Original article

Tick species diversity and population dynamics of *Ixodes ricinus* in Galicia (north-western Spain)

Susana Remesar[a], Pablo Díaz Fernández[a,*], Jose Manuel Venzal[b], Ana Pérez-Creo[a],
Alberto Prieto[a], Agustín Estrada-Peña[c], Ceferino Manuel López[a], Rosario Panadero[a],
Gonzalo Fernández[a], Pablo Díez-Baños[a], Patrocinio Morrondo[a]

[a] *Departamento de Patología Animal (Grupo INVESAGA), Facultad deVeterinaria, Universidade de Santiago de Compostela, Lugo, Spain*
[b] *Laboratorio de Vectores y enfermedades transmitidas, Facultad de Veterinaria, Universidad de la República, CENUR Litoral Norte, Salto, Uruguay*
[c] *Department of Parasitology, Faculty of Veterinary Medicine, Miguel Servet, Zaragoza, Spain*

ARTICLE INFO

ABSTRACT

Ticks are the major arthropod vectors of animal and human pathogens in Europe; thus, information of tick species and their phenology allows identifying those areas and periods involving a high risk of exposure to particular tick-borne pathogens. In order to assess the diversity of exophilic tick species in north-western Spain, questing ticks were collected by flagging in 17 different locations; ticks were microscopically and molecularly identified at the 16S rDNA loci. In addition, phenology of questing ticks in the same region was studied in representative sampling areas of three different ecological regions, where ticks were monthly collected in 300-m transects during two complete years. The possible influence of different climate variables on the normalized accumulated fraction of nymphal and adult *Ixodes ricinus* was assessed using a generalized linear mixed model (GLMN).

I. ricinus was the most frequently and widely distributed tick species; *Dermacentor marginatus*, *Dermacentor reticulatus*, *Ixodes frontalis* and *Ixodes acuminatus* were sporadically found. Nymphs were the most commonly collected stage. Both nymphs and adults of *I. ricinus* presented a unimodal activity pattern; density showed a peak in late spring-early summer whereas the lowest levels were found at late summer-early autumn. GLMN showed that both nymphal and adult *I. ricinus* phenology was directly associated with the accumulated temperature, photoperiod and average daily maximum temperature and inversely associated with average daily maximum relative humidity.

This information could be used to predict the annual periods associated with a higher exposure and risk of transmission of pathogens and to complete the global map of distribution of tick species.

1. Introduction

Ticks are considered the major arthropod vectors in Europe and the USA due to the wide variety of human and animal pathogens they can transmit (Jongejan and Uilenberg, 2004). Ixodid ticks are the main vectors of zoonotic pathogens in Europe (Gilbert, 2010) where *Ixodes ricinus* is of major concern since it is the most widely distributed tick (Estrada-Peña et al., 2004; Rizzoli et al., 2014) and the main vector of important zoonotic pathogens such as Lyme borreliosis (LB) spirochetes, the tick-borne encephalitis virus or *Babesia* spp. (Pérez et al., 2012). A significant proportion of Ixodidae, including *I. ricinus*, are three-host ticks in which each stage quests for a host so that the availability of different hosts facilitates the completion of the tick life cycle (Medlock et al., 2013). Since a particular pathogen is usually more efficiently transmitted by certain tick species, the information of their distribution patterns and phenology is key to identify those areas and periods involving a high risk of exposure to particular tick-borne pathogens, promoting prevention measures (Parola and Raoult, 2001; Barandika et al., 2011).

The spread of some species of ticks has been reported in the last years in Europe (Jaenson and Lindgren, 2011; Medlock et al., 2013; Mierzejewska et al., 2017). Micro-climate features and relative abundance of some key hosts impact the seasonal patterns of tick activity, their distribution, and the persistence of foci of pathogens (Alonso-Carné et al., 2015). Both temperature and relative humidity affect tick questing and survival rates (Parola and Raoult, 2001; Randolph, 2004;

Lindgren and Jaenson, 2006; Ruiz-Fons et al., 2012) and determine the duration of questing periods as well as the territory with suitable environmental conditions. Although extensive information on the presence and distribution of exophilic ticks in Spain is available (Estrada-Peña et al., 2004; García-Sanmartín et al., 2008; Márquez, 2008; Barandika et al., 2011; Fernández de Mera et al., 2013; Espí et al., 2017; Requena-García et al., 2017), data from Galicia (north-western Spain) are still limited.

This study assesses the diversity of questing tick species in northwestern Spain, from data obtained by flagging in 17 different locations. In addition, a two-year longitudinal study based on monthly sampling of three ecologically different areas was performed, aiming to capture the phenology of *I. ricinus* and the probable regulatory variables. This information is essential to understand the reasons behind the reported increase in LB incidence and to issue adequate self-protection measures in humans, improving the control of transmission tick-borne pathogens.

2. Material and methods

2.1. Study area

This study was performed in Galicia (43°47′N–41°49′N, 6°42′W–9°18′W), a 29,574 km^2 region located in north-western Spain (Fig. 1). The climate of the study area is Oceanic-Atlantic, characterized by mild temperatures (annual mean = 13.3 °C) and high precipitations (annual mean = 1180 mm) without a clear seasonality (Naranjo and Pérez-Muñuzuri, 2006). The landscape is mainly composed of meadows (*Poa pratensis*, *Lolium perenne* and *Festuca pratensis*) and large autochthonous forest areas, with *Quercus robur* and *Castanea sativa* as the more common species (Pato et al., 2013). Many areas of natural forests were, however, replaced by non-autochthonous *Eucalyptus* sp.

