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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

Alberto Espí[a,*], Ana Del Cerro[a], Aitor Somoano[b], Verónica García[c], José M. Prieto[a], José F. Barandika[d], Ana L. García-Pérez[d]

[a] Servicio Regional de Investigación y Desarrollo Agroalimentario (SERIDA), Área de Sanidad Animal, Centro de Biotecnología Animal, Deva, Gijón, Asturias, Spain
[b] Servicio Regional de Investigación y Desarrollo Agroalimentario (SERIDA), Área de Cultivos Hortofrutícolas y Forestales, Villaviciosa, Asturias, Spain
[c] Servicio de Salud del Principado de Asturias, Hospital de Cabueñes, Gijón, Asturias, Spain
[d] NEIKER - Instituto Vasco de Investigación y Desarrollo Agrario, Derio, Bizkaia, Spain

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ABSTRACT

To determine the prevalence and diversity of *Borrelia burgdorferi* sensu lato (s.l.) in an endemic Nature Reserve (Sierra del Sueve) in North-Western Spain, and the risk of human exposure to infected ticks in Asturias, 1013 questing ticks and 70 small mammals were collected between 2012 and 2014. A retrospective descriptive analysis was also carried out on human Lyme borreliosis (LB) cases reported to the local hospital (Cabueñes).

Samples were screened for *B. burgdorferi* s.l. presence by a nested PCR assay, and genospecies were confirmed by sequencing. *B. burgdorferi* s.l. was detected in 1.4% (12/845) of *I. ricinus* questing nymphs, 9.1% (2/33) of questing adults, and 12.9% (9/70) of small mammals, as well as in the other tick species. PCR positive samples of 17 questing tick and 6 small mammals were sequenced. Four genospecies were identified: *B. afzelii*, *B. garinii*, *B. lusitaniae*, and *B. valaisiana*. Phylogenetic analyses based on the *fla*B gene showed the heterogeneity of *B. afzelii* in this area.

The detection of *B. burgdorferi* s.l. among questing ticks and small mammals in the study area, as well as the abundance of ticks and of large wild and domestic mammals, indicate a high risk of infection by *B. burgdorferi* s.l. in the area. Reporting of LB cases to the local hospital support this, and shows the need of thorough monitoring of *B. burgdorferi* infection in ticks and hosts in the area. More investigations are needed to assess the role of different wildlife species and the risk of transmission to humans.

Prevalencia y diversidad de *Borrelia burgdorferi* sensu lato en garrapatas y pequeños mamíferos de una Reserva Natural del noroeste de España donde la borreliosis de Lyme es endémica. Incidencia en las poblaciones humanas circundantes

Palabras clave:
Borreliosis de Lyme
Borrelia burgdorferi sensu lato
PCR
Garrapatas
Pequeños mamíferos

RESUMEN

Entre 2012 y 2014 se recogieron 1.013 garrapatas de la vegetación y 70 pequeños mamíferos en la Reserva Natural de la Sierra del Sueve (Asturias) y zonas colindantes, con el fin de determinar la prevalencia de *Borrelia burgdorferi* sensu lato (s.l.) y el riesgo de exposición humana a garrapatas infectadas en Asturias, área endémica de borreliosis de Lyme. También se incluye un estudio descriptivo y retrospectivo de pacientes diagnosticados de borreliosis en un hospital local (Hospital de Cabueñes, Gijón).

* Corresponding author.
E-mail address: aespi@serida.org (A. Espí).

