

Title

IDENTIFICATION AND DISTRIBUTION OF HUMAN-BITING TICKS IN NORTHWESTERN SPAIN

Running Title

HUMAN-BITING TICKS IN SPAIN

Keywords

Tick bites; Ixodidae; epidemiology; emerging diseases; Spain

Authors

1. María Carmen Vieira Lista. Infectious and Tropical Diseases Group (e-INTRO). IBSAL-CIETUS (Biomedical Research Institute of Salamanca-Research Center for Tropical Diseases at the University of Salamanca), Faculty of Medicine, University of Salamanca, Salamanca, Spain. carmelilla@usal.es

2. Moncef Belhassen-García*. Department of Internal Medicine. Infectious Diseases Unit. University Hospital of Salamanca. Infectious and Tropical Diseases Group (e-INTRO). IBSAL-CIETUS (Biomedical Research Institute of Salamanca-Research Center for Tropical Diseases at the University of Salamanca), Faculty of Pharmacy, University of Salamanca, Salamanca, Spain. belhassen@usal.es

3. María Belén Vicente Santiago. Infectious and Tropical Diseases Group (e-INTRO). IBSAL-CIETUS (Biomedical Research Institute of Salamanca-Research Center for Tropical Diseases at the University of Salamanca), Faculty of Pharmacy, University of Salamanca, Salamanca, Spain. belvi25@usal.es

4. Javier Sánchez-Montejo. Infectious and Tropical Diseases Group (e-INTRO). IBSAL-CIETUS (Biomedical Research Institute of Salamanca-Research Center for Tropical Diseases at the University of Salamanca), Faculty of Pharmacy, University of Salamanca, Salamanca, Spain. s.montejo@usal.es

5. Carlos Pedroza Pérez. Infectious and Tropical Diseases Group (e-INTRO). IBSAL-CIETUS (Biomedical Research Institute of Salamanca-Research Center for Tropical Diseases at the University of Salamanca), Faculty of Pharmacy, University of Salamanca, Salamanca, Spain. carlospedroza@usal.es

6. Lía Carolina Monsalve Arteaga. Assistant Physician, Internal Medicine Department. Ensemble Hospitalier de la Côte, Morges, Switzerland. liacma@usal.es

7. Zaida Herrador. National Centre for Tropical Medicine, Health Institute Carlos III (ISCIII in Spanish), Madrid, Spain. zherrador@isciii.es

8. Rufino del Álamo-Sanz. Consejería de Sanidad Junta Castilla y León, Valladolid, Spain. AlaSanRu@jcyll.es

9. Agustín Benito. National Centre for Tropical Medicine, Health Institute Carlos III (ISCIII in Spanish), Madrid, Spain. abenito@isciii.es

10. Julio David Soto López. Infectious and Tropical Diseases Group (e-INTRO). IBSAL-CIETUS (Biomedical Research Institute of Salamanca-Research Center for Tropical Diseases at the University of Salamanca), Faculty of Pharmacy, University of Salamanca, Salamanca, Spain. Jdjuliosoto@gmail.com

11. Antonio Muro*. Infectious and Tropical Diseases Group (e-INTRO). IBSAL-CIETUS (Biomedical Research Institute of Salamanca-Research Center for Tropical Diseases at the University of Salamanca), Faculty of Pharmacy, University of Salamanca, Salamanca, Spain. ama@usal.es

*Corresponding author and correspondence address for proofs.

Acknowledgements

We want to thank the Junta de Castilla y León.

Funding

Consejería de Sanidad, Junta de Castilla y León; ISCIII: RICET RD16/0027/0018; IPI16/01784

Abstract

Ticks drive a wide diversity of pathogens to a great variety of hosts, including humans. We conducted a tick surveillance study in northwestern Spain between 2014 and 2019. Ticks were removed from people and were identified. Tick numbers, species, development stages, evolution over time, seasonal and geographical distribution, and epidemiological characteristics of people bitten by ticks were studied. We collected ticks from 8143 people. Nymphs of *Ixodes ricinus* were the most frequently

collected. *Rhipicephalus bursa*, *Rhipicephalus sanguineus* sensu lato (s.l), *Hyalomma marginatum*, *Hyalomma lusitanicum*, *Dermacentor marginatus*, *Dermacentor reticulatus* and *Haemaphysalis punctata* were also found, with adults as the main stage. *Hyalomma* spp. and *R. bursa* have been progressively increasing over time. Although bites occurred throughout the year, the highest number of incidents were reported from April to July. The distribution patterns of the tick species were different between the north and the south of the region, which was related to cases detected in humans of the pathogens they carried. Adult men were more likely to be bitten by ticks than women. Ticks were most frequently removed from adults from the lower limbs, while for children they were mainly attached to the head. Epidemiological surveillance is essential given the increase in tick populations in recent years, mainly of species potentially carrying pathogens causing emerging diseases in Spain, such as the Crimean Congo Hemorrhagic Fever (CCFH).

1. Introduction

Ticks are hematophagous parasites distributed worldwide, and they are of great importance from an epidemiological and clinical point of view. They can transmit a wide variety of pathogens, such as viruses, bacteria and protozoa (Estrada-Peña and Jongejan, 1999). Furthermore, they have a great impact in the veterinary field due to economic losses due to morbidity and mortality, affecting 80% of the world's cattle population (Yitayew and Samuel, 2015) with a total cost of tick-borne diseases (TBDs) estimated to be between 14 and 19 billion dollars globally per year (Ghosh et al., 2007). Tick-borne diseases are a growing public health concern, and their incidence is clearly increasing worldwide- just look at the increase in the USA of Lyme and human ehrlichiosis and of TBE and haemorrhagic fever cases in Europe and Asia (Rochlin and Toledo, 2020)-due to several interacting factors (Dantas-Torres et al., 2012)(Estrada-Peña et al., 2017a). The ecological characteristics of these vectors affect their epidemiology, with their activity cycles closely related to environmental factors, such as temperature and relative humidity, which are fundamental for their survival. Climate change

or weather variability are just two of the many factors that determine tick population abundance, but there are also other variables of importance, such as composition, host community abundance and landscape features ([Gortazar et al., 2014](#)) ([Estrada-Peña and De La Fuente, 2014](#)) ([Estrada-Peña et al., 2004](#)).

Ticks from 896 species are known around the world, and the most prevalent Ixodidae family comprises 702 species in 14 genera. Moreover, Ixodid ticks are the main vectors of zoonotic pathogens in Europe ([Gilbert, 2010](#)), for which we found five genera (*Ixodes*, *Dermacentor*, *Haemaphysalis*, *Rhipicephalus* and *Hyalomma*) and 54 species, with *I. ricinus* being the most widely distributed tick ([Estrada-Peña et al., 2004](#)) ([Rizzoli et al., 2014](#)) ([Boulanger et al., 2019](#)). Lyme borreliosis is the most prevalent tick-borne disease in Europe and it is caused by *Borrelia burgdorferi* sensu lato (s.l.) complex ([Cull et al., 2019](#)). On the Iberian Peninsula, we found 4 main genera of ticks that bite humans: *Ixodes*, *Dermacentor*, *Rhipicephalus* and *Hyalomma*, which are potential transmitters of *B. burgdorferi* sensu lato, several genospecies of *Rickettsia*, *Anaplasma phagocytophilum* and the Crimea-Congo virus.

