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The distribution of ticks (Acari: Ixodidae) of domestic livestock in Portugal

AGUSTÍN ESTRADA-PEÑA[1,*] and MARIA MARGARIDA SANTOS-SILVA[2]
[1]*Department of Parasitology, Veterinary Faculty, Miguel Servet, 177, 50013 Zaragoza, Spain;* [2]*Centre for Vector and Infectious Diseases Research, National Institute of Health, Av. Liberdade, 5, 2965-575 Aguas de Moura, Portugal; *Author for correspondence (e-mail: aestrada@unizar.es)*

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Abstract. This paper introduces the first countrywide faunistic study of the tick parasites on ruminants in Portugal. The aim of this study was to map accurately the distribution of the ticks *Dermacentor marginatus*, *Rhipicephalus (Boophilus) annulatus, R. bursa*, *Hyalomma m. marginatum*, *H. lusitanicum* and *Ixodes ricinus* in Portugal. Additional information about the abiotic preferences of these species has been obtained through the use of abiotic (temperature- and vegetation-derived) variables have been recorded from remotely sensed information at a nominal resolution of 1.1 km^2. A further aim was the development of predictive models of distribution using Classification and Regression Trees (CART) methodologies. Four species (*R. annulatus*, *R. bursa*, *D. marginatus* and *H. m. marginatum*) are mostly restricted to south-eastern parts of the country, under hot and dry climate conditions of Mediterranean type. *H. lusitanicum* has been collected almost only in the southern half of Portugal. *I. ricinus* has a very patchy distribution and is mainly associated with vegetation of *Quercus* spp., found in southern zones of the country, but it is present also in the more humid western part. A variable number of abiotic variables, mainly temperature derived, are able to describe the preferences of the tick species. It is remarkable that variables derived from maximum values of the Normalized Derived Vegetation Index (yearly or summer-derived) only apply to discriminate areas where *I. ricinus* has been collected. CART models are able to map the distribution of these ticks with accuracy ranging within 75.3 and 96.4% of actual positive sites.

Introduction

In recent years research about the effects of climatic factors affecting the interactions between vectors, hosts and pathogens had increased, mainly because the medical importance and economic losses associated with vector-borne diseases. In this sense, studies have been conducted to understand the tick species composition, ecosystem peculiarities and host–vector–pathogen relationships in different countries. Faunistic studies concerning ticks in the Mediterranean region are of interest to evaluate the distribution and composition of species affecting livestock, as a previous step in the knowledge of the pathogens they may transmit and the economic effects of these on animal production and public health (Yousfi-Monod and Aeschlimann 1986; Papadopoulos et al. 1996; Bouattour et al. 1999; Aktas et al. 2004).

Portugal is the most western country of continental Europe. It presents a climate differentiated in three types: Mediterranean in the southern part of the country; Oceanic along the littoral and northern and Continental in some inlands of the northeast and central east region of the territory. Animal farming is the most common activity associated to the agricultural system in Portugal. In recent years the cattle number of *Bos taurus* breeds were about 2,112,181, 5,358,702 *Ovis aries* and 993,672 *Capra hircus*, most of them located in the southern half part of the country (INE 1999). Most of the cattle are reared using a traditional management system characterized by extensive grazing on natural pastures, which is favourable to maintenance of tick populations. Other silvatic animals like deer, wild boars, foxes, rabbits, rodents and wild birds are widespread and are also used as hosts for these arthropods (Caeiro 1992; Dias 1994; Silva et al. 2001).

The presence of ticks on domestic animals in Portugal is documented (Caeiro 1999). However, a countrywide study of the distribution, together with climate and vegetation preferences of the main species affecting livestock is lacking. To understand the abiotic preferences of the most important tick species and to establish a better approach to the expected eco-climate limits of ticks and tick-borne pathogens, the use of statistical methods has been applied to other European countries (Estrada-Peña 1999; Randolph 2000; Rogers and Randolph 2000). This study is intended to map the distribution of the most prominent tick species affecting domestic livestock in Portugal, using preliminary surveys in the country, with an overview of the abiotic preferences and the association between species. Statistical analyses have been performed to recognize the main discriminatory climate variables responsible of the observed distribution of these tick species. These variables have been used to build Classification and Regression Tree (CART) models.