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B. burgdorferi s.l. se detectó, mediante una PCR anidada, en el 1,4% (12/845) de las ninfas y en el 9,1% (2/33) de los adultos de la garrapata *I. ricinus*, en porcentajes variables de las restantes especies y en el 12,9% (9/70) de los pequeños mamíferos. Se secuenciaron un total de 17 muestras de garrapatas de la vegetación y 6 de pequeños mamíferos detectándose 4 genoespecies causantes de la borreliosis de Lyme: *B. afzelii*, *B. garinii*, *B. lusitaniae*, y *B. valaisiana*. Los análisis filogenéticos basados en el gen *fla*B mostraron la heterogeneidad de *B. afzelii* en el área de estudio.
La detección de *B. burgdorferi* s.l. en garrapatas de la vegetación y pequeños mamíferos de la zona de estudio, así como la gran abundancia de garrapatas y la presencia de grandes poblaciones de animales silvestres y domésticos, son indicativos de que el riesgo de infección en esta área es relevante. Este hecho está en consonancia con los casos de borreliosis de Lyme descritos en este estudio, mostrando la necesidad de establecer un seguimiento continuado de la enfermedad.

Introduction

Lyme borreliosis (LB) is a tick-transmitted disease of humans and animals distributed worldwide and caused by spirochetes of the *Borrelia burgdorferi* s.l. group. LB has been reported throughout Europe where it is the most common tick-borne infection, as well as it is in the USA.[1] Recent surveys showed that the overall prevalence of LB may be stabilizing, but its geographical distribution is increasing.[2] The genus *Borrelia* contains several major human and animal pathogens, among which some species cause LB. In Europe, at least five species of *B. burgdorferi* s.l. (*B. afzelii*, *B. garinii*, *B. burgdorferi* sensu stricto, *B. spielmanii*, and *B. bavariensis*) can cause this disease and three species (*B. bissettii*, *B. lusitaniae*, and *B. valaisiana*) have occasionally been detected in patients, but are not recognized as important pathogens.[3] *B. garinii* is the genospecies mostly involved in clinical cases in Spain.[4]

Three tick species, *Ixodes ricinus*, *I. hexagonus* and *I. uriae*, are considered vectors of LB spirochetes in Europe,[1] being *Ixodes ricinus* the species that more often bites humans. LB spirochetes perpetuate through cycles involving rodent reservoir hosts, such as *Apodemus* spp. mice in Eurasia.[5] Despite the existence of past and present clinical reports of human LB in Asturias (North-Western Spain),[6–10] there is scarce quantitative data on the prevalence of *B. burgdorferi* s.l. in ixodid ticks and potential reservoirs in this area, except for the[11] study on immature-stage *I. ricinus* ticks. The study area displays suitable environmental conditions to maintain the complex life-cycle of *B. burgdorferi*, including abundant reservoir and tick populations. Therefore, the aim of this preliminary study was to determine the presence, prevalence and genetic characteristics of *B. burgdorferi* s.l. in questing ticks and small mammals in Asturias. We also report several recent cases of Lyme disease in humans diagnosed in this area as indicative survey.

Materials and methods

Study area

The study was carried out in Asturias region, an Oceanic climate region in North-Western Spain (Fig. 1). Rainfall in the region is abundant and evenly distributed along the year. Average seasonal temperature is mild, even in winter. The main study area is the "Sierra del Sueve" Natural Reserve (43°28′48″N, 5°14′32″W), an 80-km^2 pre-coastal mountain range (maximum altitude 1167 m) where livestock and wildlife are abundant, including cattle, horses, wild boar (*Sus scrofa*), red deer (*Cervus elaphus*), a significant number of fallow deer (*Dama dama*) and small mammals. Some orchards were also prospected as sample points outside the Natural Reserve in neighbor municipalities.

Questing tick collection

Ticks were collected by dragging a blanket (1 × 1 m) over the vegetation in grazing lawns, shrubs and woods since these constitute the main biotopes in the study area. Sampling at each site was conducted twice a month from January 2012 to December 2014. Tick-drags were inspected every 10 m, then gathered specimens were transported to the laboratory, counted and identified using a taxonomic key[12] and stored at −80 °C until being tested. Only adults and nymphs were selected for analysis because transovarial infection of LB is rare or non-existent.[13]