2.2. Study 1. Diversity of tick species

The purpose of this part of the study was to ascertain the diversity of exophilic tick fauna in the region. Questing ticks were collected in April and May 2015, by flagging at 17 different locations. A 1-m^2 piece of white cotton flannel was used and the ticks on the flag were removed every 2–5 m, depending on tick density, and stored in plastic tubes with ethanol 70%. The total flagging time, excluding the removal of sampled ticks from the flag, was 30 min at each site. Collections were always performed by the same two trained investigators.

Collected ticks were microscopically identified using reference keys (Pérez-Eid, 2007). To confirm the microscopic identifications, a subset of each morphologically-identified species was further molecularly characterized based on the sequence of *16S* rRNA gene (Simon et al., 1994; Norris et al., 1996). DNA was previously extracted using a commercial kit (High Pure PCR Template Preparation Kit, Roche Diagnostics GmbH®, Mannheim, Germany) following the manufacturers' instructions. Selected 16S rDNA fragments were purified and sequenced on an ABI 3730xl sequencer (Applied Biosystems, Foster City, CA, USA) at the Sequencing and Fragment Analysis Unit of the Santiago de Compostela University. Sequences were aligned and edited using ChromasPro (Technelysium, Brisbane, Australia) and consensus sequences were then scanned against the GenBank database using BLAST. Unique sequences identified in this study were deposited in GenBank under accession numbers MH645512-MH645522.

2.3. Study 2. Tick population dynamics

The study of the phenology of questing ticks was addressed in three different ecological regions (Coast, Plateau and Mountain) previously described by Lago et al. (2012). One single representative sampling
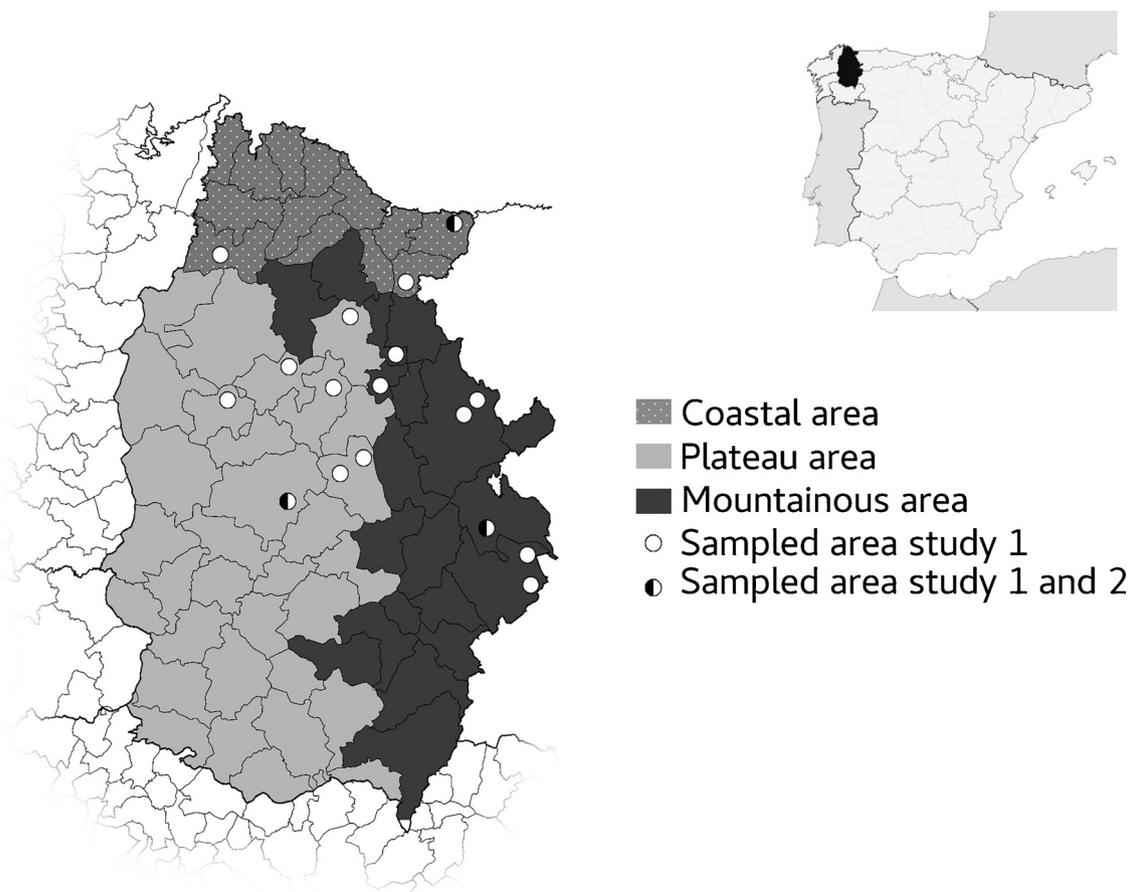

Fig. 1. Map of the sampling locations and defined geographic areas in the study (Galicia, NW Spain).

Table 1
Number of ticks captured in the monthly collections (study 2), including the species, development stage, sampling area and year of sampling.

