Citizen science on Lyme neuroborreliosis in Spain reveals disease associated risk factors and control interventions

Running title: Lyme neuroborreliosis in Spain

Authors: José de la Fuente[1,2,*], Agustín Estrada-Peña[3,4], Christian Gortázar[1], Rita Vaz-Rodrigues[1], Isabel Sánchez[5], Juan Carrión Tudela[5]

1. Health and Biotechnology (SaBio). Instituto de Investigación en Recursos Cinegéticos IREC-CSIC-UCLM-JCCM, Ronda de Toledo 12, 13071 Ciudad Real, Spain.
2. Department of Veterinary Pathobiology, Center for Veterinary Health Sciences, Oklahoma State University, Stillwater, OK 74078, USA.
3. Department of Animal Health, Faculty of Veterinary Medicine, Zaragoza, Zaragoza, Spain.
4. Group of Research on Emerging Zoonoses, Instituto Agroalimentario de Aragón (IA2), Zaragoza, Spain.
5. Asociación de Enfermedades Raras D'Genes, San Cristóbal 7, 30850Totana-Murcia, Spain.

*Corresponding author: Prof. José de la Fuente. SaBio, IREC, CSIC-UCLM-JCCM. Ronda de Toledo 12. Ciudad Real 13005, Spain. Tel. 0034 926295450. jose_delafuente@yahoo.com / josedejesus.fuente@uclm.es

Abstract

Lyme neuroborreliosis (LNB) caused by *Borrelia burgdorferi* sensu lato complex spirochetes is one of the tick-borne diseases with high prevalence and social/economic burden in Spain and other European countries. Due to limited information available about the incidence, prevalence, and symptoms of LNB, current prevention and treatment interventions are not very effective against this disease. To address these limitations, in this study we used a citizen science approach to evaluate the LNB-associated risks and implementation of control interventions in Spain. Despite limitations of the study associated with response to the questionnaire and information on tick species, the results evidenced the effect of factors such as age, gender, tick bites, disease clinical signs, comorbidities such as alpha-gal syndrome, healthcare services and treatment effectiveness affecting LNB. The main conclusions of the study are that (a) better surveillance is required for the identification of tick infestations, pathogen infection and diagnosis of LNB and related comorbidities, and (b) new interventions need to be developed and implemented in both public and private healthcare services to improve prevention, diagnosis, and treatment of LNB.

Keywords: Lyme disease; Borrelia; Tick; Risk Factors; Citizen Science

Introduction

Lyme neuroborreliosis (LNB) or Lyme disease (LD) is a tick-borne disease caused by *Borrelia burgdorferi* sensu lato complex spirochetes with high prevalence and social/economic burden in Europe and the United States (van den Wijngaard et al., 2017; Hook et al., 2022). Possible expansion of LNB has been also reported in Asia (Rollins et al., 2022). Ticks of the *Ixodes ricinus* complex are the main vectors of *B. burgdorferi* s.l. in Europe and multiple vertebrate species contribute to pathogen circulation (Estrada-Peña and de la Fuente, 2017). The geographical range of the tick vector is affected by temperature in northern and humidity in southern Europe, which translates into higher tick prevalence during May-June and September-October in central Europe (Estrada-Peña et al., 2012).

The main symptoms associated with LNB may be non-disease specific and include localized and self-limiting skin manifestations, erythema migrans, and infection disseminates to the central nervous system (Dessau et al., 2015; Hansen et al., 2013; Hansen and Lebech, 1992; Stanek et al., 2012). The most common neurological manifestations include facial nerve palsy, headache, polyradiculitis, and encephalitis (van Samkar et al., 2023). Serology testing of *B. burgdorferi* s.l. is the primary laboratory tool employed to diagnose LNB (Kenyon and Chan, 2021). Over time, *B. burgdorferi* s.l. is often controlled by innate and adaptive immune responses (Steere et al., 2016) with improvement after antibiotic treatment in most patients (Dersch et al., 2016). However, LNB is frequently under- and over-diagnosed with social and economic implications (Kobayashi et al., 2022; Geebelen et al., 2022). Recent studies have shown that social factors such as insurance payor, primary care status, and diagnostic setting are risk factors of LNB suggesting that lower socioeconomic status and less health care access may be associated with disseminated disease stage (Moon et al., 2021).