Materials and methods

This study has been performed as a compilation of data from collections of ticks, originally carried out in 1988–1998 and referred to the locality of capture. Some 97% of tick specimens were obtained from captures on hosts, while a few were obtained by standard dragging. Original collections were done systematically in neither space nor time. Surveys used in this study were made under very different conditions and with different purposes, done on a presence/absence criterion and without sampling a statistically significant number of hosts. Although several tick species from different groups of hosts are represented in these collections, this study is restricted to the adult stages of ticks collected on ruminants because they represent the most accurate set of captures. These collections were coded as present/absent for the smallest administrative divisions (ADs) available in Portugal (the 'freguesia') and used to map the known distribution of each tick. No attempts were done to evaluate the abundance of the ticks involved, as collections were done with a presence/

absence criterion, without information about the abundance of every tick species and stages. Therefore, total tick numbers as produced for this study cannot be included. Anyway, a total of 912 collections, representing the ticks included in this study, were evaluated.

The main purpose of this study, other than provide a map of distribution of the ticks at the highest administrative resolution available, has been to obtain the preferences of abiotic (climate) and vegetation variables for each tick species. This was done using remotely sensed information, from the NOAA-AVHRR series of satellites, at a nominal 1.1 km^2. A set of monthly images over Portugal was obtained for the period 1995–1998. Table 1 lists the name and the abbreviation of the variable of temperature and vegetation as used throughout this paper, obtained from this set of remotely sensed information. The Normalized Derived Vegetation Index (NDVI) is an indicator of vegetation stress, indirectly related to the rainfall and relative humidity. Because the difficulty to obtain accurate rainfall or humidity estimates at the scale of work, it was preferred to use this surrogate of ground humidity, as indicator of the preferences of ticks. No attempts were done to use the type of vegetation as indicator of tick presence/absence, as captures are referred to AD. Because it was impossible to accurately map the site of capture of the tick in the original surveys, and because each AD may have several types of dominant vegetation, this feature cannot help in the evaluation of the habitat. Anyway, coarse indications about dominant vegetation features of each AD were the tick is present are included in the results below.

To obtain the variables discriminating the habitat of each tick species, an analysis of variance (ANOVA) has been performed using the presence/absence data for each AD as dependent variable, with the climate and vegetation variables obtained for each AD as independent ones. The range of preferences of climate and vegetation features has been determined by the 95% range of the mean in the ANOVA analysis on the different variables used to separate sites where the species have been found. Other statistical test performed was the χ^2 test on contingency tables to know the association between species. Using data about presence/absence, χ^2 test has been performed to know what pairs of species have been significantly collected together at the level of AD.

CART models were built up and used to predict the countrywide distribution of the species involved this study, using the set of climate and vegetation variables listed in Table 1. CART models are a category of tree-based classification models with bootstrap aggregation known as bagging (Furlanello et al. 1997). These procedures allow the improvement of tree-based models, in which sets of variables are combined to obtain the greater accuracy of classification. The bagging reduces the model error in classifiers with high variance, i.e., when using climate variables for a whole country or wide territory. These procedures have been used herein with the whole set of variables to produce models of distribution (presence/absence) of the tick species involved for the whole territory of Portugal. Models were validated independently for each tick species adopting the methodology outlined by Rizzoli et al. (2002). The original

Table 1. Variables used in this study as obtained from remotely sensed imagery, their abbreviations, and the significance in discriminating positive and negative sites for the species of ticks involved in the current study.