Micromammal collection and processing

A total of 70 small mammals were captured between 2011 and 2013, deploying 917 Sherman live traps (H.B. Sherman Traps, Tallahassee, FL) placed at the same biotopes as tick survey in Sierra del Sueve (6 points), and 50 snap traps (Topcat® Andermatt Biocontrol, Switzerland) placed in orchards from neighbor municipalities (10 points). Experimental procedures were approved by the Regional Animal Ethics Committee (Consejería de Agroganadería y Recursos Autóctonos del Principado de Asturias) and were conducted in accordance with the guidelines established by the current laws of the country. Captured live animals were anaesthetized with ketamine hydrochloride (Imalgene: Merial) at a dose of 10 mg/kg intramuscularly, euthanized by intraperitoneal injection of T-61 solution (Intervet) and processed by a competent person. Alternatively, cervical dislocation or concussion to the head (Directive 2010/63/UE) was carried out in field if the captured animal was in poor health. Dead animals and traps were thoroughly examined for ticks. Any tick found was collected into tubes containing 75% ethanol. Then, all animals were aseptically dissected and several tissues were collected (ear, lung, heart, liver, spleen, kidney and urinary bladder) for PCR analysis.

DNA extraction and PCR amplification

Total DNA was extracted from ticks and small mammals using DNeasy Blood and Tissue Kit (Qiagen, Valencia, Spain). Adult ticks were analyzed individually and nymphs were pooled into groups of 5 individuals belonging to the same species and collected at the same time and from the same sampling sites. Small mammal's tissues of each individual were pooled and DNA was extracted according to the manufacturer's protocol. In each DNA extraction round (19 samples or less per round) a negative control sample free of any template was included. Detection of *B. burgdorferi* s.l. DNA in ticks and rodents was carried out using a nested PCR assay, with specific primers for a 389-pb portion of the flagellin gene (*flaB*) as described previously by Clark et al.[14]

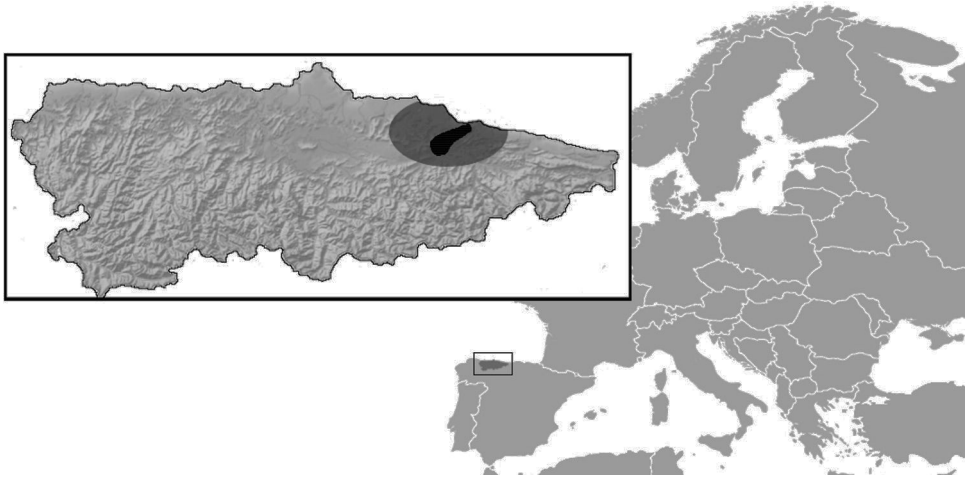

Figure 1. Location of Asturias in North Spain, and the area where study was conducted (43°28′N, 5°14′O). Natural Reserve of "Sierra del Sueve" and surroundings are indicated in dark gray and light gray correspondently.