Citizen science is the society involvement in the systematic collection and analysis of data, development of technology, testing and dissemination of investigations by researchers on a primarily avocational or voluntary basis (Iii and Mims, 1999). Citizen science has been applied to the study of infectious diseases including tick-borne diseases like LNB and other co-existing diseases such as COVID-19 (Hines and Sibbald, 2015; Seifert et al., 2016; Nieto et al., 2018; Lewis et al., 2018; Lernout et al., 2019; Porter et al., 2021a; 2021b; de la Fuente et al., 2021; Shutikova et al., 2021; Azagi et al., 2022; Sgroi et al., 2022). Additionally, citizen science has proven impact and shown limitations on studying current and future distribution of tick vector species (Porter et al., 2019; 2021a; 2021b; Tran et al., 2021; Eisen and Eisen, 2021; Sgroi et al., 2022). In this way, partnership between social community and researchers provides a powerful tool for studying prevalence and educating society about the risks associated with tick-borne diseases and in encouraging tick bite prevention and better disease diagnosis by healthcare system.

To face challenges associated with LNB in Spain (Garcia-Vozmediano et al., 2022), herein we used citizen science to evaluate the disease-associated risks and implementation of control interventions.

Material and Methods

Characteristics of the survey and data transformation and statistical analysis

Responses to the questionnaire were received during January – July 2022. The questionnaire contained qualitative questions: consent, sex, residence zip code, province, exposure to tick bites, identified tick species, estimated place and month of tick bite, LNB (No and no signs; No but reports signs; Yes – clinical; Yes – tested positive), date of disease diagnosis, symptoms compatible with LNB (fever, sweat, chills, fatigue, tiredness, throat pain, gastric or intestinal, respiratory, muscular, vision, hearing, nervous; for each one: No response; Never; Sporadic; Frequent; Very frequent), disease treatments and duration, type and results of treatment, role of public and private health system, allergic reactions to mammalian meat consumption, and their perceptions regarding their general and mental health after disease diagnosis and treatment, and quantitative questions: birthyear, age and age class (<30; 30-60; >60) (Supplementary Data).

The questionnaire was anonymous and circulated via Google (https://forms.gle/Jwu3Jcg6eZ6utNfY8) with privacy protection. By July 2022 we received 412 responses, of which 405 participants were included in the study and agreed in the use and publication of this anonymous information. Main details on participants included in the study are disclosed in Table 1. Regarding their spatial distribution, respondents' location zip codes were mapped using eSpatial (https://maps.espatial.com; accessed in January 2023).

We designed a binomial generalized linear model with a logit link function to test the statistical effect of categorical predictors (age) on the probability of having been diagnosed with LNB (dependent variable). We used a stepwise backward strategy to obtain the final model. Homogeneity among binary variables was analyzed using a Fisher's exact test. The significance level was set at $p < 0.05$ using a SPSS statistical software.

Results and Discussion

Age, sex, history of tick bites, and LNB

A total of 405 participants in the survey were included in the analysis. Lyme neuroborreliosis was diagnosed in 61% of the male respondents and in 81% of female respondents ($p < 0.001$). Respondents aged between 30 and 60 years were less likely to have been diagnosed with LNB (34.1%) than younger (<30) or older (>60) respondents (49.2%; $p = 0.015$). The spatial distribution showed that both respondents with LNB diagnosed and/or symptoms or LNB not diagnosed and without symptoms were located across Spain. Most of the respondents with LNB diagnosed and symptoms were located in Madrid, Murcia, and Pamplona (Figure 1). However, most cases without LNB or symptoms were located in Madrid and Algorta (Figure 1). These results agree with higher abundance of *I. ricinus* tick vector in northern Spain (Estrada-Peña et al., 2012; Vieira Lista et al., 2022).