Variable	Abbreviation	*R. bursa*	*I. ricinus*	*R. annulatus*	*H. lusitanicum*	*H. marginatum*	*D. marginatus*
Yearly mean temperature	MeanT	< **0.001**	< **0.001**	< **0.001**	< **0.001**	< **0.001**	< **0.001**
Mean of monthly maximum temperatures	MeanTMax	< **0.001**	< **0.001**	< **0.001**	< **0.001**	< **0.001**	< **0.001**
Absolute maximum temperature	MaxTMax	0.159	0.122	0.413	0.221	0.468	0.761
Maximum of monthly mean temperatures	MaxTMean	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	< **0.001**
Yearly mean NDVI values	VMean	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	< **0.001**
Mean of monthly maximum NDVI values	MeanVMax	**0.001**	**0.001**	**0.016**	< **0.001**	**0.002**	< **0.001**
Absolute maximum NDVI	MaxVMax	0.122	**0.024**	0.318	0.502	0.309	0.441
Maximum of monthly mean NDVI	MaxVMean	0.202	**0.005**	0.207	0.306	0.277	< **0.001**
Mean temperature in summer	TMean-Sum	0.173	< **0.001**	< **0.001**	< **0.001**	< **0.001**	< **0.001**
Maximum of monthly temperatures in summer	MaxTMean-Sum	< **0.001**	< **0.001**	< **0.001**	< **0.001**	< **0.001**	< **0.001**
Mean of monthly maximum temperatures in summer	MeanTMax-Sum	0.452	0.412	0.514	0.231	0.446	0.641
Maximum of monthly temperatures in winter	MaxTMean-Win	0.841	0.615	0.488	0.357	0.348	0.312
Absolute maximum temperature in winter	MaxTMax-Win	0.512	0.221	0.238	0.349	0.444	0.199
Mean of minimum temperatures in winter	MeanTMin-Win	< **0.001**	< **0.001**	< **0.001**	< **0.001**	< **0.001**	< **0.001**
Mean NDVI in summer	VMean-Sum	0.268	0.315	0.465	0.544	0.411	0.512
Maximum of monthly NDVI in summer	MaxVMean-Sum	0.315	**0.044**	0.278	0.199	0.201	0.206
Absolute maximum NDVI in summer	MaxVMax-Sum	0.441	**0.016**	0.266	0.203	0.155	0.801

dataset was split randomly into one training and one evaluation sets, containing approximately 60 and 40% of the total cases, respectively. The training dataset was used to generate the model that was then tested with the independent evaluation dataset. Variables were combined and the arrangement producing the best discriminating response for each tick species was used to build the final model for that species.

Results

Figure 1 shows the main biophysical variables of Portugal. Figure 1a displays the altitude of the country, being the maximum heights (around 1800–2000 m) located around north and central parts. Mean temperatures (Figure 1b) are low in north as a consequence of elevation, while maximum average temperatures are recorded in southern portions of the country. Inversely, highest average NDVI values were recorded in western parts of the territory, being low in northeast and southern portions of the country (Figure 1c).

Six tick species were collected over the study period from cattle, sheep and goats namely *Dermacentor marginatus* (30% of the total AD), *Rhipicephalus (Boophilus) annulatus* (15%), *Rhipicephalus bursa* (22%), *Hyalomma m. marginatum* (19%), *H. lusitanicum* (27%), *Ixodes ricinus* (23%) together with specimens of the *R. sanguineus* group (63%). Because the difficulty to establish the actual specific status of the samples in the *sanguineus* group, no further analysis were done with these specimens. *Haemaphysalis punctata* has been also collected in some points of the country, but its scarcity precludes further analysis about distribution and ecological preferences. Distribution