	Sampling sites			Year	
	1 (Plateau)	2 (Mountain)	3 (Coast)	Year 1	Year 2
I. ricinus					
Larvae	143	64	445	297	355
Nymphs	747	1,368	3,652	2,868	2,899
Males	33	114	80	108	119
Females	54	126	80	145	115
TOTAL	**977**	**1,672**	**4,257**	**3,418**	**3,488**
D. marginatus					
Males	0	1	3	3	1
Females	0	12	0	8	4
TOTAL	**0**	**13**	**3**	**11**	**5**
D. reticulatus					
Males	7	0	0	5	2
Females	10	3	0	10	3
TOTAL	**17**	**3**	**0**	**15**	**5**
I. frontalis					
Larvae	0	0	1	0	1
Nymphs	10	2	2	5	9
TOTAL	**10**	**2**	**3**	**5**	**10**
I. acuminatus					
Males	0	0	1	0	1
TOTAL	**1,004**	**1,690**	**4,264**	**3,449**	**3,509**

area was chosen in each region (Fig. 1).

2.4. Tick collection and identification

Ticks were collected monthly in each of the 3 selected areas during two complete years (November 2015-November 2017) using the same protocol as in the previous part of the study, but instead of a 30 min sampling, transects of 300 m were used. Thus, tick density was calculated on the basis of the number of specimens collected in transects of 300 m. Tick identification was performed as previously described.

Tick density was re-scaled to the monthly accumulated fraction of nymphs and adult ticks, for each month, species, year and sampling area as described elsewhere (Alonso-Carné et al., 2016). This procedure allows normalizing the variability between years and sites as well as detecting the recruitment of active ticks.

2.5. Meteorological and remotely sensed data collection

The effect of climate parameters on tick phenology was explored using data from climate recording stations representative for each sampling site. The station nearest to the sampling area and showing a similar altitude was selected; thus, selected stations were 1–7 km away from the flagging areas. For each site, daily values of mean, maximum and minimum air temperature (ºC) and relative humidity (%), dew point (ºC) and evapo-transpiration (l/m^2) as well as days of ground frost were obtained from Meteogalicia (http://www.meteogalicia.gal/web/index.action); average of daily values calculated between samplings were used. Saturation deficit (SD, in mm Hg) was calculated using both temperature (T, in degrees Celsius) and relative humidity (RH, in %) with the formula: $SD = (1\text{-}RH/100)*4.9463e^{0.0621T}$. NDVI data was obtained from the MODIS series of satellites using the MOD44 product available at the Global Agriculture Monitoring (GLAM) Project (http://pekko.geog.umd.edu/usda/test/); NDVI data obtained were the mean of an area of 5 km^2 (the maximum resolution provided) during a period of 16 days. Data on daily photoperiod were calculated from the NOAA Sunrise/Sunset and Solar Position Calculators, available in the webpage of the Earth System Research Laboratory (https://www.esrl.noaa.gov/gmd/grad/solcalc/calcdetails.html). Photoperiod data were expressed

as the number of daylight hours of each sampling day according to the coordinates of the site.

2.6. Statistical methods

Data on tick density, weather data and remotely sensed NDVI values were analysed for correlations using R statistical software (R Core Team, 2017). The number of nymphs and adults of *I. ricinus* were firstly tested for normality using the Shapiro-Wilk normality test. Since data did not follow a normal distribution, they were then log-normalized using the formula log (1 + x). To assess the possible influence of the different variables studied on the normalized accumulated fraction of nymphs and adult ticks, Generalized Linear Mixed Models (GLMN) were performed using the R-packages "lme4″ and "lmerTest". Both month of sampling and ecological region were considered as random variables. Factors were removed based on AIC (Akaike Information Criteria) using a backward conditional method from the initial model until the best one was built. T-values for each variable included in the final model as well as the correlation between them were also obtained.

3. Results

For the study 1 (diversity of tick faunal composition), a total of 2458 ticks were collected from vegetation. *Ixodes ricinus* (86 larvae, 2017 nymphs, 182 female and 162 male ticks) was the most frequently and widely distributed identified tick species (99.55% of the total of specimens), since it was found in all sampling areas. In addition, seven females and three males of *Dermacentor marginatus* (0.41%) were only found in the mountain area, whereas a single male of *Dermacentor reticulatus* (0.04%) was also collected in the plateau area.

For the study 2 (tick population dynamics), a total of 6958 ticks were collected in the three selected locations (Table 1). Five ixodid species were found: *I. ricinus* was the most abundant (99.25%) followed by *D. marginatus* (0.29%), *D. reticulatus* (0.23%), *Ixodes frontalis* (0.22%) and *Ixodes acuminatus* (0.01%). Molecular analysis confirmed the morphological species identification, although most isolates showed single nucleotide polymorphisms when compared to reference sequences deposited in GenBank except for *I. acuminatus* (Supplementary Table 1). The sequence of *I. acuminatus* showed a 99% identity (440/445 bp) with a single sequence of *I. ricinus* (JN248424). Nevertheless, it showed a 95% (421/443) identity with *Ixodes laguri* (KR870981) and no more than 93% (415/446) with most of *I. ricinus* sequences deposited in GenBank.

Only adults of *D. reticulatus* and *D. marginatus* were collected by flagging, mostly during the first year of study (Table 1). The male/female ratio remained roughly constant in both species and during both years; *D. marginatus* male/female ratio was 1:2.7 and 1:4 in the first and second year respectively, whereas a ratio of 1:2 and 1:1.5 was observed for *D. reticulatus* in each year. *Dermacentor marginatus* was consistently found in the mountain and at the coast, whereas *D. reticulatus* was only detected in the plateau area (Table 1).

All but one specimen of *I. frontalis* were nymphs collected in all sampling areas mainly during the second year of study (Table 1). One single male of *I. acuminatus* was collected in September 2017.