Gene sequencing and phylogenetic analysis

PCR products were purified using QIAquick Gel Extraction Kit (Qiagen, Valencia, Spain). Amplicons from *Borrelia* specific reactions were cloned in pGEM T-easy plasmid vector (Promega, Madrid, Spain) before sequencing or were sequenced directly for species identification. Amplicons or plasmids inserts were sent to SECUGEN (Madrid, Spain) for Sanger sequencing using internal forward PCR primer or T7 primer (5′-TAA TAC GAC TCA CTA TAG GG-3′). At least two independent clones of a PCR product were sequenced. Sequences obtained were compared with known sequences from databases (GeneBank) by using the Basic Local Alignment Search Tool (BLASTN) at the National Center for Biotechnology Information (NCBI). For phylogenetic analyses, sequences were aligned with Clustal W algorithm[15] using the MEGAN 6 package. The phylogenetic tree was constructed using the Neighbor-Joining method[16] based on the Kimura 2-parameter distance method,[17] with bootstrap analysis of 1000 replicates.[18]

Study of human borreliosis in Asturias

A ten-year (2004–2014) descriptive and retrospective study of patients treated of borreliosis from Cabueñes Hospital (the main hospital of eastern Asturias) was carried out. Epidemiological, clinical diagnostic and therapeutic parameters were compiled. Patients with diagnosis confirmed by ELISA (IgM: Vidas Lyme IgM. France; IgG: Liaison Borrelia IgG, Italy) and subsequently by Western Blot (IgM e IgG: Sekisui Virotech Line ImmunoBlot, Germany) were included. Suspected cases with clinical symptoms and evolution, but without conclusive ELISA results, were also taken into account.

Statistical analysis

A 2×2 chi-squared test or Fisher's exact test (when $n < 10$) were performed to compare *B. burgdorferi* prevalence regarding season, geographic orientation, vegetation type and tick stage (nymphs and adults). The differences were considered statistically significant at $p \leq 0.05$. These analyses were performed using R package (free software environment available at http://www.r-project.org/).

Results

Questing ticks

From 2012 to 2014 a total of 39,386 ticks were collected from vegetation cover; 22,239 were larvae, 16,726 were nymphs and 421 were adults. Tick abundance – as the number of ticks collected per 100 m^2 transect – was 65.0 for larvae, 48.9 for nymphs and 1.2 for adults. Seven tick species of the genera *Ixodes*, *Haemaphysalis*, *Dermacentor* and *Rhipicephalus* were collected from vegetation. *I. ricinus* accounted for 59.6% (251/421 [95%CI: 54.9–64.2]) and *H. concinna* for 20.4% (86/421[95%CI: 16.8–24.5]) of adult ticks identified, whereas other species as *H. punctata*, *D. reticulatus*, *H. inermis* and *R. bursa* accounted for 8.0–4.0%, and *I. frontalis* was only occasionally found. Table 1 shows the distribution of 878 unfed *I. ricinus* ticks randomly selected for LB analysis and analyzed in 202 pools. Evidence of presence of *B. burgdorferi* (PCR-positive ticks) was found in *I. ricinus* questing nymphs (1.4% [95%CI: 0.8–2.5] minimum expected prevalence) and adults (6.1% [95%CI: 1.7–19.6]) collected from all sampling sites. PCR-positive ticks belong to all vegetation types and all geographic sites taken into account in the study area. Prevalence of infection between different geographic orientation, vegetation type and tick stage was no significantly different (Fisher exact test p value > 0.05). Another 135 non-*Ixodes* ticks were also selected for LB analysis in 47 pools. *B. burgdorferi* PCR-positive ticks were found in 3.9% (5/127) *Haemaphysalis* spp., 25.0% (1/4) *D. reticulatus* and 25.0% (1/4) *R. bursa.*

Small mammals

Twenty six out of a total of 70 small mammals examined were captured in "Sierra del Sueve" Natural Reserve and belonged to six species (Table 2). A total of 55 *I. ricinus* larvae were collected from 65.4% (17/26 [95%CI: 46.2–80.6]) trapped mammals. No adults or nymphs were found feeding on them. *B. burgdorferi* s.l. was detected in 11.5% (3/26 [95%CI: 4.0–29.0]) of the small mammals tested by PCR, all of them belonging to the species *Apodemus sylvaticus*. Another 44 specimens belonging to five species of small mammals were gathered in experimental and commercial orchards located in a 20 km radius around the Natural Reserve (Table 2). No ticks were found feeding on all of them despite being captured all along the year and mainly in summer when larvae in Asturias are more abundant. However, *Arvicola scherman* (40.0% [95%CI: 16.8–68.7]) and *Microtus lusitanicus* (18.2% [95%CI: 5.1–4.8]) yielded positive results to *Borrelia* infections.