The association between global warming and the emergence of TBD is well documented in Europe and Eurasia ([El-Sayed and Kamel, 2020](#)). The geographical expansion of tick species is directly related to the emergence of new infectious diseases, which makes it more necessary than ever to understand the dynamics and distribution of ticks. Although there is much previous information about the distribution of ticks in several areas of Spain, the data come mainly from the study of captured ticks in the environment, either on vegetation or on animals (wild and domestic) ([Estrada-Peña et al., 2004](#)) ([Barandika et al., 2008](#)) ([Fernández de Mera et al., 2013](#)) ([Requena-García et al., 2017](#)) ([Remesar et al., 2019b](#)) ([Estrada-Peña et al., 2017b](#)). There is also a very exhaustive study of ticks removed from people in Castilla y León carried out by our group ([Fernández-Soto, 2003](#)) in the period corresponding to 1997–2002. A long time has passed since then and given the emergence of new species of ticks and the

introduction of new TBDs, such as Crimean Congo haemorrhagic fever (CCHF) and DEBONEL (Dermacentor-borne necrosis erythema and lymphadenopathy), it seems essential to re-examine the current situation in this area of Spain. Thus, the purpose of this work is to update data about species of ticks removed from people and their spatial and temporal patterns. This will allow us to identify the risk areas, activity peaks and dynamics of these pathogen carriers.

2. Methods

2.1. Study site/site selection

This study was performed in Castilla y León (41°23'0" N, 4°27'0" W), an area located in northwestern Spain. It covers a surface of 94.224 km² and is the most extensive region of the European Union. Although there is a marked continental climate in most of the territory, characterised by cold winters and hot summers with short periods of spring and autumn, regional variations in both temperatures and rainfall allow us to distinguish different climatic domains in the region. Continentalised Mediterranean in the centre, with semi-arid enclaves in some areas, mountain Mediterranean in mountainous areas in the north-east, east and south, and Atlantic in the north (Fig. 1). This great geomorphological and bioclimatic variety together with its vast extension gives rise to a great range of climatological conditions and ecosystems which undoubtedly condition the geographical distribution of the different tick species. Castilla y León is rich in forests and ample wooded areas and the privileged geographical situation of the northern subplateau makes it a region of special interest as an area of passage, breeding and wintering of birds from central and northern Europe and the African continent.

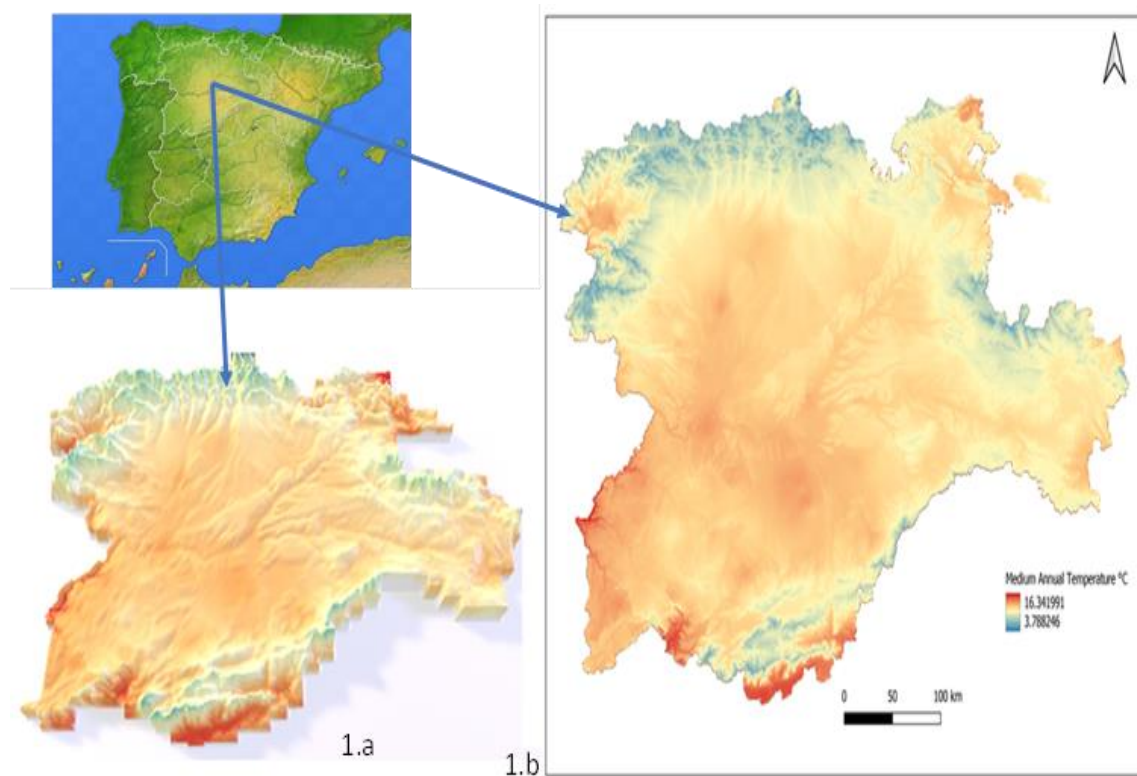


Figure 1. Map of the area of study (Castilla y León, NW Spain). 1a. Altitude. 1b. Medium Annual Temperature.

2.2. Tick collection and identification

During 2014–2019, ticks were collected from people who went to Primary Healthcare Centres and Hospital Emergency Services in Castilla y León for their removal through a programme of the Junta de Castilla y León for the prevention and control of tick-borne anthroponoses. The ticks were removed from the hosts with tweezers and sent to our laboratory (Laboratory of the Faculty of Pharmacy at the University of Salamanca) at room temperature in a container. Each tick was morphologically identified under a binocular lens as to life stage and species using taxonomic reference keys (Gil-Collado, J., Guillén, J. L., y Zapatero, 1979)(Estrada-Peña et al., 2004)(Estrada-Peña, A.; Mihalca, A.D.; Petney, 2017)(Apanaskevich et al., 2008). Each tick was classified to species except in the case of *R. sanguineus* sensu lato ticks which were identified only to "group" level as the re-definition of *R. sanguineus* sensu stricto (Nava

et al., 2018) was not available during most of the study. *Rhipicephalus turanicus* specimens were also reported as *R. sanguineus* s.l. Tick species, developmental stages (larva, nymph, adult), sex and feeding degree were recorded, as well as the epidemiological characteristics of the patients (age, sex, geographical location) and anatomic location of the attachment of the tick on the patient. A unique individual identification number was assigned to each tick and its corresponding file.

2.3. Geopositioning and Data analysis

Information about tick bites were obtained from the database of the center of reference of the Biomedical Research Institute of Salamanca-Research Center for Tropical Diseases at the University of Salamanca. To obtain the geographical coordinates of every tick bite we geocode the locations of tick collections using “Batch geocoder for journalists” (<https://geocode.localfocus.nl>), prior data curation. The latitude and longitude then were projected to the coordinate reference system ETRS89 to map the distribution of the species of interest through the years and the median temperature in the provinces of the autonomous community of Castilla y León, using pretty breaks as data classification method. All the maps were done in QGIS 3.18.3 (QGIS Geographic Information System, 2022).

Absolute frequencies were used to summarize the number of ticks of every species and show visual tendencies across 1: years, 2: seasons. Relative frequencies converted in percentages were used to show visual tendencies between sex and the stages collected of the specimens, and both frequencies were used to show epidemiological characteristics of people bitten by ticks.

