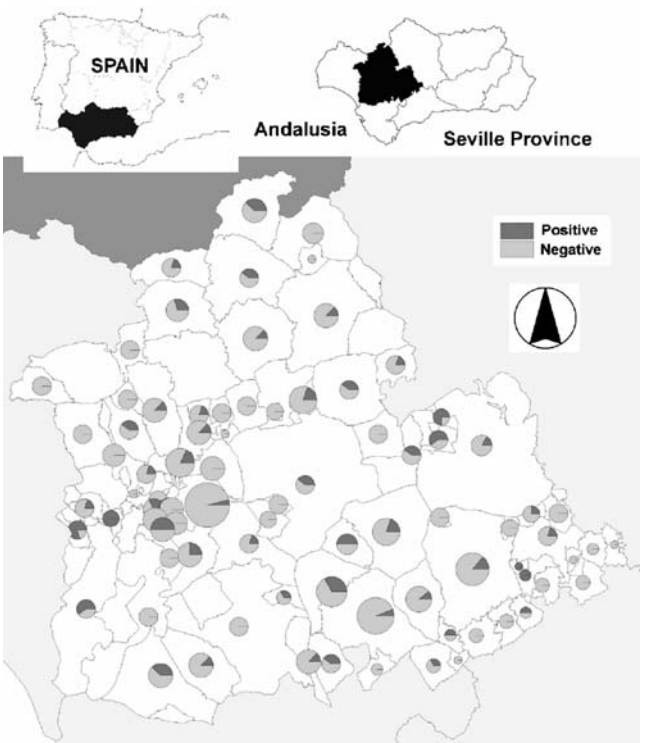

Fig. 1 Geographical distribution of R. massiliae positive ticks in Seville province. Diameter of circles is proportional of number of samples studied in each area

Mediterranean spotted fever is well known to be endemic in the whole Iberian Peninsula (Herrero-Herrero et al. 1989; Cardeñosa et al. 2003, 2006; Bartolomé et al. 2005; Guerrero et al. 2006) and particularly western Andalusia (Bernabeu-Wittel et al. 2006a, b). Nevertheless, to our knowledge, no studies evaluating the presence of Rickettsia spp. in ticks from domestic dog has been made yet in Andalusia.

The most striking finding of our study was the exclusive detection of R. massiliae (including Bar-29 strain), together with the absence of R. conorii. R. massiliae has been detected in R. sanguineus and/or R. turanicus in France (Beati and Raoult 1993), Portugal (Bacellar 1999), Spain (Beati et al. 1996; Merino et al. 2005), Switzerland (Bernasconi et al. 2002), Greece (Psaroulaki et al. 2006), and Algeria (Bitam et al. 2006) and recently was signaled in the US (Eremeeva et al. 2006). Our results are in accordance with those obtained by Merino et al. (2005) and Fernández-Soto et al. (2006a). They studied 4,049 ticks removed from asymptomatic patients in Castilla–León (north western Spain) belonging to 14 ixodid and one argasid species; R. conorii was detected exclusively in a single specimen of R. sanguineus over a sample of 132 R. sanguineus and 388 R. turanicus. In contrast, R. massiliae was detected in 37 R. turanicus, six R. sanguineus, one Rhipicephalus pusillus, and 4 of 1,799 Ixodes ricinus Fernández-Soto et al. (2006a, b). In Portugal, Bacellar (1999) found a prevalence of SFG rickettsiae of 1.8% among 2,207 R. sanguineus tested for the presence of rickettsiae, from vegetation, dogs, and sylvatic mammals. Of 25 Rickettsia spp. Isolates, 22 were identified as R. massiliae and three corresponded to R. conorii.

In Seville province, the absence of R. conorii in R. sanguineus ticks contrasts with the elevated seroprevalence (8.7% globally) found recently in humans by Bernabeu-Wittel et al. (2006a, b). The prevalence of R. massiliae-BAR29 past infections in the same population was 3.4% (Bernabeu-Wittel et al. 2006b). In natural conditions, R. massiliae could be transmitted by several species of R. sanguineus group (Matsumoto et al. 2005a, b). R. sanguineus is well adapted to human rural and urban environments, with an elevated specificity for canine hosts, mainly dogs. In usual conditions, despite the high prevalence of R. massiliae detected in the studied R. sanguineus, the risk of transmission to humans associated to the bite of this tick is low; in fact, these ticks feed on humans only when domestic or sylvatic canids are not available (or protected with tick repellents; Brouqui et al. 2007). Recently, R. massiliae has been confirmed as human pathogen, causing a disease that mimics entirely the clinical and biological features of MSF (Vitale et al. 2006). Hence, the exact etiologic agent of an unknown number of MSF or MSF-like cases, as well as the detected past human infections in our area, remains unclear. In this sense, future clinical studies specifically focused in the molecular detection of MSF etiology are needed to definitively answer this issue.

The above mentioned seroepidemiological study carried out recently by Bernabeu-Wittel et al. (2006a, b) in Andalusia indicated the possible existence of several SFG rickettsiae transmitted by ticks (R. conorii and R. massiliae sensu lato) or by flea (R. felis). The prevalence of past infections due to of R. massiliae-Bar29 and R. conorii in this region was highest in rural areas (about 10–11%) with respect to suburban and urban areas, in oldest population segments (because of prolonged–repeated exposure time with respect to younger populations), in people with high-risk professions, and in those with close contact with various animal species (Bernabeu-Wittel et al. 2006b). These findings clearly reflect the close correlation between SFG rickettsiae past infections and the intensity of host–vector exposure.

In conclusion, our data show the presence of high R. massiliae infection rates in brown ticks in south western Spain and a total absence of R. conorii. This, together with clinical and recent human seroprevalence studies carried out, raises the question of the real etiologic agent of an unknown number of MSF or MSF-like cases that clinicians attend frequently in this area.

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