Nymphs were the most commonly collected stage of *I. ricinus*. The total number of *I. ricinus* collected as well as the proportions of each developmental stage were almost identical in both sampling years (Table 1); thus, the male/female/nymph/larvae ratio was 1:1.3:26.6:2.8 during the first year and 1:0.9:24.4:3.0 during the second year. *Ixodes ricinus* was always present in the surveys, independently of the month and location, except for the samplings carried out in March 2016 and January 2017 in the mountain area.

Regardless the area of collection, the density of all questing stages of *I. ricinus* dramatically rose in late spring-early summer (Fig. 2); thus, the number of ticks collected in May represented the 20–39% of the total nymphs and adults for each site and year of sampling. The number of
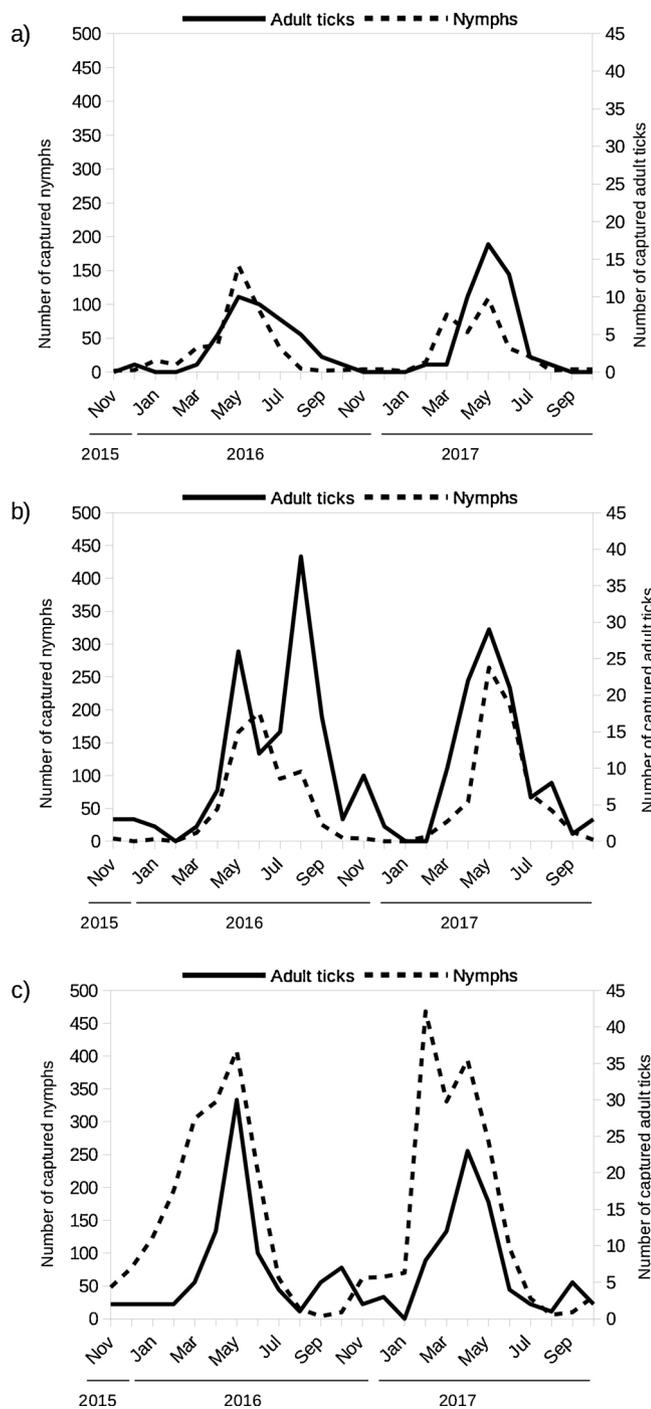
a)

b)

c)

Fig. 2. Number of nymphs and adults of *I. ricinus* when considering the month of collection and sampling area. a) Plateau, b) Mountain, c) Coast.

questing nymphs and adults began to decrease in summer, achieving its lowest levels at late summer-early autumn. During 2017, the nymphs of *I. ricinus* followed a bimodal peak of abundance. In the plateau area (Fig. 2), these peaks were observed in March and in May (representing the 24.6% and 31.6% of the specimens captured that year, respectively). At the coast, the two peaks showed by the nymphs appeared in February and April (25.3% and the 21.3% of all nymphal *I. ricinus* collected that year, respectively). During 2016, both nymphs and adults from the mountain showed a first peak in June (29.6%) and May (20.2%), respectively, and a higher second peak in August (16.0% and 30.2% of the total number of nymphs and adults, respectively).

The analysis of the effect of the climate parameters on the

Table 2
GLMN results for the density of nymphs and adults of *I. ricinus*. Factors were removed following the AIC until the best model was built.

	Estimate	Std.Error	Df	T-value	Pr(> | t |)
Model for questing adult *I. ricinus*					
(Intercept)	0.0314	0.1604	43.420	0.196	0.84546
Accumulated temperature	0.6709	0.0376	37.110	17.834	< 2e-16
Photoperiod	0.0004	0.0001	32.480	5.616	3.15e-06
Maximum temperature	0.0114	0.0023	46.480	4.764	1.90e-05
Maximum relative humidity	− 0.0053	0.0017	45.630	− 3.100	0.00331
Model for questing *I. ricinus* nymphs					
(Intercept)	− 0.1740	0.1727	64.540	− 1.008	0.31732
Accumulated temperature	0.6512	0.0463	43.770	14.053	< 2e-16
Photoperiod	0.0006	0.0001	35.050	6.535	1.52e-07
Maximum temperature	0.0101	0.0025	66.560	4.095	0.00012
Maximum relative humidity	− 0.0040	0.0018	66.100	− 2.300	0.02461

phenology of tick population was addressed only for nymphs and adults of *I. ricinus* ticks, since the number of *Dermacentor* spp., *I. frontalis* and *I. acuminatus* was insufficient to perform further statistics. Larvae tend to have an irregular distribution and are therefore not suitable for these analyses. The results of the Generalized Linear Mixed Models showed that both nymphal and adult *I. ricinus* phenology was mainly associated with the accumulated temperature, photoperiod, average daily maximum temperature and average daily maximum relative humidity (Table 2).