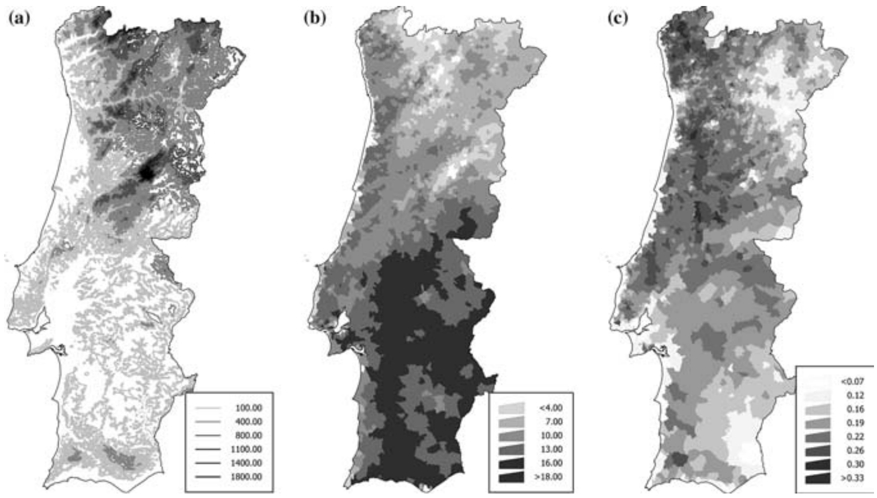

Figure 1. Summary of biophysical attributes of Portugal. (a) Altitude in meters; (b) mean yearly temperature in ° C; (c) mean NDVI yearly values (unit less).

maps for these species, together with the predictive output obtained from CART analysis for each species (see below), are included in Figure 2. Four species are mostly restricted to south-eastern parts of the countries. These are *R. annulatus*, *R. bursa*, *D. marginatus* and *H. m. marginatum*. In these areas, isolated patches of *Quercus* spp., together with natural pasture constitute the predominant vegetation. However, both *D. marginatus* and *R. bursa* are also found in some points of central and northern parts of the country in colder

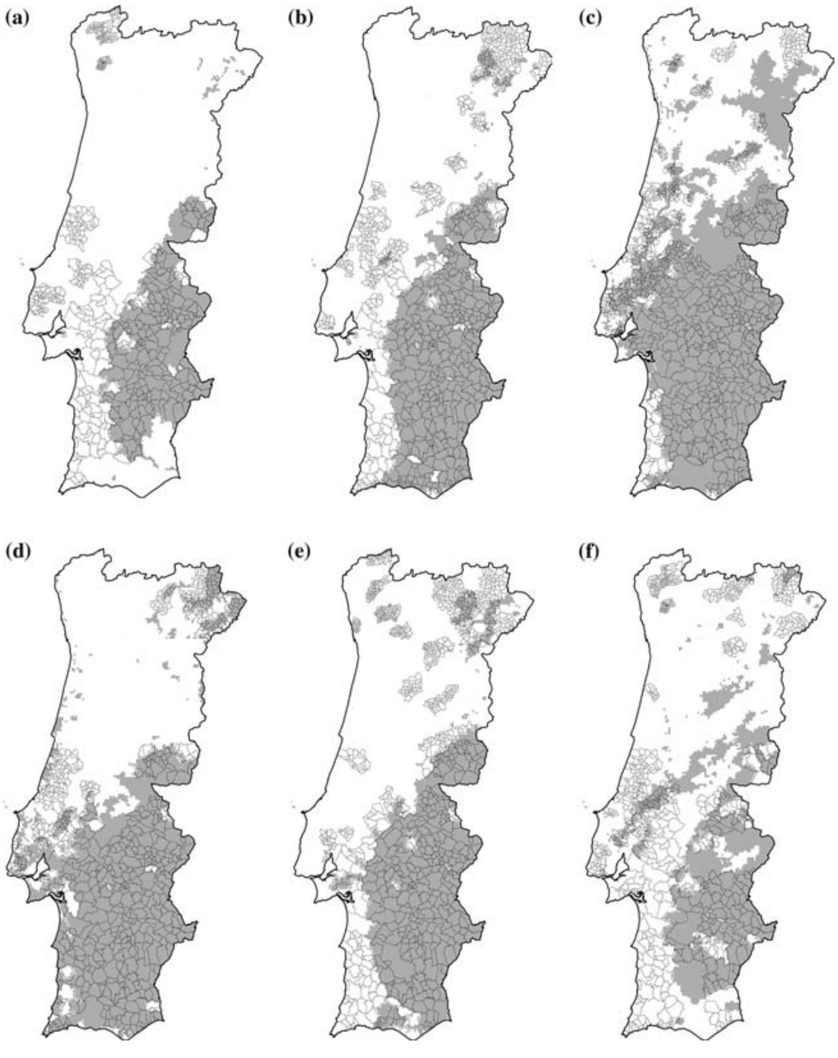

Figure 2. The known and predicted distribution of the seven tick species included in this study. The known distribution is mapped as the contour of the administrative division, while the predicted one is mapped with a grey shadow. (a) *R. annulatus*; (b) *R. bursa*; (c) *H. lusitanicum*; (d) *D. marginatus*; (e) *H. m. marginatum*; (f) *I. ricinus*.