In addition, we search for signals of displacement in the geographic distribution of the collects. For the visual pattern we used geographic coordinates of the previous geocode step. The comparative figures were done in the package *ggplot2* (Wickham, H., 2016), in the statistical environment R (R Core Team, 2021). Kruskal-Wallis rank sum test and Pairwise comparisons

using Wilcoxon rank sum test with continuity correction were used for statistical support in R. A chi square analysis was done to evaluate differences in sex specimens. P-values were adjusted using false discovery rate and Bonferroni methods, considered less than or equal to 0.05 as statistically significant.

3. Results

3.1. Tick numbers, species, development stages and evolution over time

A total of 8143 ticks were collected over 6 years of which 7345 of them were identified to the species level, and their stage (larvae, nymph and adult) was determined. The remaining 798 were discarded as their morphological identification was impossible due to the structural and conservation state in which they were received. Ixodid ticks belonging to 5 genera and 8 species were found; *I. ricinus* was the most frequent species infecting humans (50.1%), followed by *R. bursa* (12.6%), *R. sanguineus* s.l (10.2%), *H. marginatum* (10%), *D. marginatus* (8.6%), *H. lusitanicum* (4.2%), *D. reticulatus* (2.7%), and *H. punctata* (1.6%). Although this general trend has been maintained over the six years of the study, during recent years, there has been a clear increase in *H. lusitanicum* and *Rhipicephalus bursa*.

Regarding the temporal distribution, while some species remained stable over time (*D. reticulatus*, and *H. punctata*), others underwent changes. *H. lusitanicum* is a clear example of how a specie has increased over time, especially since 2015. Similarly, this occurred with *R. bursa*, with a huge growth spike in 2019. *D. marginatus* and *H. marginatum* had roughly identical temporal distributions with clear downward trends in 2017 and 2018 before recovering in 2019. *R. sanguineus* and *I. ricinus* have both of them their maximum peak in 2015. Despite this, both species exhibit a downward trend ([Figure 2](#)).

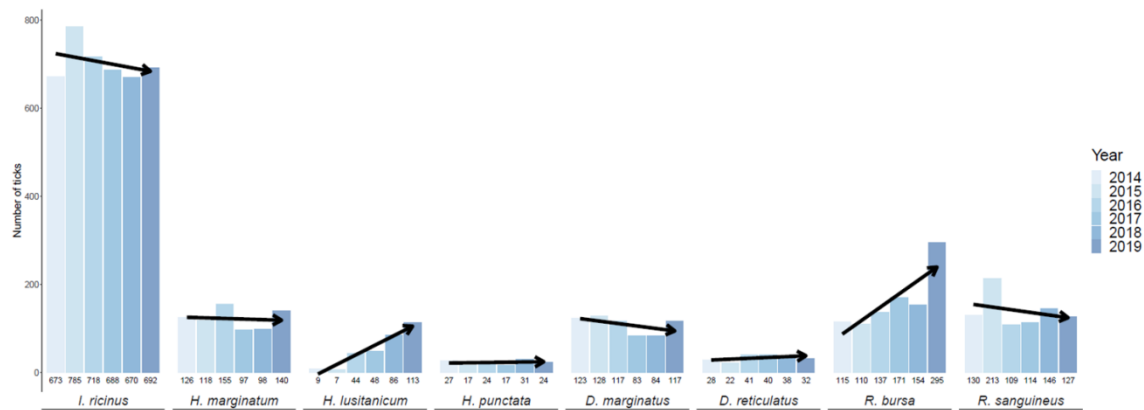


Figure 2. Number of ticks recovery and evolution over time. Eight species were included in the study carried out between 2014-2019. Ticks belonged to *Ixodes ricinus*, *Hyalomma marginatum*, *Hyalomma lusitanicum*, *Haemaphysalis punctata*, *Dermacentor marginatus*, *Dermacentor reticulatus*, *Rhipicephalus bursa* and *Rhipicephalus sanguineus*.

The adult stage (male and female) was the most frequent in all species except for *Ixodes*, where nymphs (57.3%) were collected in much larger numbers. For *Dermacentor*, *Haemaphysalis* and *Rhipicephalus*, females were the predominant stage (64.8%, 58.4% and 56.3%, respectively), while for *Hyalomma*, the adults recovered were mainly males (66%). These sex differences were statistically significant ($P < 0.05$). When we analysed the interspecies percentage of females and males, we observed no intra-species variability. (Figure 3).

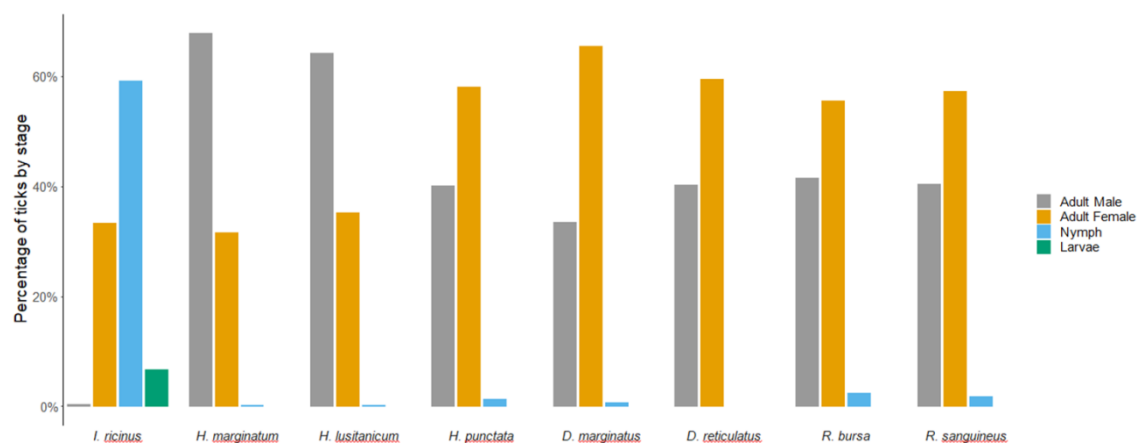


Figure 3. Number of tick species according to developmental stages (larvae, nymph and adult). Predominant stages were adult females for *Dermacentor*, *Haemaphysalis* and *Rhipicephalus*, while for *Hyalomma* were mainly adult males. *Ixodes* was the exception with nymphs as the predominant stage. These sex differences were statistically significant ($P < 0.05$).

3.2. Seasonal and geographical distribution of ticks

The highest number of tick bites (69% of the total) was reported during the months of April to July. *Ixodes* was the most frequent and widely distributed tick throughout the year, independent of the month, while all other species showed marked seasonality (Figure 4). Although *Ixodes* spp. was detected throughout the year, its highest detection levels were in spring, autumn and late winter for the adult stage and spring and summer for the nymphs (June mainly). *Hyalomma* was infrequent in winter and autumn, with peak activity in spring and early summer.