The models for both nymphs and adults identified the accumulated temperature as the main trait influencing the phenology of *I. ricinus*. The photoperiod and the average daily maximum temperature also have a positive effect on the phenology of both stages of *I. ricinus*. In contrast, the density of *I. ricinus* decreases with increasing average daily maximum relative humidity (Table 2).

4. Discussion

This study addressed both the faunal diversity of exophilic ticks and the annual distribution of questing *I. ricinus* in an area of north-western Spain. The faunal diversity of ticks in the surveyed region was low, probably because of the uniform climate, type of vegetation, and host faunal composition present in the sampling sites. The results of both studies reveal that *I. ricinus* is the main tick species in the surveyed area, being clearly predominant over others. These results are consistent with those reported in other northern areas of Spain (García-Sanmartín et al., 2008; Barandika et al., 2011; Espí et al., 2017) as well as in northern and central European countries (Gilot et al., 1975; Estrada-Peña et al., 2004). Moreover, *I. ricinus* was also the main tick species found on roe deer (*Capreolus capreolus*) in the same study area (Vázquez et al., 2011). Some species such as *Haemaphysalis concinna, H. punctata, H. inermis* and *Rhipicephalus bursa* were previously detected in other northern regions of Spain (Oteo et al., 2006; García-Sanmartín et al., 2008; Barandika et al., 2011; Espí et al., 2017). Since *I. ricinus* requires high relative humidity for survival in the environment (Medlock et al., 2013), other tick species such as *R. bursa, Rhipicephalus sanguineus* s.l., *Hyalomma lusitanicum* and *Dermacentor marginatus* have been reported as being more prevalent than *I. ricinus* in drier regions of Spain (Márquez, 2008; Fernández de Mera et al., 2013; Requena-Garcia et al., 2017). These results imply a potential exposure for both humans and animals, since *I. ricinus* is considered the main vector of important pathogens such as *Borrelia burgdorferi* s.l., *Anaplasma phagocytophilum* and tick-borne encephalitis virus (Pérez et al., 2012). Thus, the high number of questing *I. ricinus* found in this survey, together with the high

prevalence of *B. burgdorferi* s.l. in ticks detected in a previous survey (Díaz et al., 2017) are consistent with the reported incidence of LB in Galicia (Vázquez-López et al., 2015).

Two *Dermacentor* species were also identified, but only adult ticks were found. It has been reported that *D. marginatus* shows a wide distribution in Spain (Márquez, 2008; Toledo et al., 2009; Fernández de Mera et al., 2013; Requena-Garcia et al., 2017), being mainly associated with dry regions with dense bush (Estrada-Peña et al., 2004). In contrast, *D. reticulatus* has been usually related to humid open-type habitats in northern and southern Europe with its southern distribution in the south of Portugal (Estrada-Peña et al., 2004; García-Sanmartín et al., 2008; Barandika et al., 2011; Földvári et al., 2016; Rubel et al., 2016; Espí et al., 2017). Both *Dermacentor* species have a different vectorial role than *I. ricinus*, being mainly identified as vectors of *Rickettsia* species (Masala et al., 2012). Two other species of the genus *Ixodes* (*I. frontalis* and *I. acuminatus*) were sporadically found. Since they may have an exophilic behaviour, they have also been occasionally found by flagging in previous investigations (Schorn et al., 2011; Bona and Stanko, 2013; Espí et al., 2017). Regarding the molecular identification of *I. acuminatus*, the close match with a single *I. ricinus* sequence (JN248424) is probably due to incorrect identification of the GenBank entry.

It has been reported that *I. ricinus* displays two different patterns of activity in Europe, namely unimodal and bimodal (Cayol et al., 2017). We found a large variability in these patterns in the three collection sites and year of collection, probably related with the temperature of the winter previous to collections (Alonso-Carné et al., 2016). Thus, a single peak was observed between late spring and early summer, agreeing with most investigations carried out in European regions without marked seasonal climatic variations (Kurtenbach et al., 2006). Nevertheless, this was not consistently observed in the two years of collections. In regions from central Europe with low winter temperature, *I. ricinus* displays a bimodal questing activity reaching its maximum density in early spring and autumn for all stages of development (Kurtenbach et al., 2006). In our study, however, the bimodal pattern was not consistent in both years of study and the autumn peak was absent, suggesting that it may be a casual event rather than a repeated pattern.