Tick bites were reported more frequently by male respondents (80/118, 67.8%) than by females (140/284, 49.3%; $p < 0.001$) and without difference between age classes. Tick bites were recorded in all months except February with highest rates (9-20 respondents/month) between April and October and consistently low rates from

November to March (0-3 respondents/month) (Figure 1A). Exposure to tick bites was not associated with LNB diagnostic and symptoms. However, respondents who reported frequent tick bites were more likely to have been diagnosed with LNB (20/25, 80%) than those reporting few or no tick bites (239/381, 62.7%; marginally significant, $p = 0.087$). Regarding tick species, 9/11 (82%) respondents were identified as infested with *I. ricinus* and one each with *Hyalomma* sp. (9%) and *Dermacentor reticulatus* (9%) (Figure 2A). Of them, 8 respondents with identified tick species were reported in April-June and 4 respondents in September-October (Figure 2A). Although with limited evidence, these results agree with higher tick prevalence during May-June and September-October in central Europe (Estrada-Peña et al., 2012). Three of the respondents, all with *I. ricinus*, were not diagnosed with LNB.

Clinical signs and therapies

Seventy three of the 405 respondents (17.7%) reported specific clinical signs attributed to LNB while only 3 (0.7%) reported no clinical signs and 336 (81.5%) did not respond to the individual clinical sign-related questions. Fatigue was the most reported clinical sign (69 respondents), followed by muscular signs (65), digestive and nervous system signs (57 each), fever, sweat, or shiver (56), sore throat (48), vision and respiratory signs (46 each), and auditive signs (35). The signs most often rated as "very frequent" were fatigue, digestive, muscular, and nervous (Figure 2B).

A total of 47 respondents reported erythema. All of them have been diagnosed with LNB. Of these, 4 belonged to the age class <30, 36 to the class 30-60, and 6 to the class >60 (excluding respondents who did not declare age class). Fifteen were males and 32 females. Mammalian meat allergy was reported by 25 respondents, all of which were diagnosed with LNB. Of these, 24 belonged to the age class 30-60 (3 males and 21 females), and one did not declare age. Based on the association between allergic reactions to mammalian meat consumption and alpha-gal syndrome (AGS) (Vaz-Rodrigues et al., 2022), these results suggest that LNB and AGS may be comorbidities in response to tick bites.

The sample included 302 cases having a LNB diagnosis or with compatible clinical signs. Of these, only 7 (2.3%) reported total recovery, 143 (47.3%) reported partial healing and normal life, and 72 (23.8%) stated that their clinical signs remained as bad as when the treatment started. A total of 222 of 412 (53.9%) respondents answered the questions regarding their general health and mental health perception after LNB. Of these, 203 (91.5%) reported that their general health had not returned to pre-diagnosis levels while 19 (8.5%) reported being back to normality. Regarding mental health, 173 (77.9%) reported that their mental health had not returned to pre-diagnosis levels while 49 (22.1%) reported being back to normality. Most of the respondents used private healthcare services (88%) and were either fully or partially cured (68%). Natural therapies were more commonly used among respondents (80%) but without differences in recovery associated with these therapies.

Study limitations and conclusions

This study has several limitations that may be considered. Not all participants provided response to the full questionnaire and thus reduced the number of entries for analysis. The information on tick species is very limited, which highlights the need to improve interventions for the identification of ticks infesting cases with LNB.

We conclude that a better surveillance is required for the identification of tick infestations, pathogen infection and diagnosis of LNB and related comorbidities. Tick-borne pathogens such as *B. burgdorferi* s.l and *Anaplasma phagocytophilum* contain surface-exposed alpha-gal modifications triggering antibody response to this glycan, which may be associated with acquired tick resistance, allergic klendusity and AGS (Cabezas-Cruz et al., 2021). Although it has been reported that IgE to alpha-gal did not correlate to previous LNB (Tjernberg et al., 2017), the characterization of anti-alpha-gal IgE and IgM/IgG antibody levels should be considered to evaluate risks for AGS associated with LNB as with other infectious diseases (Dupont et al., 2022; Vaz-Rodrigues et al., 2023). In agreement with a recent report (Oteo et al., 2023), new interventions need to be implemented in both public and private healthcare services to improve prevention, diagnosis, and treatment of LNB. These interventions include identification and analysis of pathogen DNA in ticks collected from infested individuals (e.g., Clark et al., 2023), microbiological diagnosis using molecular tests, education for reducing exposure to tick bites, especially in high risk zones and environments and guiding tick removal, appropriate therapeutic and prophylaxis management, the development of effective vaccines to control pathogen infection and reduce disease symptomatology, and the support for research to advance in the development and implementation of effective vaccines and other interventions for the control of tick vector infestations in key host species and the prevention of pathogen infection and transmission.