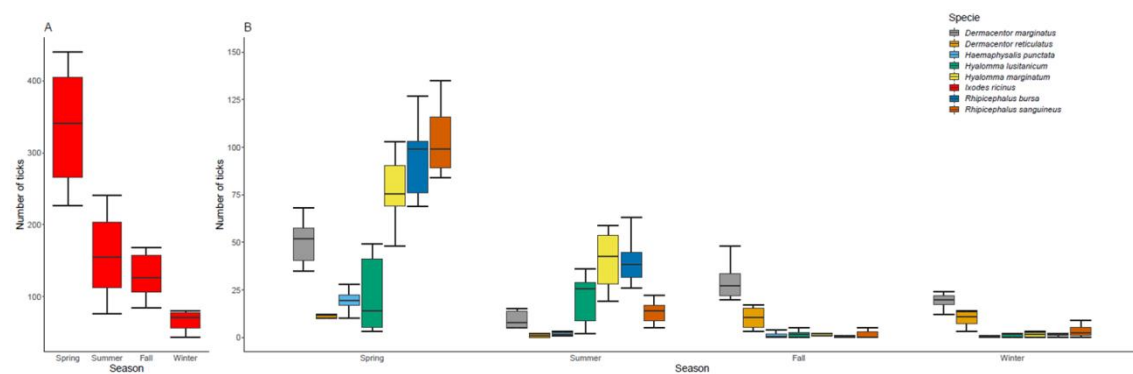


Figure 4. **Seasonality of tick bites on humans.** Species sampled by season and year: winter (December–February), spring (March–May), summer (June–August), and autumn (September–November). **A.** *Ixodes ricinus*. **B.** *Hyalomma marginatum*, *Hyalomma lusitanicum*, *Haemaphysalis punctata*, *Dermacentor marginatus*, *Dermacentor reticulatus*, *Rhipicephalus bursa* and *Rhipicephalus sanguineus*. All ticks belonging to each genus showed a repetitive pattern over time.

Rhipicephalus had two peaks of activity in spring and summer and then practically disappeared in autumn and winter. *Dermacentor* showed pronounced seasonality with two annual peaks of maximum activity in spring and autumn for *D. marginatus* (47.2%-26.16%) and autumn and winter for *D. reticulatus* (34.3%-32.8%) although it also has an important presence in spring (30%) with very little or no presence in summer (8.7%-2.8%). Most *D. marginatum* bites were in April and May with slightly lower peaks in October and March, while most bites by *D. reticulatus* occurred in December and March, with a smaller peak in April. *Haemaphysalis* had a very low occurrence and was limited to spring (May and June).

The geographical distribution showed variations among the different species of ticks

(Figure 5).

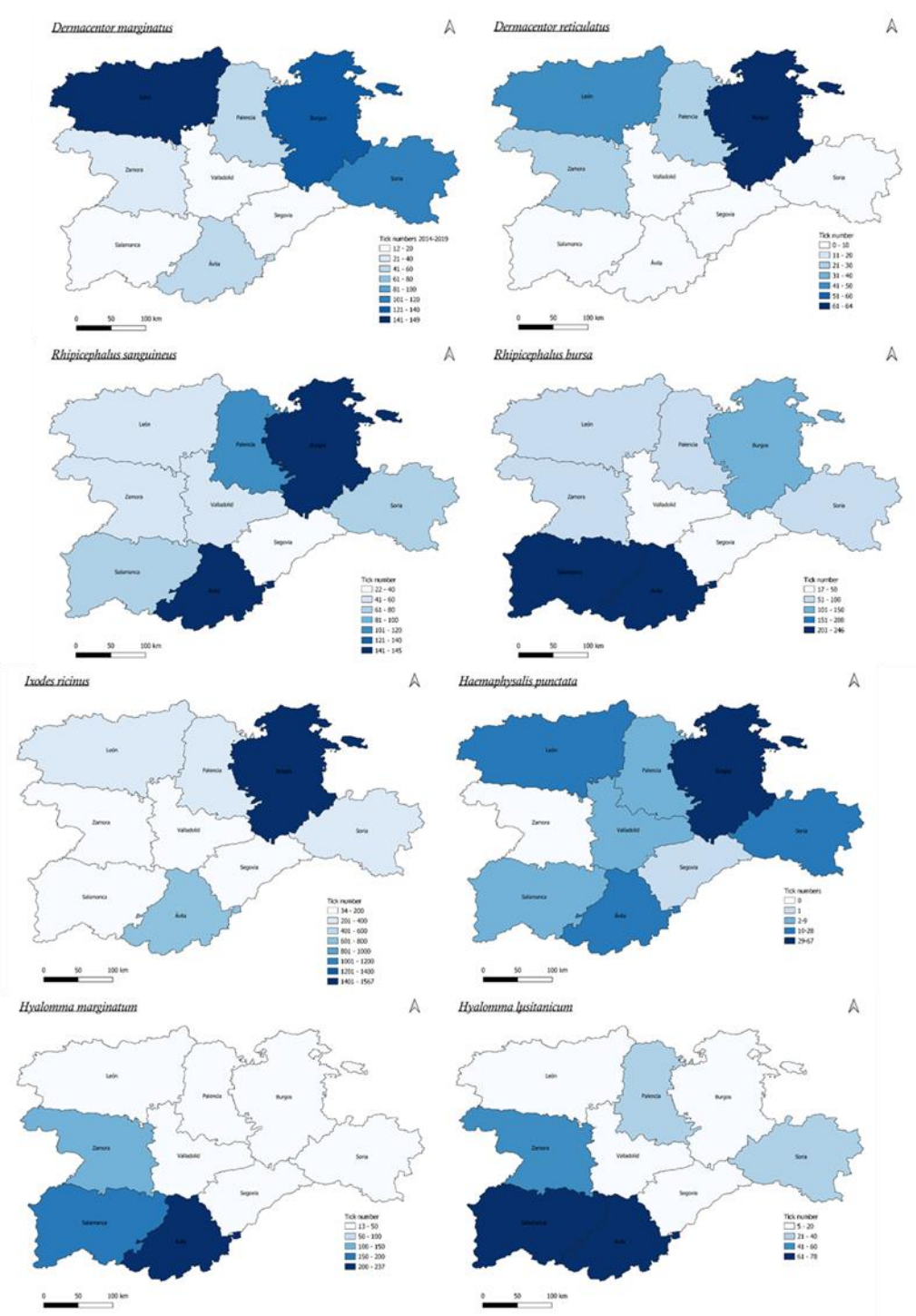


Figure 5. **Geographical distribution of ticks.** *Dermacentor* spp., *Ixodes ricinus* and *Haemaphysalis punctata* are mainly distributed in the North. *Hyalomma* spp. in the South and *Rhipicephalus* spp. shows a somewhat different distribution with interspecies variations although the largest number of specimens is still in the South.

Ixodes ricinus was present in all areas, with a higher prevalence in the northeast. *Dermacentor* spp. was also mainly distributed in the northern areas for both species. The two species of *Hyalomma* were detected in all provinces but mainly in the south.

Rhipicephalus spp. showed differences in the distribution of the species. *R. bursa* is mainly distributed in the south, while *R. sanguineus* is distributed in both the south and the north, with more presence in the northeast. *Haemaphysalis* was the less represented genus, mostly found in the northeast, with little or null presence in the other areas.

Looking at how the position of the ticks has varied over the years by analysing latitude and longitude (Figure 6), we can see that when analysing the data as a group there is a tendency to move northwards and westwards. In the case of longitude, this is statistically significant both at the group level and by year, specially between 2014 and 2015 with 2019 ($P < 0.05$ pairwise comparison).