Table 1
Borrelia burgdorferi s.l. in *I. ricinus* ticks collected in Sierra del Sueve, Spain, 2012–2014.

Epidemiological factors	Ticks tested	Ticks infected		Genospecies
	No. (pools)	No. (pools)	%[a]	
Geographic orientation				
North	337 (73)	7–31 (7)	2.08	*B. afzelii*, *B. garinii*, *B. valaisiana*
South	541 (129)	7–31 (7)	1.29	*B. afzelii*
Vegetation type				
Grassland	244 (52)	4–20 (4)	1.64	*B. afzelii*, *B. garinii*, *B. valaisiana*
Shrubs	330 (78)	6–22 (6)	1.82	*B. afzelii*, *B. garinii*
Woodland	304 (72)	4–20 (4)	1.32	*B. afzelii*
Seasons				
Winter	234 (50)	2–10 (2)	0.85	*B. afzelii*
Spring	287 (71)	5–21 (5)	1.74	*B. afzelii*, *B. garinii*, *B. valaisiana*
Summer	114 (26)	4–16 (4)	3.51	*B. afzelii*
Autumn	243 (55)	3–15 (3)	1.23	*B. afzelii*
Tick stage:				
Adult	33	2	6.06	*B. afzelii*
Nymph	845 (169)	12–60 (12)	1.42	*B. afzelii*, *B. garinii*, *B. valaisiana*

[a] "Minimum expected prevalence": percentage of positives is calculated assuming that any pool would contain one infected tick.

Gene sequencing and phylogenetic analysis

In 17 of 22 PCR-positive tick samples, *B. burgdorferi* s.l., genospecies were identified based on the sequencing of the *fla*B gene PCR product. Nucleotide sequences obtained in this study were submitted to GenBank under accession numbers from KT347437 to KT347458. *B. afzelii* was the most prevalent genospecies (76.5%; $n = 13$), and was detected in *I. ricinus* (61.5%), *H. concinna* (30.8%) and *R. bursa* (7.7%) ticks. *B. garinii* (11.8%; $n = 2$), *B. lusitaniae* (5.9%; $n = 1$) and *B. valaisiana* (5.9%; $n = 1$) were only found in *I. ricinus* ticks. One sequence, corresponding to an *I. ricinus* tick, was considered too short (207 pb) to be included in the phylogenetic analysis and hence was removed. In small mammals, 6 out of 9 PCR positive samples were successfully sequenced but the other three samples could not be identified at the genospecies level due to the low quality of the sequences (Table 2). These 22 sequences plus 27 retrieved from GenBank were aligned and a phylogenetic tree was constructed by the NJ method (Fig. 2). The tree topology, showed that the strains belonging to the same genospecies clustered together, in three main clades (bootstrap > 70%) defined by the reference strains included for comparison. Small mammals-derived sequences clustered with *B. afzelii* strains, whereas tick-derived sequences clustered with *B. afzelii*, *B. garinii*, *B. lusitaniae* and *B. valaisiana* strains. Within the *B. afzelii* clade, two subgroups could be differentiated. Fourteen of the *B. afzelii* positive samples (10 ticks and 4 small mammals) predominated in the subgroup I. In contrast, *fla*B sequences from small mammals AT1304 and AT1303 grouped with the reference sequences included in the analysis (subgroup II). The tick-derived sample 282b Ir is closely related to *B. afzelii* but it forms a subgroup separated from the other *Borrelia* strains. Based on pairwise distances, *fla*B sequences from tick and small mammal *B. afzelii* strains were 97.6–100% similar. Samples 282b Ir, AT1304 and AT1303 shared high similarity rates (99.7–100%) with all the reference sequences. *fla*B sequences from tick samples 214a Ir and 263b Ir displayed 98.8–99.9% homogeneity with *B. valaisiana* and *B. lusitaniae* reference strains, respectively. The similarity among the *B. garinii fla*B sequences was 99.0%, and was between 97.9 and 99.7% identical to sequences derived from GenBank.