The analysis of the phenology of *I. ricinus* within its distribution range has revealed a relationship between tick activity and features of the habitat (mainly the prevailing weather and the photoperiod) and the faunal composition of hosts (Estrada-Peña et al., 2004). Generalized Linear Mixed Models revealed that photoperiod and accumulated values of mean temperature had the most significant effect on *I. ricinus* populations in north-western Spain. It has been reported that temperature and relative humidity have a significant effect on questing, survival and development of *I. ricinus*, being the principal factors limiting their geographical range (Gray, 2008; Alonso-Carné et al., 2016). Since exophilic ticks must maintain their water content, unsaturated atmosphere leads to a loss of water from ticks and, as a result, they return to the moist lower levels of the vegetation to absorb water from the environment (Rudolph and Knülle, 1974). It has been suggested that photoperiod has a marked effect on tick populations. The increase in daylight hours stimulates the questing activity and interrupts both the developmental and behavioural diapause in engorged and unfed ticks, respectively (Belozerov, 1982; Estrada-Peña et al., 2013; Gray et al., 2016). In addition, the accumulated temperature was identified in other studies as the best predictor of questing larval activity in vegetation (Alonso-Carné et al., 2016) due to its positive effect on the development of *I. ricinus* eggs (Gray, 2008). Statistical analyses also showed a positive association between the density of nymphs and adults of *I. ricinus* and the maximum mean temperature, whereas an increase in the maximum mean relative humidity had a negative effect on *I. ricinus* populations. Those results may be due to the combined effect of both variables; thus, the smallest density of *I. ricinus* overlaps with periods with low maximum mean temperature and high maximum

mean humidity levels (mid-winter). The relative humidity has been identified as one of the major factors limiting the geographic range of *I. ricinus* (Gray et al., 2009; Alonso-Carné et al., 2015) and it has been suggested that ticks are vulnerable to extreme values of humidity when they are questing or in diapause (Estrada-Peña, 2008).

We did not find any relationship between values of NDVI and tick phenology. In this sense, Bisanzio et al. (2008) considered this variable as not suitable for determining the questing activity of *I. ricinus* nymphs. We found that SD, relative humidity and mean temperature did not have a significant influence on the phenology of *I. ricinus*, in contrast to other studies (Perret et al., 2000, 2003; Randolph et al., 2002; Estrada-Peña et al., 2004). The calculation of SD integrates the temperature and relative humidity, and it is related to the atmospheric drying power; for this reason, a number of studies included SD in modelling. Since *I. ricinus* can actively take up water from air when the mean relative humidity is greater than 85% (Kahl and Knülle, 1988), the lack of relationship between SD and density of both nymphs and adults might be due to the presence of mean relative humidity values above 85% during most of the study, thereby reducing the negative effect of SD over tick population.

5. Conclusions

Our results reveal that *I. ricinus* is the dominant ixodid tick in all sampled sites in Galicia (north-western Spain); in addition, the tick species diversity was low in that area. Since *I. ricinus* is one of the most important tick vectors of pathogens to both human and animals in Europe, further studies on the prevalence of pathogens transmitted by *I. ricinus* should be addressed in the region.

Nymphs and adults of *I. ricinus* present a unimodal activity pattern in the study area, showing the highest density from late spring to early summer. Its density is mainly correlated with both photoperiod and accumulated temperature. These clues could result in a framework to pinpoint the annual periods of critical temperatures in which populations of the tick are recruited, resulting in a higher exposure and risk of transmission of pathogens.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.ttbdis.2018.09.006.

References

Alonso-Carné, J., Garcia-Martin, A., Estrada-Pena, A., 2015. Assessing the statistical relationships among water-derived climate variables, rainfall, and remotely sensed features of vegetation: implications for evaluating the habitat of ticks. Exp. Appl. Acarol. 65, 107–124.

Alonso-Carné, J., Garcia-Martin, A., Estrada-Pena, A., 2016. Modelling the phenological relationships of questing immature *Ixodes ricinus* (Ixodidae) using temperature and NDVI data. Zoonoses Public Health 63, 40–52.

Barandika, J.F., Olmeda, S.A., Casado-Nistal, M.A., Hurtado, A., Juste, R.A., Valcarcel, F., Anda, P., Garcia-Perez, A.L., 2011. Differences in questing tick species distribution between Atlantic and Continental climate regions in Spain. J. Med. Entomol. 48, 13–19.

Belozerov, V.N., 1982. Diapause and biological rythms in ticks. In: Obenchain, F.D., Galum, R. (Eds.), Physiology of Ticks. Pergamon Press, New York.

Bisanzio, D., Amore, G., Ragagli, C., Tomassone, L., Bertolotti, L., Mannelli, A., 2008. Temporal variations in the usefulness of normalized difference vegetation index as a predictor for *Ixodes ricinus* (Acari:Ixodidae) in a *Borrelia lusitaniae* focus in Tuscany, central Italy. J. Med. Entomol. 45, 547–555.

Bona, M., Stanko, M., 2013. First records of the tick *Ixodes frontalis* (Panzer, 1795) (Acari,

Ixodidae) in Slovakia. Tick Tick Borne Dis. 4.

Cayol, C., Koskela, E., Mappes, T., Siukkola, A., Kallio, E.R., 2017. Temporal dynamics of the tick *Ixodes ricinus* in northern Europe: epidemiological implications. Parasit. Vectors 10.

Díaz, P., Arnal, J.L., Remesar, S., Perez-Creo, A., Venzal, J.M., Vazquez-Lopez, M.E., Prieto, A., Fernández, G., López, C.M., Panadero, R., Benito, A., Diez-Banos, P., Morrondo, P., 2017. Molecular identification of *Borrelia* spirochetes in questing *Ixodes ricinus* from northwestern Spain. Parasit. Vectors. 10.

Earth System Research Laboratory, 2018. https://www.esrl.noaa.gov/ Last access: 04/04/2018.

Espí, A., Del Cerro, A., Somoano, A., Garcia, V., Prieto, J.M., Barandika, J.F., Garcia-Perez, A.L., 2017. B*orrelia 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. Enferm. Infecc. Microbiol. Clin. 35, 563–568.