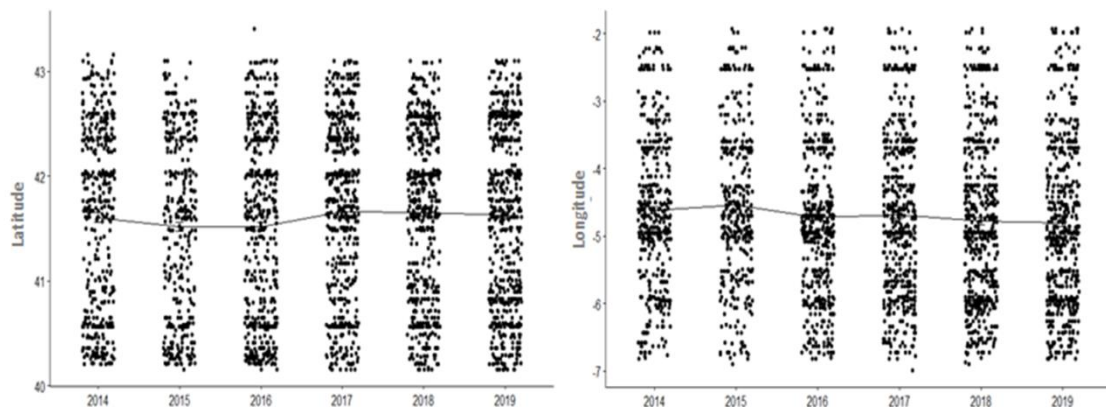


Figure 6. Geographical displacement. Towards northwards and westwards

Kruskal-Wallis rank sum test. p -value < 0.05

3.3. Epidemiological characteristics of people bitten by ticks

Data on sex, age and sites of tick bites in people are shown in Table 1. Regarding the sex of people bitten by ticks, 65.3% were male and 34.7% female. Ticks were collected from people of all ages: 27% of those bitten were children (<14 years), and the remaining 73% were

adults. The least number of bites was found in the 15–35 age group. In contrast, the group most frequently bitten by ticks was the 55+ age group.

Table 1. Epidemiological characteristics of people bitten by ticks (2014-2019). Sex, age and bite sites are specified for each tick species.

Specie	<i>I. ricinus</i>	<i>R. bursa</i>	<i>R. sanguineus</i>	<i>D. marginatus</i>	<i>D. reticulatus</i>	<i>H. marginatum</i>	<i>H. lusitanicum</i>	<i>H. punctata</i>	Total
Sex: male (%)	2247 (67.5%)	633 (73.4%)	411(59.13%)	292 (50.8%)	100 (56.8%)	495 (75.7%)	218 (75.7%)	7 (54.7%)	4466 (65.7%)
female (%)	1170 (34.24%)	229 (26.6%)	284 (40.86%)	283 (49.2%)	76 (43.2%)	159 (24.3%)	70 (24.3%)	58 (45.3%)	2329 (34.3%)
Age:									
0-14	673	163	214	126	34	29	13	44	1296
15-35	505	140	108	51	40	81	35	12	972
36-55	840	190	101	134	37	192	61	21	1576
55+	882	228	147	185	26	269	124	31	1892
Site: adult (%)	2478 (75.7%)	615 (75.6%)	408 (60.8%)	403 (73.5%)	124 (75.6%)	580 (94.6%)	240 (93.7%)	70 (58.3%)	4918 (76.1%)
child (%)	797 (24.3%)	198 (24.3%)	263 (39.2%)	145 (26.4%)	40 (24.4%)	33 (5.4%)	16 (6.2%)	50 (41.7%)	1542 (23.9%)
Head	42/132	54/94	74/151	182/102	28/19	17/9	11/5	16/32	424/544
Neck	37/59	43/19	46/33	26/8	13/3	29/9	15/5	6/7	215/143
Thorax	458/159	120/27	60/22	20/7	9/6	100/19	50/9	8/2	825/251
Upper limbs	284/76	41/12	32/14	20/4	12/8	36/8	11/2	6/1	442/125
Lower limbs	681/188	98/9	39/9	119/5	17/8	125/19	52/8	6/1	1037/188
Back	149/47	46/15	31/11	14/4	8/3	44/8	21/2	4/1	317/91
Pelvis	147/44	42/14	16/5	4/3	0/0	69/8	18/5	5/1	301/80

If we look at each of the species separately, we observed that *I. ricinus*, *R. bursa*, *D. marginatus*, *H. marginatum* and *H. lusitanicum* predominantly bit the 55+ age group, while *R. sanguineus* and *H. punctata* predominantly bit the children's age group (0–14).

Ticks were most frequently removed from adults from the lower limbs (28.8%), and children were mainly bitten on the head (40.8%). Particularly, *Ixodes ricinus* and *Hyalomma* spp. bit mainly on the lower limbs, while *Rhipicephalus* spp., *Dermacentor* spp. and *H. punctata* had a preference for the head.

4. Discussion

The emergence of novel tick-borne diseases in recent years makes it essential to understand the distribution of tick populations. Although there are already studies on the distribution of ticks in different areas of Spain (Barandika et al., 2008)(Fernández de Mera et al., 2013)(Requena-García et al., 2017)(Remesar et al., 2019b)(Estrada-Peña et al., 2017a)(Merino et al., 2005)(Fernández-Soto, 2003)(Fernández-Soto et al., 2006), data on tick

species, their distribution and activity have probably undergone changes and evolution in recent years. The present study provides epidemiological information on the diversity, relative abundance and seasonal and geographic distribution of human-biting ticks in Castilla y León (northwest Spain) over a six-year span (2014–2019), allowing us to understand tick activity and the periods of highest risk to humans.

Ixodes ricinus was the most predominant and widely distributed species, both spatially and temporally, with a clear dominance over the others, followed by *R. bursa*. These results coincide with those observed in other studies carried out by our group seventeen years ago ([Fernández-Soto, 2003](#)), in which *I. ricinus* and *R. bursa* were also the prevalent species. However, data about other ticks have changed over the years. *H. marginatum* is currently the fourth most frequently removed species from humans, almost even with *R. sanguineus* s.l., whereas in a previous study (1996-2002), it was ranked fifth behind *D. marginatus*. Nevertheless, the percentage of recovered ticks belonging to *D. marginatus* was higher in the 1997–2002 period. The most evident case of a change in distribution patterns and abundance is found for *H. lusitanicum*, which currently accounts for 4.2% of the ticks removed from humans, compared to 0.85% in the previous study. In this sense, keep in mind that ticks of the genus *Hyalomma* are the main vectors of CCHFV, which is currently considered an emerging or possibly a re-emerging pathogen in southern Europe. Although the presence of this virus was already reported in Spain in 2010 ([Estrada-Peña et al., 2012](#)) an increase in human CCHFV cases in Spain and, more specifically, in Castilla y León has been detected, as reported by our group ([Monsalve Arteaga et al., 2021](#))([Monsalve-Arteaga et al., 2020](#)). The increase in human cases matched the increase in *Hyalomma* ticks observed in this area. All observed changes in both distribution patterns and frequency of ticks are determined not only by biotic factors such as climate or abiotic factors (vegetation), but also by the accessibility of hosts.

