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Original article

Species of ticks and carried pathogens in owned dogs in Spain: Results of a one-year national survey

Agustín Estrada-Peña[a,*], Xavier Roura[b], Angel Sainz[c], Guadalupe Miró[d], Laia Solano-Gallego[e]

[a] Department of Animal Health, Faculty of Veterinary Medicine, Zaragoza, Spain
[b] Hospital Clínic Veterinari, Universitat Autònoma de Barcelona, Barcelona, Spain
[c] Department of Animal Medicine and Surgery, Veterinary Clinic Hospital, Faculty of Veterinary Medicine, Universidad Complutense de Madrid, Madrid, Spain
[d] Department of Animal Health, Veterinary Clinic Hospital, Faculty of Veterinary Medicine, Universidad Complutense de Madrid, Madrid, Spain
[e] Department of Animal Medicine and Surgery, Faculty of Veterinary Medicine, Universitat Autònoma de Barcelona, Barcelona, Spain

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ABSTRACT

This study presents the results of a national survey in Spain on the distribution of ticks on owned dogs, their phenology and the associated pathogens over one year. In the study, 1628 adult ticks were collected on 660 dogs presented to 26 veterinary practices, of which 507 dogs (76.8%) carried at least one adult tick. The primary species of ticks were *Rhipicephalus sanguineus* s.l. (53%), *Dermacentor reticulatus* (9%), *Ixodes ricinus* (9%), and *I. hexagonus* (4%). Parasitism by two species of these ticks was rare. The four species showed a clear association with the biogeographical features of the country, with *I. ricinus* associated with the wettest northern regions, *I. hexagonus* and *D. reticulatus* associated with the north of the territory, and *R. sanguineus* s.l. prevalent throughout the entire country. Dogs living in rural areas had a higher prevalence of all species, but *R. sanguineus* s.l. was the most prevalent; however, this tick was also more common on dogs living indoors. *R. sanguineus* s.l. adults were active throughout the year, with a maximum peak from March to July. The other tick species were collected throughout the year, with an autumn-winter peak of *D. reticulatus*, but without clear seasonality for either *I. ricinus* or *I. hexagonus*. Combined real-time PCR and conventional PCR of the feeding ticks recorded Piroplasmida (*Hepatozoon canis*, *Babesia canis*, *B. gibsoni*, and several sequences compatible with *Theileria* spp.), *Rickettsia* spp. (*R. massiliae*, *R. sibirica mongolitimonae*, *R. monacensis*), *Ehrlichia canis*, *Anaplasma platys*, *A. phagocytophilum* and *Borrelia* spp. (*B. afzelii*, *B. garinii*, *B. valaisiana*). *Hepatozoon canis* and *B. canis* were the most frequently detected pathogens, with variable rates of infection according to the region. Other than a close association of *Borrelia* spp. with *I. ricinus* (and therefore to the wet northern areas of the territory), the other tick-borne pathogens were recorded throughout the country. Although a potential transmission role for ticks carrying unusual pathogens cannot be attributed to these results, these findings introduce a change of paradigm on the tick-borne pathogen distribution in Spain and emphasize the importance of performing active surveys to understand the complex patterns of tick-borne pathogen distributions and their vectors.

1. Introduction

Canine tick-borne diseases are a group of infections that are globally distributed and are rapidly spreading, and therefore, the awareness is increasing that these diseases are an important threat to animal health (Otranto et al., 2009a). Dogs are hosts of several species of ticks of which *Ixodes ricinus*, *Ixodes hexagonus*, *Dermacentor reticulatus* and *Rhipicephalus sanguineus* s.l. are most likely the most prominent in western Europe (Petney et al., 2012). Other than the concerns about human and animal health because of tick-transmitted pathogens, there is obvious interest in determining the eco-epidemiological patterns of their transmission cycles, the areas in which ticks develop permanent populations, and how these patterns change through time. A baseline of data regarding which species of ticks and pathogens are circulating in a territory is an essential requirement, both as basic epidemiological background and as support for practitioners involved in the diagnosis of these diseases.

* Corresponding author.
E-mail address: aestrada@unizar.es (A. Estrada-Peña).

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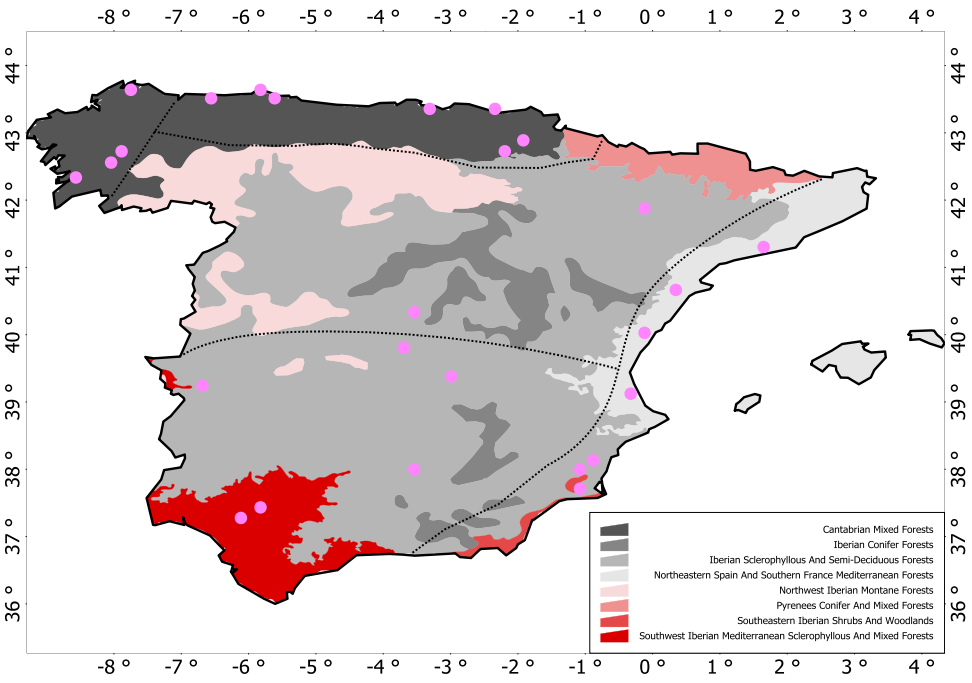