Authors' contributions
JF, AEP, IS and JCT designed the study. CG, RVR and JF analyzed the data. JF, CG and AEP interpreted the data and wrote the manuscript. All authors revised the manuscript and approved the final version.

Declaration of interests
We declare no competing interests.

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Ethical approval
The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to. No ethical approval was required as this study was based on consented interviews. We used no individual patient data and performed no animal sampling. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Data availability statement
The data that support the findings of this study are available in the Supplementary Data.

ORCID
José de la Fuente https://orcid.org/0000-0001-7383-9649
Christian Gortázar https://orcid.org/0000-0003-0012-4006
Agustín Estrada-Peña https://orcid.org/0000-0001-7483-046X
Rita Vaz-Rodrigues https://orcid.org/0000-0002-0411-7738

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Table 1. Main characteristics of respondents related to LNB.

Respondent characteristics*	**LNB diagnosed and symptoms (n = 302)**	**LNB not diagnosed and without symptoms (n = 103)**
Sex	Males = 72; Females = 229; Other = 1	Males = 46; Females = 55; Other = 2
Age (years) mean (range)	45.39 (11-76)	47.46 (21-70)
Exposed to tick bites	Yes = 159; No = 142; Unknown = 1	Yes = 62; No = 40; Unknown = 1
Healthcare service	Public = 26; Private = 196; No response = 80	Not Applicable
Results of treatment	Fully cured = 7; Partially cured = 143; Not cured = 72; No response = 80	Not Applicable
Allergic reactions to mammalian meat consumption	Yes = 25; No = 197; No response = 80	Not Applicable
Use of natural therapies	Yes = 178; No = 44; No response = 80	Not Applicable
Cure related to natural therapies	Yes = 80; No = 98; No response = 124	Not Applicable
Health status after diagnosis and treatment	Recovered = 19; Not recovered = 203; No response = 80	Not Applicable
Mental health status after diagnosis and treatment	Recovered = 49; Not recovered = 173; No response = 80	Not Applicable

*A total of 405 participants in the survey were included in the analysis.