The predominance of *I. ricinus* over the other species is not unique to the study area. The same pattern has been observed in studies carried out in different locations in Spain (Requena-García et al., 2017)(Espí et al., 2017)(JF et al., 2011)(Fernández-Soto, 2003)(Remesar et al., 2019a)(Fernández-Soto et al., 2006) and in European countries such as Belgium, Italy, Sweden, the Netherlands, the United Kingdom, Finland, Norway, Romania and Germany (Lernout et al., 2019),(Battisti et al., 2019),(Lindblom et al., 2016), (Jahfari et al., 2016),(Cull et al., 2019),(Lindblom et al., 2014), (Jahfari et al., 2016), (Hjetland et al., 2013), (Briciu et al., 2011), (Faulde et al., 2014). The nymph is the predominant stage of *I. ricinus*, which is also consistent with what has been observed in a study previously conducted by our group (Fernández-Soto, 2003). Our results compared with those obtained across Europe are lower than those reported in Belgium and Great Britain, higher than those in Italy and similar to those in Sweden (Lernout et al., 2019), (Cull et al., 2018).

Although we cannot conclude that ticks have a preference among human hosts according to their sex, of the 7862 participants included in this study, 65.7% were men and 34.3% were women. This preference for males was very similar to what was observed by Fernández Soto in 2003 (62% men and 38% women). The fact that this pattern remains the same as it was almost 20 years ago may indicate that the occupational and behavioural habits in terms of outdoor activities among men and women have not changed. Thus, 23% of tick-bite victims are in the age group 14 years old or younger, while the 15–35 group has the lowest number of tick bites at 16%, in line with northern Europe (Wilhelmsson et al., 2013) and Belgium (Lernout et al., 2019).

Differences in anatomical sites of attachment were observed for both children and adults. Tick bites occurred most frequently on the lower limbs and thorax in adults and on the head in children. Similar results have been observed in other studies in western and northern Europe, with a predominance of bites on the legs in adults and the head and neck of children

(Wilhelmsson et al., 2013)(Cull et al., 2019)(Hügli et al., 2009)(Robertson et al., 2000) (Pańczuk et al., 2019). These apparent preferences, as suggested by some authors, are probably due to the morphological, behavioural and physiological differences between men and women and adults and children. Adult legs and children's heads are the most accessible places for ticks since they are at the height of the vegetation where ticks are searching for a host.

Although ticks are removed during all months of the year, the highest number of tick bites are recorded during spring and summer, with a peak of tick activity in June and July, as observed in this same area in previous studies. These data are also in accordance with several studies carried out in Europe (Briciu et al., 2011) (Weisshaar et al., 2006) (Wilhelmsson et al., 2013) (Cull et al., 2019) (Hügli et al., 2009)(MT et al., 1999)(Robertson et al., 2000). When looking at the temporal distribution of the different genera, we observed that adults of *Ixodes* were detected throughout the year but mostly in spring, autumn and later winter (March), which differed somewhat from previous years in the same study area where adults were biting mainly in autumn and spring but not in winter (Fernández-Soto, 2003). These activity patterns are similar to those in several countries of northern Europe (Cull et al., 2019) (Lindblom et al., 2016) (Briciu et al., 2011) (Lernout et al., 2019). *Hyalomma* is infrequent in winter, with its peak activity in spring and summer, with June and July being the months with the most reported cases for both species. In recent years, we have seen that *H. marginatum* bites are being brought forward to May. The activity pattern of *Rhipicephalus* has been maintained in northwestern Spain over time, with two peaks in spring and summer, although as with *Hyalomma*, in the case of *R. bursa*, there are many reports of bites as early as May. *Dermacentor* shows a particular seasonality being practically absent in summer for both species which could lead us to think that it does not feel comfortable with hot temperatures. *D. marginatus* presents a bimodal activity pattern with peaks in spring (April-May) and autumn, and the peak number of bites always in April. In the case of *D. reticulatus* bites occur

similarly in autumn, winter and spring (from October to March). This differs from that observed in Belgium, where the highest peaks of activity occur in spring (March-May and the late summer and autumn (August-November) (Lernout et al., 2019). *Haemaphysalis* is practically limited to spring. Although previous studies have reported cases in summer, we have not seen them.

While studying the geographical distribution of the ticks, we observed quite stable and different distribution patterns between the north and the south. These distribution patterns were determined by various climatic (temperature and humidity) and ecological factors. *D. marginatus*, *D. reticulatus* and *H. punctata* were mainly distributed in the north of the study area, although the greatest increase in *D. marginatus* has been seen in the south-west. On the other hand *H. marginatum*, *H. lusitanicum* and *R. bursa* were found in greater numbers in the south. *H. marginatum* is expanding its habitat to south-western areas and *H. lusitanicum* in recent years to arid or semi-arid areas in the west. The wider distribution of *Hyalomma* in the south could explain the increasing occurrence of Crimea Congo observed in this area in recent years. Finally, *I. Ricinus*, and *R. sanguineus* were distributed in both areas. However, the greatest number of specimens was found in the northeast for both of them especially *I. ricinus*.

5. Conclusions

Our results show that humans from northwestern Spain are mainly bitten by *I. ricinus* nymphs. Ticks bite particularly on the legs of adults and on the head of children. Therefore, although the peak season for tick bites is spring and summer, tick bites are becoming increasingly frequent in autumn and even winter. Seasonal tick patterns have changed in recent years, both in Spain and elsewhere in Europe so that many species of ticks have expanded their distribution. The increased period of activity increases the likelihood of being

bitten and therefore of being infected by a tick-borne pathogen. This study is essential for proper epidemiological surveillance. Moreover, knowledge of tick populations and human exposure to tick bites could suggest ways to reduce the risk of tick-borne diseases.

References

- Apanaskevich, D.A., Santos-Silva, M.M., Horak, I.G., 2008. The genus *Hyalomma* Koch, 1844. IV. Redescription of all parasitic stages of *H. (Euhyalomma) lusitanicum* Koch, 1844 and the adults of *H. (E.) franchinii* Tonelli Rondelli, 1932 (Acari: Ixodidae) with a first description of its immature stages. *Folia Parasitol. (Praha)*. 55, 61–74. <https://doi.org/10.14411/fp.2008.009>
- Barandika, J.F., Hurtado, A., García-Sanmartín, J., Juste, R.A., Anda, P., García-Pérez, A.L., 2008. Prevalence of tick-borne zoonotic bacteria in questing adult ticks from Northern Spain. *Vector-Borne Zoonotic Dis.* 8, 829–835. <https://doi.org/10.1089/vbz.2008.0023>
- Battisti, E., Zanet, S., Boraso, F., Minniti, D., Giacometti, M., Duscher, G.G., Ferroglio, E., 2019. Survey on tick-borne pathogens in ticks removed from humans in Northwestern Italy. *Vet. Parasitol. Reg. Stud. Reports*. <https://doi.org/10.1016/j.vprsr.2019.100352>
- Boulanger, N., Boyer, P., Talagrand-Reboul, E., Hansmann, Y., 2019. Ticks and tick-borne diseases. *Med. Mal. Infect.* <https://doi.org/10.1016/j.medmal.2019.01.007>
- Briciu, V.T., Titilincu, A., Țățulescu, D.F., Cârștina, D., Lefkaditis, M., Mihalca, A.D., 2011. First survey on hard ticks (Ixodidae) collected from humans in Romania: Possible risks for tick-borne diseases. *Exp. Appl. Acarol.* 54, 199–204. <https://doi.org/10.1007/s10493-010-9418-0>
- Cull, B., Pietzsch, M.E., Gillingham, E.L., McGinley, L., Medlock, J.M., Hansford, K.M., 2019. Seasonality and anatomical location of human tick bites in the United Kingdom. *Zoonoses Public Health* zph.12659. <https://doi.org/10.1111/zph.12659>
- Cull, B., Pietzsch, M.E., Hansford, K.M., Gillingham, E.L., Medlock, J.M., 2018. Surveillance of British ticks: An overview of species records, host associations, and new records of *Ixodes ricinus* distribution. *Ticks Tick. Borne. Dis.* <https://doi.org/10.1016/j.ttbdis.2018.01.011>