Fig. 1. Official definition and distribution of biogeographical regions in Spain, with the location of the veterinary clinics enrolled in this study (dots).

Table 1
Pathogens detected by Real-Time PCR, target region, length of sequences, and reverse and forward primers used for amplification.

Pathogen(s)	Target region	Seq. length	Reverse primers	Forward primers
Piroplasmida (general)	*18S rRNA*	114	GACGATCAGATACCGTCGTAGTCC	CAGAACCCAAAGAACTTTGATTTCTCTC
Ehrlichia spp./*Anaplasma* spp.	*16S rRNA*	146	GCAAGCYTAACACATGCAAGTCG	GGATTATACAGTATTACCCAYCATTTCTARTG
Rickettsia spp.	*ITS2*	300	GCTCGATTGRTTTACTTTGCTGTGAG	CATGCTATAACCACCAAGCTAGCAATAC
Borrelia burgdorferi s.l.	*16S rRNA*	192	GCGAGTTCGCCGGGAGAGTA	TGGTCAAAGTAATAAG
Hepatozoon canis	*18S rRNA*	373	CCAGCAGCCCGGGTAATTC	AGATTTGTTAAAGACAAACACTGCGAAAG

The increasing prevalence of tick-transmitted pathogens in owned dogs appears to be associated with increased accessibility to traditional environments in which the contact with wild host species is common (Otranto et al., 2009b). Wild animals including foxes (Zimen, 2013), roe deer (Jaenson et al., 2012) and wild boar (Massei et al., 2015) now have closer contact with owned dogs as a consequence of the increase in their populations. Furthermore, the increased availability and rapid evolution of molecular-based techniques, together with increased awareness, have produced a clear resurgence in interest towards the ticks affecting pets (Stich et al., 2014).

Nationwide surveys have been conducted in some European countries (i.e., Abdullah et al., 2016; Claerebout et al., 2013; Földvari and Farkas, 2005; Jaenson et al., 1994, 2012; Jameson and Medlock, 2009, 2011; Nijhof et al., 2007; Papadopoulos et al., 1996; Smith et al., 2011). Meta-analyses on published information have produced georeferenced data sets of the reported distributions of ticks (Estrada-Peña et al., 2013a, 2016; Rubel et al., 2016). These surveys produce a preliminary baseline of data to compare further results with the aim to check the progression of permanent populations or determine the association of ecological factors with strains of pathogens. For example, the spread of *D. reticulatus*, which is one of the most important tick species affecting carnivores in Europe and the primary vector of *Babesia canis* (Pantchev et al., 2015), has been notable in several European countries (Mierzejewska et al., 2015). In the 1950's, Hungary reported *D. reticulatus* in two isolated areas (Janisch, 1959), but the tick is currently the second most common tick species in the country (Sréter et al., 2005; Széll et al., 2006). *Dermacentor reticulatus* was unreported in active surveys until 1975 in Austria (Hinaidy, 1976), but the populations of this tick have now expanded their historical limits (Rubel et al., 2016). The same phenomenon is recorded in Germany (Dautel et al., 2006; Heile et al., 2006), Poland (Mierzejewska et al., 2015; Szymanski, 1986; Zahler et al., 2000), Slovakia (Bullová et al., 2009), The Netherlands (Nijhof et al., 2007) and the United Kingdom (Abdullah et al., 2016; Medlock et al., 2011). Similar findings about the recent spread of ticks are also reported for *I. ricinus* (Medlock et al., 2013) and for *R. sanguineus* s.l. (Gray et al., 2014) for which different traits act in combination to promote changes in the pattern of distribution (Gray et al., 2009).

The patterns of tick-borne diseases are not well understood because of the central problem that systematic surveillance of animals is not routine (Abdullah et al., 2016). Currently, no formal tick surveillance program exists in Spain, although limited contemporary data on the distribution of ticks and the prevalence rates of transmitted pathogens have resulted from local surveys (i.e., Barral et al., 2002; Castellà et al., 2001; Encinas, 1985; Estrada-Peña, 2001; Estrada-Peña et al., 1991, 2004; Márquez, 2008). These studies help to fill the information gaps at a regional level, but systematic surveillance remains essential to provide a complete overview of the ranges of ticks at a countrywide scale. With the increased significance of tick-borne diseases in Spanish veterinary practices, the data collected on ticks and pathogens seasonally and surveyed over large territories are a fundamental aspect of the control of ticks on pets, in addition to providing a valuable source of information to prepare a rational control approach.

The aim of this study was to conduct a national survey of ticks feeding on owned dogs presented to veterinary practices in Spain to provide a comprehensive understanding of their distribution.