Study of human borreliosis in Asturias

From 2004 to 2014 a total of 18 LB cases were diagnosed. The mean age of patients was 37.2 ± 4.9 (SD) years (4 cases under 12 years of age), with a slight male predominance (1.25:1). 72.2% recalled the body region of the bite, being the most frequent place extremities, however only 38.9% of the cases they visualized the tick. We considered that tick bites have happened in the same place as patients live, according with their proof: 38.9% Gijón, Villaviciosa 27.9%, Ribadesella, Colunga Carreño Arriondas 5.6% 5.6% 5.6% 5.6% and a case imported from USA. The symptoms were: erythema migrans (66.7%), cephalea (5.6%), chest pain (5.6%), cervical pain (5.6%), extremities pain (38.9%), articular pain (11.1%), malaise (50.0%), fever (44.4%), facial paralysis (11.2%), paresthesia (11.2%), dysarthria (5.6%) and palpitations (5.6%). General physical examination was non-specific. The duration of symptoms before diagnosis was registered in 66% of cases, with an average duration of 19.6 ± 9.3 (SD) days. Hemogram and biochemical analyses

Table 2
Small mammals captured and PCR/sequencing results.

Animal species	Common name	Captured no. (%)[a]		PCR/Sequencing no. (%)[b]		Genospecies
		Natural reserve	Surroundings	Natural reserve	Surroundings	
Apodemus sylvaticus	Wood mouse	19 (73.1)	17 (38.6)	3 (15.8)	–	*B. burgdorferi* s.l.
Apodemus flavicollis	Yellow-necked field mouse	1 (3.8)	–	–	–	–
Microtus lusitanicus	Lusitanian pine vole	3 (11.5)	11 (25.0)	–	2 (18.2)	*B. afzelii*
Microtus agrestis	Field vole	1 (3.8)	–	–		–
Crocidura russula	White-toothed shrew	1 (3.8)	3 (6.8)	–	–	–
Crocidura suaveolens	Lesser white-toothed shrew	1 (3.8)	–	–		–
Sorex coronatus	Crowned shrew	–	3 (6.8)		–	–
Arvicola scherman	Montane water vole	–	10 (23.8)		4 (40.0)	*B. afzelii*
Total		26	44	3 (11.5)	6 (13.6)	

[a] Percentages of captured animals from the total number of captured animals.
[b] Percentages of positive animals from the total number of each animal species.