- Dantas-Torres, F., Chomel, B.B., Otranto, D., 2012. Ticks and tick-borne diseases: A One Health perspective. *Trends Parasitol.* 28, 437–446. <https://doi.org/10.1016/j.pt.2012.07.003>
- El-Sayed, A., Kamel, M., 2020. Climatic changes and their role in emergence and re-emergence of diseases. *Environ. Sci. Pollut. Res.* 27, 22336–22352. <https://doi.org/10.1007/s11356-020-08896-w>
- Espí, A., Del Cerro, A., Somoano, A., García, V., M. Prieto, J., Barandika, J.F., García-Pérez, A.L., 2017. *Borrelia burgdorferi* sensu lato prevalence and diversity in ticks and small mammals in a Lyme borreliosis endemic Nature Reserve in North-Western Spain. Incidence in surrounding human populations. *Enfermedades Infecc. y Microbiol. Clin. (English ed.)* 35, 563–568. <https://doi.org/10.1016/j.eimce.2016.06.003>
- Estrada-Peña, A.; Mihalca, A.D.; Petney, T.N., 2017. No Title Ticks of Europe and North Africa: A guide to species identification.
- Estrada-Peña, A., De La Fuente, J., 2014. The ecology of ticks and epidemiology of tick-borne viral diseases. *Antiviral Res.* 108, 104–128. <https://doi.org/10.1016/j.antiviral.2014.05.016>
- Estrada-Peña, A., de la Fuente, J., Cabezas-Cruz, A., 2017a. Functional redundancy and ecological innovation shape the circulation of tick-transmitted pathogens. *Front. Cell. Infect. Microbiol.* 7, 1–11. <https://doi.org/10.3389/fcimb.2017.00234>
- Estrada-Peña, A., Jongejans, F., 1999. Ticks feeding on humans: A review of records on human-biting Ixodoidea with special reference to pathogen transmission. *Exp. Appl. Acarol.* 23, 685–715. <https://doi.org/10.1023/A:1006241108739>
- Estrada-Peña, A., Martinez, J.M., Sanchez Acedo, C., Quilez, J., Del Cache, E., 2004. Phenology of the tick, *Ixodes ricinus*, in its southern distribution range (central Spain). *Med. Vet. Entomol.* 18, 387–397. <https://doi.org/10.1111/j.0269-283X.2004.00523.x>
- Estrada-Peña, A., Palomar, A.M., Santibáñez, P., Sánchez, N., Habela, M.A., Portillo, A., Romero, L., Oteo, J.A., 2012. Crimean-Congo Hemorrhagic Fever Virus in Ticks, Southwestern Europe, 2010. *Emerg. Infect. Dis.* 18, 179–180. <https://doi.org/10.3201/eid1801.111040>

- Estrada-Peña, A., Roura, X., Sainz, A., Miró, G., Solano-Gallego, L., 2017b. Species of ticks and carried pathogens in owned dogs in Spain: Results of a one-year national survey. *Ticks Tick. Borne. Dis.* 8, 443–452. <https://doi.org/10.1016/j.ttbdis.2017.02.001>
- Faulde, M.K., Rutenfranz, M., Hepke, J., Rogge, M., Görner, A., Keth, A., 2014. Human tick infestation pattern, tick-bite rate, and associated *Borrelia burgdorferi* s.l. infection risk during occupational tick exposure at the Seedorf military training area, northwestern Germany. *Ticks Tick. Borne. Dis.* <https://doi.org/10.1016/j.ttbdis.2014.04.009>
- Fernández-Soto, P., 2003. Garrapats que parasitan a personas en Castilla y León, determinación por serología de su parasitismo y detección molecular de los patógenos que albergan.
- Fernández-Soto, P., Pérez-Sánchez, R., Álamo-Sanz, R., Encinas-Grandes, A., 2006. Spotted fever group rickettsiae in ticks feeding on humans in northwestern Spain: Is *Rickettsia conorii* vanishing? *Ann. N. Y. Acad. Sci.* 1078, 331–333. <https://doi.org/10.1196/annals.1374.063>
- Fernández de Mera, I.G., Ruiz-Fons, F., de la Fuente, G., Mangold, A.J., Gortázar, C., de la Fuente, J., 2013. Spotted fever group rickettsiae in questing ticks, central Spain. *Emerg. Infect. Dis.* 19, 1163–1165. <https://doi.org/10.3201/eid1907.130005>
- Ghosh, S., Azhahianambi, P., Yadav, M.P., 2007. Upcoming and future strategies of tick control: A review. *J. Vector Borne Dis.* 44, 79–89.
- Gil-Collado, J., Guillén, J. L., y Zapatero, L.M., 1979. Claves para la identificación de los Ixodoidea españoles (adultos). *Rev. Ibérica Parasitol.* 39, 107–118.
- Gilbert, L., 2010. Altitudinal patterns of tick and host abundance: A potential role for climate change in regulating tick-borne diseases? *Oecologia* 162, 217–225. <https://doi.org/10.1007/s00442-009-1430-x>
- Gortazar, C., Reperant, L.A., Kuiken, T., de la Fuente, J., Boadella, M., Martínez-Lopez, B., Ruiz-Fons, F., Estrada-Peña, A., Drosten, C., Medley, G., Ostfeld, R., Peterson, T., VerCauteren, K.C., Menge, C., Artois, M., Schultsz, C., Delahay, R., Serra-Cobo, J., Poulin, R., Keck, F., Aguirre, A.A., Henttonen, H.,

- Dobson, A.P., Kutz, S., Lubroth, J., Mysterud, A., 2014. Crossing the Interspecies Barrier: Opening the Door to Zoonotic Pathogens. *PLoS Pathog.* 10. <https://doi.org/10.1371/journal.ppat.1004129>
- Hjetland, R., Eliassen, K.E., Lindbæk, M., Nilsen, R.M., Grude, N., Ulvestad, E., 2013. Tick bites in healthy adults from western Norway: Occurrence, risk factors, and outcomes. *Ticks Tick. Borne. Dis.* <https://doi.org/10.1016/j.ttbdis.2013.02.003>
- Hügli, D., Moret, J., Rais, O., Moosmann, Y., Erard, P., Malinverni, R., Gern, L., 2009. Tick bites in a Lyme borreliosis highly endemic area in Switzerland. *Int. J. Med. Microbiol.* 299, 155–160. <https://doi.org/10.1016/j.ijmm.2008.06.001>
- Jahfari, S., Hofhuis, A., Fonville, M., van der Giessen, J., van Pelt, W., Sprong, H., 2016. Molecular Detection of Tick-Borne Pathogens in Humans with Tick Bites and Erythema Migrans, in the Netherlands. *PLoS Negl. Trop. Dis.* 10, 1–15. <https://doi.org/10.1371/journal.pntd.0005042>
- JF, B., SA, O., MA, C.-N., A, H., RA, J., F, V., P, A., AL, G.-P., 2011. Differences in questing tick species distribution between Atlantic and continental climate regions in Spain. *J. Med. Entomol.* 48, 13–19. <https://doi.org/10.1603/ME10079>
- Lernout, T., De Regge, N., Tersago, K., Fonville, M., Suin, V., Sprong, H., 2019. Prevalence of pathogens in ticks collected from humans through citizen science in Belgium. *Parasit. Vectors* 12, 1–11. <https://doi.org/10.1186/s13071-019-3806-z>
- Lindblom, A., Wallménus, K., Sjöwall, J., Fryland, L., Wilhelmsson, P., Lindgren, P.E., Forsberg, P., Nilsson, K., 2016. Prevalence of rickettsia spp. in ticks and serological and clinical outcomes in tick-bitten individuals in Sweden and on the Åland Islands. *PLoS One* 11, 1–14. <https://doi.org/10.1371/journal.pone.0166653>
- Lindblom, P., Wilhelmsson, P., Fryland, L., Sjöwall, J., Haglund, M., Matussek, A., Ernerudh, J., Vene, S., Nyman, D., Andreassen, Å., Forsberg, P., Lindgren, P.E., 2014. Tick-borne encephalitis virus in ticks detached from humans and follow-up of serological and clinical response. *Ticks Tick. Borne. Dis.* <https://doi.org/10.1016/j.ttbdis.2013.07.009>