areas at higher altitude, mainly in zones with *Q. rotundifolia* and *Quercus* spp. *H. lusitanicum* has been collected almost only in the southern half of Portugal, associated with variable vegetation types, with some scattered collections in areas of central and northern Portugal. *I. ricinus* has a very patchy distribution and is mainly associated with vegetation of *Quercus* spp., found in southern zones of the country, but also in the humid western part, associated with forests of *Q. suber* and *Eucalyptus* spp. It is also present in forests of *Pinus* spp., providing they are in the higher relative humidity environment of the western coast of the country. This species has also been collected in northern parts of the country.

The set of abiotic variables used to discriminate positive and negative sites is included in Table 1. As expected, some variables may explain the distribution of several tick species, while others are specific to elucidate the distribution of only one species. It is interesting to note that the more restricted species *I. ricinus* has a wide range of discriminating variables (12) while other more widely distributed species have fewer variables available for habitat discrimination (8–9). All the tick species involved in this study are sensitive to temperature-derived yearly variables, like MeanT, MeanTMax and MaxTMean. However, vegetation-derived (NDVI) features are not always suitable to discern an area as positive or negative for some tick species. Furthermore, some abiotic variables defining conditions in a given season of the year can be also applied to the definition of zones where ticks are present or absent. In this sense, the variables that characterize climate in summer and winter (Tmean-Summer, MaxTMean-Summer and MeanTMin-Winter) can be used as discriminatory of habitat for all the species. It is a remarkable fact that variables derived from maximum values of NDVI (yearly or summer-derived) are of applicability only to determine areas where *I. ricinus* is present or absent. Other vegetation-derived variables at defined seasons of the year are useless for the rest of the species.

The range of abiotic features preferences has been determined by the 95% range of the mean in the ANOVA analysis of some variables used to separate positive and negative sites for each species, selecting the values for ADs where the species have been recorded. These values are good markers to know the climatic features to which species are associated and are included in Figure 3 as the lower and upper limits of the 95% range. *R. annulatus* and *H. marginatum* are the species with preferences towards highest values of MeanT, while *H. lusitanicum* and *R. bursa* are intermediate and *I. ricinus*, *D. marginatus* displayed preferences towards the lower scale of the range. The situation is similar for values concerning MeanTMax. This picture changes for MaxT-Mean, as *R. bursa* and *H. marginatum* displayed preferences towards areas with the highest maximum temperature range. Concerning MeanTMin, *R. annulatus* and *H. marginatum* cannot tolerate areas with low values of minimum temperatures, being preferences of *H. lusitanicum, R. bursa* and *I. ricinus* located around the medium range of the scale. Collections of *D. marginatus* are placed in the lowest range of the scale.

Figure 3. Diagrams of the 95% range of preferences of the tick species. Variables with significant differences between species have been included. The charts show the 95% lower and upper range of the variables in the sites where the ticks have been collected. Nomenclature of variables follows the outline in Table 1.

As mentioned, association between species has been measured at the AD level as accurate coordinates for every collection point were not determined. Table 2 shows the number of common occurrences obtained for the association between pairs of species, as well as the different combinations of presence/absence for each pair of species. Strong associations have been obtained for *R. bursa* as collected together with *I. ricinus* and *D. marginatus*; both species of *Hyalomma* are also positively associated as they occur together in more than 56% of the sites where species of this genus have been collected. It is interesting to note that *R. annulatus* is associated with *H. m. marginatum* but not with *H. lusitanicum*. Other associations are not significant at the 0.05% level.