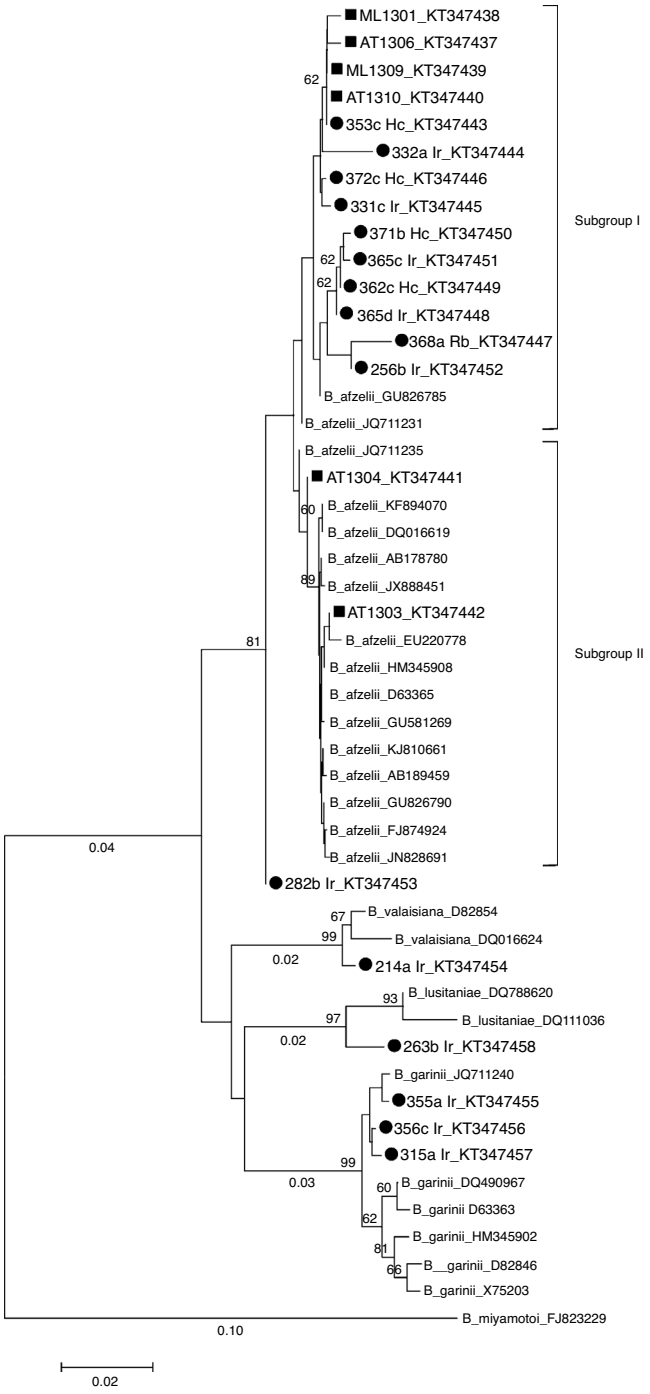

Figure 2. Phylogenetic tree (Neighbor-Joining) based on the partial sequence of *B. burgdorferi* s.l. *fla*B gene (338 bp). Sequences used in the analysis included *B. afzelii* AB178780, GU826785, JQ711231, JQ711235, KF894070, DQ16619, JX888451, EU220778, HM345908, D63365, GU581269, KJ810661, AB189459, GU826790, FJ874924, JN828691; *B. garinii* DQ490967, HM345902, X75203, D82846, D63363, JQ711240; *B. valaisiana* D82854, DQ016624; *B. lusitaniae* DQ788620, DQ016624, and *B. miyamotoi* FJ823229 as outgroup. Scale bar indicates an evolutionary distance of 0.02 nucleotides per position in the sequence. Bootstraps (1000 replicates) values >than 60% are shown below the branches. *B. burgdorferi* sensu lato sequences obtained in the present study are labeled by a solid circle (tick) or by a square (rodent). Ir: *I. ricinus*; Hc: *H. concinna*; Rb: *R. bursa*; ML: *M. lusitanicus*; AT: *Arvicola*

were performed in all cases, with an average of 10,738 ± 1757.4 (SD) leukocytes and of 6833 ± 1406.3 (SD) neutrophils. C-reactive protein analysis was performed in 83% of cases, with an average value of 19.36 ± 6.45 (SD) mg/L. In half of the cases the Erythrocyte Sedimentation Rate speed was assessed with an average value of 40.89 ± 9.39 (SD) mm/hour. Only in one patient the cerebrospinal fluid was affected (lymphocytic pleocytosis). In two cases the electrocardiogram was abnormal. ELISA for *B. burgdorferi* was performed in all cases, being positive in 15 of them, dubious in 2 cases and negative in one case. Diagnosis was: 61.1% LD, 22.3% suspected LD, 11.1% atrioventricular blockade secondary to borreliosis and 5.6% neuroborreliosis. We confirmed 77.8% of the cases, those with clinical and evolution compatible, with positive serology confirmed by Western Blot. Despite the inconclusive results of the serology, the rest of the cases appeared under suspicion and they have been considered and treated as borreliosis, given their compatible symptoms and clinical evolution. The 72.2% of cases required hospitalization, with an average duration of 16 ± 2.7 (SD) days. Antibiotic therapy was the treatment in all cases: doxycycline 38.9%, amoxicillin 22.2%, ceftriaxone 22.2%, 11.1% cefuroxime, amoxicillin-clavulanic acid 5.6%. The average duration of treatment was 19 ± 1.6 (SD) days. Only two cases suffered long-term neuropathy sequelae.