Estrada-Peña, A., 2008. Climate, niche, ticks, and models: what they are and how we should interpret them. Parasitol. Res. 1, 87–95.

Estrada-Peña, A., Martinez, J.M., Acedo, C.S., Quilez, J., Del Cacho, E., 2004. Phenology of the tick, *Ixodes ricinus*, in its southern distribution range (central Spain). Med. Vet. Entomol. 18, 387–397.

Estrada-Peña, A., Estrada-Sanchez, A., Estrada-Sanchez, D., de la Fuente, J., 2013. Assessing the effects of variables and background selection on the capture of the tick climate niche. Int. J. Health. Geogr. 12.

Fernández de Mera, I.G., Ruiz-Fons, F., de la Fuente, G., Mangold, A.J., Gortazar, C., de la Fuente, J., 2013. Spotted fever group *Rickettsiae* in questing ticks, Central Spain. Emerg. Infect. Dis. 19, 1163–1165.

Földvári, G., Siroky, P., Majoros, G., Szekeres, S., Sprong, H., 2016. *Dermacentor reticulatus*: a vector on the rise. Parasit. Vectors 9, 314.

García-Sanmartín, J., Barandika, J.F., Juste, R.A., García-Pérez, A.L., Hurtado, A., 2008. Distribution and molecular detection of *Theileria* and *Babesia* in questing ticks from northern Spain. Med. Vet. Entomol. 22, 318–325.

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.

Gilot, B., Pautou, G., Moncada, E., Ain, G., 1975. Première contribution à l'ètude èlogique d' *lxodes ricinus* (Linnè, 1758) (Acarina, lxodoidea) dans le Sud-Est de la France. Acta Trop. 32 (4), 340–347.

Global Agriculture Monitoring, 2018. http://glad.umd.edu/projects/global-agriculture-monitoring-glam-project Last Access: 04/04/2018.

Gray, J.S., 2008. *Ixodes ricinus* seasonal activity: implications of global warming indicated by revisiting tick and weather data. Int. J. Med. Microbiol. 298, 19–24.

Gray, J.S., Dautel, H., Estrada-Pena, A., Kahl, O., Lindgren, E., 2009. Effects of climate change on ticks and tick-borne diseases in Europe. Interdiscip. Perspect. Infect. Dis. 2009, 593232.

Gray, J., Kahl, O., Lane, R., Levin, M.L., Tsao, J.I., 2016. Diapause in ticks of the medically important *Ixodes ricinus* species complex. Tick Tick Borne Dis. 7, 992–1003.

Jaenson, T.G.T., Lindgren, E., 2011. The range of *Ixodes ricinus* and the risk of contracting Lyme borreliosis will increase northwards when the vegetation period becomes longer. Tick Tick Borne Dis. 2, 44–49.

Jongejan, F., Uilenberg, G., 2004. The global importance of ticks. Parasitology 129, S3–S14.

Kahl, O., Knülle, W., 1988. Water vapour uptake from subsaturated atmospheres by engorged immature ixodid ticks. Exp. Appl. Acarol. 4, 73–83.

Kurtenbach, K., Hanincova, K., Tsao, J.I., Margos, G., Fish, D., Ogden, N.H., 2006. Fundamental processes in the evolutionary ecology of Lyme borreliosis. Nat. Rev. Microbiol. 4, 660–669.

Lago, N., López, C., Panadero, R., Cienfuegos, S., Pato, J., Prieto, A., Díaz, Mourazos, P.N., Fernández, G., 2012. Seroprevalence and risk factors associated with Visna/Maedi virus in semi-intensive lamb-producing flocks in northwestern Spain. Prev. Vet. Med. 103, 163–169.

Lindgren, E., Jaenson, T., 2006. Lyme Borreliosis in Europe: Influences of Climate and Climate Change, Epidemiology, Ecology and Adaptation Measures. WHO Regional Office for Europe, Copenhagen.

Márquez, F.J., 2008. Spotted fever group *Rickettsia* in ticks from southeastern Spain natural parks. Exp. Appl. Acarol. 45, 185–194.

Masala, G., Chisu, V., Satta, G., Socolovschi, C., Raoult, D., Parola, P., 2012. *Rickettsia slovaca* from *Dermacentor marginatus* ticks in Sardinia, Italy. Tick Tick Borne Dis. 3, 392–394.

Medlock, J.M., Hansford, K.M., Bormane, A., Derdakova, M., Estrada-Pena, A., George, J.C., Golovljova, I., Jaenson, T.G.T., Jensen, J.K., Jensen, P.M., Kazimirova, M., Oteo, J.A., Papa, A., Pfister, K., Plantard, O., Randolph, S.E., Rizzoli, A., Santos-Silva, M.M., Sprong, H., Vial, L., Hendrickx, G., Zeller, H., Van Bortel, W., 2013. Driving forces for changes in geographical distribution of *Ixodes ricinus* ticks in Europe. Parasit. Vectors 6.

Meteogalicia, 2018. http://www.meteogalicia.gal/web/index.action. Last Access: 04/04/2018.

Mierzejewska, E.J., Estrada-Peña, A., Bajer, A., 2017. Spread of *Dermacentor reticulatus* is associated with the loss of forest area. Exp. Appl. Acarol. 72, 399–413.

Naranjo, L., Pérez-Muñuzuri, E., 2006. A variabilidade natural do clima en Galicia. Xunta de Galicia, Servicio Central, Santiago de Compostela.