Results from CART analysis are shown in Figure 2 together with the actual distribution of the tick species. The predicted distribution of *R. annulatus* (Figure 2a) identified 96.4 and 63.3% of negative and positive ADs, respectively. Modelled distribution for *R. bursa* (Figure 2b) correctly allocated 97.3 and 83.7% of negative and positive ADs. Poorer results were obtained for both *H. lusitanicum* (Figure 2c) and *H. m. marginatum* (Figure 2d), as only 88.2 and 87% of negative ADs were, respectively identified for these species, with 76.9 and 76.3% of correctly identified positive ADs, respectively. Similar data were obtained when modelling the distribution of *D. marginatus* (Figure 2e, 75.5 and 75.3% of well identified negative and positive ADs) and *I. ricinus* (Figure 2f, 80.4 and 79.5% of accurately identified negative and positive ADs).

Discussion

The occurrence of suitable hosts and favourable climate conditions in Portugal benefit distribution and maintenance of ticks and tick-borne diseases in nature. Portugal presents an interesting environment, sharing both dominant Atlantic and Mediterranean vegetation and climate features, in the north-northwest and southern parts, respectively. The distribution of ticks and their ecological preferences reflect the ecological features of the country.

All the species of ticks have been found concentrated in the southern or southeast portions of the country, where large forests of *Quercus* spp., are associated with patches of natural grass, and where grazing ruminants are abundant. Other wild animals like red deer (*Cervus elaphus*), roe deer (*Dama dama*) and wild boar (*Sus scropha ferus*) largely considered as hosts of these ticks, are also abundant in the area. Some factors may have contributed to some bias in the results presented herein. First at all, collecting efforts have been concentrated in areas where hosts are abundant or where access is easy, leaving some parts of the north of the country unsampled. Although more than 90% of ADs in Portugal have been considered in this study, the picture of distribution of ticks in the country is yet incomplete. The species included in this study are the most typical representatives of ruminants in the Mediterranean basin.

Table 2. Associations between pairs of species based on the number of administrative divisions where they have been collected together, with each of the pair absent, or both absent.

Species (0/1)		*I. ricinus*		*R. bursa*		*R. annulatus*		*H. lusitanicum*		*H. m. marginatum*		*D. marginatus*	
		0	1	0	1	0	1	0	1	0	1	0	1
R. bursa	0	2706	491										
	1	446	465*										
R. annulatus	0	2999	493	2946	546								
	1	153	463	251	365								
H. lusitanicum	0	2651	363	2727	287	2886	128						
	1	501	593	470	624	606	488						
H. m. marginatum	0	2883	437	3029	291	3108	212	2860	460				
	1	269	519	168	620	384	404*	154	634*				
D. marginatus	0	2390	475	2517	348	2614	251	2359	506	2630	235		
	1	762	481	680	563*	878	365	655	588	690	553		

Pairs marked with * are significant at the 0.05 level.

Both vegetation and temperature-derived variables are very adequate as discriminating factors in the definition of the habitat for each tick species. Statistically significant differences have been found for sets of several temperature and NDVI-derived variables for at least six of the species considered herein.

Data on distribution of ticks in the country are coherent with the previous reports about the ecology of the involved species (Caeiro 1992, 1999). Every tick species reported here has been collected in wide portions of the southern parts of the country, with a more or less patchy distribution in central and northern parts, as a consequence of lower temperatures, poor vegetation resources because the altitude and smaller prevalence of grazing ruminants. *I. ricinus* is a well known species, widely distributed in Central Europe and British isles (Gray 1991). In the Mediterranean countries it has a patchy distribution, as a consequence of the high humidity requirements for survival. Anyway, the species is able to colonize areas of Mediterranean-type environment (Gilot et al. 1975; Bouattour et al. 1999) in both European and northern African countries. It seems that the species is able to develop local races adapted to prevailing conditions in very different environments (Gilot 1985). In this study, *I. ricinus* has shown marked preferences towards a medium range of temperature, far from high extremes, with a patchy distribution in areas of the south (associated to *Quercus* spp.) of the Atlantic coast (in forests of *Pinus* spp., with high humidity) and in the north (in points with low temperatures and the highest range of humidity).