Discussion

Asturias, as well as nearby Atlantic regions in Northern Spain, is endemic for LB. Several clinical reports on LB cases diagnosed on local hospitals from Asturias and neighbor regions during the last years still support the importance of this disease.[9,10,19,20] Most of the known competent tick vectors of *B. burgdorferi* belong to the *I. ricinus-persulcatus* complex.[21] As expected, in our study *B. burgdorferi* DNA was detected in *I. ricinus*. Interestingly, it was also detected in *H. concinna*, *H. punctata*, *R. bursa* and *D. reticulatus*. The prevalence of *B. burgdorferi* s.l. in ticks in several European areas varied significantly.[22–25] Indeed, our results indicate that *B. burgdorferi* s.l. is widespread in the study area at higher infection rates in ticks that those reported in the Basque Country (Northern Spain) by Barandika[26] and at slightly lower rates than those previously reported in Asturias by Ruiz-Fons et al.[11]

The role of *A. sylvaticus* as competent reservoir and vector of the LB agent may depend on habitat features since those affect both rodent presence and activity and tick density as well. Moreover, the abundant presence of large mammals in the Natural Reserve gives ticks a chance to increase their population, whereas in the surrounding orchards large mammals are scarce. Given the high *B. burgdorferi* s.l. prevalence in voles from orchards (Table 2), the circulation of this bacterium within vole subterranean burrows should not be discarded. In that sense, relatively high densities of *Laelaps agilis*, an obligate hematophagous parasitic mite which might bear *B. burgdorferi* s.l.,[27,28] have been observed in the pinna of many specimens of *A. scherman* (Somoano, A. and Espí, A., unpublished results). Further studies focused on mite community of the nest of these voles might clarify the species involved in this cycle.[29] Nevertheless, *A. scherman* and *M. lusitanicus* could be competent reservoir hosts but not necessarily competent vectors of ticks.[30]

B. afzelii was the only genospecies detected on small mammals and the most common genospecies detected in questing ticks in our study. Nevertheless, we also detected two *B. garinii*, one *B. valaisiana* and one *B. lusitaniae* in questing ticks. Similar results were reported in the Basque Country, were *B. afzelii* was among the identified genospecies in both small mammals and questing ticks.[26] In our study, the NJ tree reveal that the *fla*B sequences derived from Asturian small mammals and questing ticks clustered with *B. afzelii*, *B. garinii*, *B. lusitaniae* and *B. valaisiana* type strains. Two main subgroups are clearly differentiate within the *B. afzelli* cluster, subgroup I clustering almost all of the sequences described in this work, and subgroup II were two small mammals sequences (AT1304 and AT1303) and the reference sequences were included.

The detection of *B. burgdorferi* s.l. among questing ticks and small mammals in "Sierra del Sueve" area, as well as the tick abundance and the presence of large populations of wild and domestic animals indicate that the risk of infection in this area is relevant. This is in agreement with clinical reports of LB from local hospitals as we observed in our descriptive and retrospective study of patients treated of borreliosis from Cabueñes Hospital, showing the relevance of establishing continuous monitoring of LB and *B. burgdorferi* s.l. prevalence in ticks and reservoir hosts. Our findings, although preliminary, provide completely new information for Asturias and supposes a starting point for further research.

Conflict of interest statement

The authors declare that they have no competing financial interests.

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