Norris, D.E., Klompen, J.S.H., Keirans, J.E., Black, W.C., 1996. Population genetics of *Ixodes scapularis* (Acari: Ixodidae) based on mitochondrial 16S and 12S genes. J. Med. Entomol. 33, 78–89.

Oteo, J.A., Portillo, A., Santibáñez, S., Pérez-Martínez, L., Blanco, J.R., Jiménez, S., Ibarra, V., Pérez-Palacios, A., Sanz, M., 2006. Prevalence of spotted fever group *Rickettsia* species detected in ticks in La Rioja, Spain. Ann. N. Y. Acad. Sci. 1078, 320–323.

Parola, P., Raoult, D., 2001. Ticks and tickborne bacterial diseases in humans: an emerging infectious threat. Clin. Infect. Dis. 7, 80–83.

Pato, F.J., Panadero, R., Vazquez, L., Lopez, C.M., Diaz, P., Vazquez, E., Diez-Banos, P., Morrondo, P., Fernandez, G., 2013. Seroprevalence of *Borrelia burgdorferi sensu lato* in roe deer (*Capreolus capreolus*) from Northwestern Spain. S. J. Zoo Wildl. Med. 44, 660–665.

Pérez, D., Kneubuhler, Y., Rais, O., Gern, L., 2012. Seasonality of *Ixodes ricinus* ticks on vegetation and on rodents and *Borrelia burgdorferi sensu lato* genospecies diversity in two Lyme Borreliosis-Endemic areas in Switzerland. Vector Borne Zoon. Dis. 12, 633–644.

Pérez-Eid, C., 2007. Les tiques. Identification, biologie, importance médicale et vétérinarire. Editions TEC & DOC, Lavoisier, Paris.

Perret, J.L., Guigoz, E., Rais, O., Gern, L., 2000. Influence of saturation deficit and temperature on *Ixodes ricinus* tick questing activity in a Lyme borreliosis-endemic area (Switzerland). Parasitol. Res. 86, 554–557.

Perret, J.L., Guerin, P.M., Diehl, P.A., Vlimant, M., Gern, L., 2003. Darkness induces mobility, and saturation deficit limits questing duration, in the tick *Ixodes ricinus*. J. Exp. Biol. 206, 1809–1815.

Randolph, S.E., 2004. Tick ecology: processes and patterns behind the epidemiological risk posed by ixodid ticks as vectors. Parasitology 129, S37–S65.

Randolph, S.E., Green, R.M., Hoodless, A.N., Peacey, M.F., 2002. An empirical quantitative framework for the seasonal population dynamics of the tick *Ixodes ricinus*. Int. J. Parasitol. Parasites Wildl. 32, 979–989.

R Core Team, 2017. R: A Language and Environment for Statistical Computing. https://www.R-project.org/.

Requena-Garcia, F., Cabrero-Sanudo, F., Olmeda-Garcia, S., Gonzalez, J., Valcarcel, F., 2017. Influence of environmental temperature and humidity on questing ticks in central Spain. Exp. Appl. Acarol. 71, 277–290.

Rizzoli, A., Silaghi, C., Obiegala, A., Rudolf, I., Hubálek, Z., Földvári, G., Plantard, O., Vayssier-Taussat, M., Bonnet, S., Spitalská, 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 Health 1, 251.

Rubel, F., Brugger, K., Pfeffer, M., Chitimia-Dobler, L., Didyk, Y.M., Leverenz, S., Dautel, H., Kahl, O., 2016. Geographical distribution of *Dermacentor marginatus* and *Dermacentor reticulatus* in Europe. Tick Tick Borne Dis. 7, 224–233.

Rudolph, D., Knülle, W., 1974. Site and mechanism of water-vapor uptake from atmosphere in Ixodid ticks. Nature 249, 84–85.

Ruiz-Fons, F., Fernandez-de-Mera, I.G., Acevedo, P., Gortazar, C., de la Fuente, J., 2012. Factors driving the abundance of *Ixodes ricinus* ticks and the prevalence of zoonotic *I. ricinus*-borne pathogens in natural foci. J. Appl. Environ. Microbiol. 78, 2669–2676.

Schorn, S., Schöl, H., Pfister, K., Silaghi, C., 2011. First record of *IxodesIxodes frontalis* collected by flagging in Germany. Tick Tick Borne Dis. 2, 228–230.

Simon, C., Frati, F., Beckenbach, A., Crespi, B., Liu, H., Flook, P., 1994. Evolution, weighting, and phylogenetic utility of mitochondrial gene-sequences and a compilation of conserved polymerase chain-reaction primers. Ann. Entomol. Soc. Am. 87, 651–701.

Toledo, A., Olmeda, A.S., Escudero, R., Jado, I., Valcarcel, F., Casado-Nistal, M.A., Rodriguez-Vargas, M., Gil, H., Anda, P., 2009. Tick-borne zoonotic bacteria in ticks collected from Central Spain. Am. J. Trop. Med. Hyg. 81, 67–74.

Vázquez, L., Panadero, R., Dacal, V., Pato, F.J., López, C., Díaz, P., Arias, M.S., Fernández, G., Diez-Baños, P., Morrondo, P., 2011. Tick infestation (Acari: Ixodidae) in roe deer (*Capreolus Capreolus*) from northwestern Spain: population dynamics and risk stratification. Exp. Appl. Acarol. 53, 399–409.

Vázquez-López, M.E., Pego-Reigosa, R., Díez-Morrondo, C., Castro-Gago, M., Díaz, P., Fernández, G., Morrondo, P., 2015. Epidemiology of Lyme disease in a healthcare area in north-west Spain. Gac. Sanit. 29, 213–216.