Dermacentor marginatus is considered a Mediterranean species (Gilot 1985) being reported from countries in Europe and northern Africa. The species is able to colonize several series of vegetation (mainly *Quercus* spp.) and can be found from the level of sea to 800–1000 m of altitude. In our study, *D. marginatus* has been found to be able to colonize areas with large oscillations of temperature towards extreme minimum. Together, with *I. ricinus*, it is the only species found in the colder sites of northern parts in Portugal. The range of distribution of *R. bursa* seems to be particularly homogeneous, as it is mainly restricted to the Mediterranean portions of Europe and Africa. It is a thermophilic species associated with areas of open vegetation (Hoogstraal and Valdez 1980). It is interesting to note that *R. bursa* is able to survive in areas with large fluctuations of temperatures towards extreme maximum. This extreme character has been also found for collections in other parts of the Mediterranean region (Papadopoulos et al. 1996). However, some patchy populations have been collected in northern Portugal, associated with zones of lower temperatures.

Three other species are well adapted to Mediterranean environments. Both *H. marginatum* and *R. annulatus* prefer high average temperatures but not extreme summer values and *H. lusitanicum* occupy an intermediate niche with low preferences towards extreme temperatures in both senses of the scale. In this sense, it should be noted that both *R. annulatus* and *H. marginatum* have a wide distribution in the African continent, as a consequence of their adaptations to survival in areas with low humidity, and are present in areas of patchy vegetation in the Mediterranean basin (Aktas et al. 2004). These three species commonly appear together on ruminants (Castellà et al. 2001) but in our study, *R. annulatus* has been found restricted to the most Mediterranean conditions of the country, with preferences towards highest temperatures and lower values of NDVI, meaning for a higher xerophily. Records of *R. annulatus* from the north of the country should be considered as an introduction by transportation by moving animals, as these foci are very small.

Results derived from the associations between species are hard to interpret, because our data have been referenced to the ADs and not to accurate coordinates. In this sense, the positive association between a given pair of species could be taken as a preference towards the same kind of habitat within some natural limits. However, it could be also inferred as the presence of both species in different environments within the range of abiotic conditions for a given AD. In this sense high levels of coincidence have been obtained for some pairs of species. While this fact could be interpreted as true associations between species, like in the case of *R. bursa* and *D. marginatus*, well established in biotopes with a preference towards colder climates, or both *Hyalomma* species, they are problematical to resolve in the case of *R. annulatus*. It is interesting to note that *R. annulatus* has been significantly collected together with *H. m. marginatum* more than with *H. lusitanicum*, as a proof of the different climate preferences of both *Hyalomma* species considered herein.

CART analyses have been already used to determine the habitat of a tick species, like *I. ricinus* (Rizzoli et al. 2002) and are a common tool in the interpretation of the climate preferences of mammals and plants populations. That reference is the only report to date about the use of CART models for mapping the habitat of a tick species, where several variables, like climate values, the kind of habitat and altitude have been used to adequately predict the range of the tick in an area of Italy. We have used only climate and vegetation-derived variables and results show an adequate ability to predict the distribution of the ticks in the country as a result of the interacting forces of variables. The relatively low resolution for some species may be a consequence of the use of ADs as mapping unit. In this sense, as outlined before, there may exist a wide range of climate and vegetation conditions within a given AD. Therefore, the averaging process performed here may have introduced some bias in the performance of the models. Anyway, it is always better to carry out that kind of predicting processes at a point-by-point resolution. This procedure is unavailable under the conditions of this study because the restrictions in getting more accurate cartography of the tick collections.

This is the first approach to a countrywide study of distribution of ticks of ruminants in Portugal. This study may have important implications when considering the tick-borne pathogens that may be transmitted to ruminants and that could affect humans. Other studies may be performed to provide more data about ecological aspects and entomological risk, to delineate strategies of control and prevention against ticks and tick-borne diseases. This will reduce our knowledge gaps and may improve the development of new models to predict ticks distribution, with significant effects on both human and animal health.

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