- Merino, F.J., Nebreda, T., Serrano, J.L., Fernández-Soto, P., Encinas, A., Pérez-Sánchez, R., 2005. Tick species and tick-borne infections identified in population from a rural area of Spain. *Epidemiol. Infect.* 133, 943–949. <https://doi.org/10.1017/S0950268805004061>
- Monsalve-Arteaga, L., Alonso-Sardón, M., Muñoz Bellido, J.L., Vicente Santiago, M.B., Vieira Lista, M.C., López Abán, J., Muro, A., Belhassen-García, M., 2020. Seroprevalence of Crimean-Congo hemorrhagic fever in humans in the World Health Organization European region: A systematic review. *PLoS Negl. Trop. Dis.* 14, e0008094. <https://doi.org/10.1371/journal.pntd.0008094>
- Monsalve Arteaga, L., Muñoz Bellido, J.L., Negro, A.I., García Criado, J., Vieira Lista, M.C., Sánchez Serrano, J.Á., Vicente Santiago, M.B., López Bernús, A., de Ory Manchón, F., Sánchez Seco, M.P., Leralta, N., Alonso Sardón, M., Muro, A., Belhassen-García, M., 2021. New circulation of genotype V of Crimean-Congo haemorrhagic fever virus in humans from Spain. *PLoS Negl. Trop. Dis.* 15, e0009197. <https://doi.org/10.1371/journal.pntd.0009197>
- MT, M., V, D., S, P., C, G., 1999. Tick species parasitizing people in an area endemic for tick-borne diseases in north-western Italy. *Parassitologia* 41, 555–560.
- Nava, S., Beati, L., Venzal, J.M., Labruna, M.B., Szabó, M.P.J., Petney, T., Saracho-Bottero, M.N., Tarragona, E.L., Dantas-Torres, F., Silva, M.M.S., Mangold, A.J., Guglielmo, A.A., Estrada-Peña, A., 2018. *Rhipicephalus sanguineus* (Latreille, 1806): Neotype designation, morphological re-description of all parasitic stages and molecular characterization. *Ticks Tick. Borne. Dis.* 9, 1573–1585. <https://doi.org/10.1016/j.ttbdis.2018.08.001>
- Pańczuk, A., Tokarska-Rodak, M., Mikuláková, W., Kendrová, L., Magurová, D., 2019. Exposure to ticks and undertaking lyme borreliosis prevention activities among students from Poland and Slovakia. *Ann. Agric. Environ. Med.* 26, 217–221. <https://doi.org/10.26444/aaem/99089>
- QGIS.org, 2022. QGIS Geographic Information System. QGIS Association. <http://www.qgis.org>
- R Core Team. 2021. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL <https://www.R-project.org/>.

- Remesar, S., Díaz, P., Portillo, A., Santibáñez, S., Prieto, A., Díaz-Cao, J.M., López, C.M., Panadero, R., Fernández, G., Díez-Baños, P., Oteo, J.A., Morrondo, P., 2019a. Prevalence and molecular characterization of *Rickettsia* spp. in questing ticks from north-western Spain. *Exp. Appl. Acarol.* 79, 267–278. <https://doi.org/10.1007/s10493-019-00426-9>
- Remesar, S., Fernández, P.D., Venzal, J.M., Pérez-Creo, A., Prieto, A., Estrada-Peña, A., López, C.M., Panadero, R., Fernández, G., Díez-Baños, P., Morrondo, P., 2019b. Tick species diversity and population dynamics of *Ixodes ricinus* in Galicia (north-western Spain). *Ticks Tick. Borne. Dis.* 10, 132–137. <https://doi.org/10.1016/j.ttbdis.2018.09.006>
- Requena-García, F., Cabrero-Sañudo, F., Olmeda-García, S., González, J., Valcárcel, F., 2017. Influence of environmental temperature and humidity on questing ticks in central Spain. *Exp. Appl. Acarol.* 71, 277–290. <https://doi.org/10.1007/s10493-017-0117-y>
- Rizzoli, A., Silaghi, C., Obiegala, A., Rudolf, I., Hubálek, Z., Földvári, G., Plantard, O., Vayssier-Taussat, M., Bonnet, S., Špitalská, E., Kazimírová, M., 2014. *Ixodes ricinus* and its transmitted pathogens in urban and peri-urban areas in Europe: New hazards and relevance for public health. *Front. Public Heal.* 2, 1–26. <https://doi.org/10.3389/fpubh.2014.00251>
- Robertson, J.N., Gray, J.S., Stewart, P., 2000. Tick bite and Lyme borreliosis risk at a recreational site in England. *Eur. J. Epidemiol.* 16, 647–652. <https://doi.org/10.1023/A:1007615109273>
- Rochlin, I., Toledo, A., 2020. Emerging tick-borne pathogens of public health importance: A mini-review. *J. Med. Microbiol.* 69, 781–791. <https://doi.org/10.1099/jmm.0.001206>
- Weisshaar, E., Schaefer, A., Scheidt, R.R.W., Bruckner, T., Apfelbacher, C.J., Diepgen, T.L., 2006. Epidemiology of tick bites and borreliosis in children attending kindergarten or so-called “forest kindergarten” in southwest Germany. *J. Invest. Dermatol.* 126, 584–590. <https://doi.org/10.1038/sj.jid.5700160>
- Wickham, H. 2016. *ggplot2: Elegant Graphics for Data Analysis*. Springer-Verlag New York.

Wilhelmsson, P., Lindblom, P., Fryland, L., Nyman, D., Jaenson, T.G., Forsberg, P., Lindgren, P.E., 2013.

Ixodes ricinus ticks removed from humans in Northern Europe: Seasonal pattern of infestation, attachment sites and duration of feeding. *Parasites and Vectors* 6, 1–11.

<https://doi.org/10.1186/1756-3305-6-362>

Yitayew, D., Samuel, D., 2015. Tick borne hemoparasitic diseases of ruminants: a review. *Adv. Biol. Res.*

(Rennes). 9, 210–224. <https://doi.org/10.5829/idosi.abr.2015.9.4.9516>