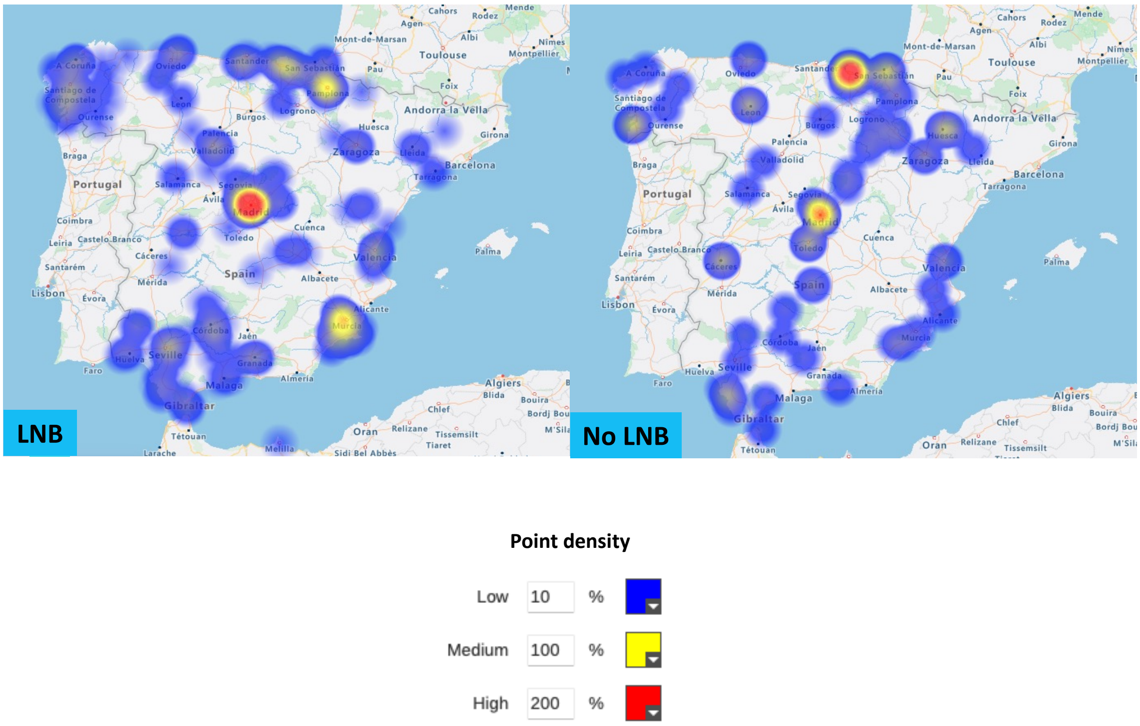

Figure 1. Heatmap of spatial distribution of respondents with LNB diagnosed and/or symptoms (LNB) or LNB not diagnosed and without symptoms (No LNB). Location zip codes were mapped using eSpatial (https://maps.espatial.com).

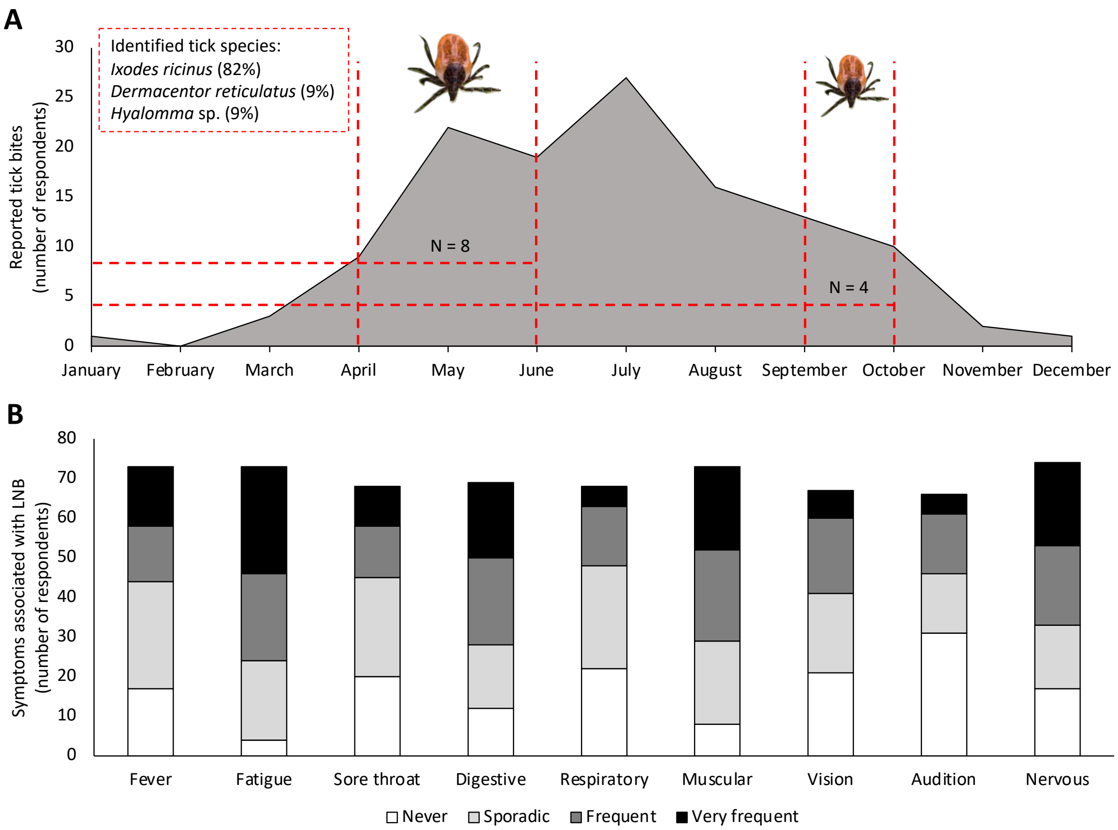

Figure 2. Symptoms and tick bites reported by respondents to a questionnaire on LNB in Spain, and their perceived frequency. (A) Tick bites throughout the year and identified tick species. (B) Clinical signs associated with LNB.

Supplementary information. Supplementary Data. Results of the survey.