

Detection of IgG antibodies against *Borrelia burgdorferi* in adults

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The aim of this study was to determine the prevalence of IgG antibodies against *Borrelia burgdorferi* in adults who were bitten by a tick at least once and did not have classical signs and symptoms of Lyme disease. A total of 88 people from different cities in Lithuania were tested for the presence of Lyme disease IgG. The results showed that the prevalence of infection among participants was 11.4%. Unit values ranged from 12.26 U to 44.78 U. A higher number of Lyme disease cases was observed in older people and those who actively spend their time outdoors. This study shows the importance of the use of serological tests in the diagnosis of Lyme disease for patients with non-specific symptoms.

Keywords: Lyme disease, IgG antibodies, outdoor activities, tick bite

INTRODUCTION

Lyme disease is the most common disease spread by ticks in Europe and the United States (Tilly et al., 2008; Marques et al., 2021). In humans, it is caused by spirochaetes of the *Borrelia burgdorferi* sensu lato complex. The complex consists of three main species: *B. burgdorferi* sensu stricto, *B. afzelii*, and *B. garinii* (Mendoza-Roldan et al., 2019; Rudenko et al., 2011). The prevalence of *B. burgdorferi* s. l. species in the world differs. *B. burgdorferi* s. s. is mainly distributed in North America, *B. afzelii* distribution is observed in Western Europe, Central Europe, and Russia, and *B. garinii* is found in Europe, Russia, and Northern Asia (Cora et al., 2017; Humair, Gern, 2000; Rauter, Hartung, 2005). Ticks are the main vectors that transmit the pathogen of *B. burgdorferi* (Hussain et al., 2021). *Ixodes ricinus* is the main

tick species that transmits Lyme disease in Europe (Marques et al., 2021; Stone et al., 2017). A metaanalysis of the prevalence of *B. burgdorferi* s. l. in ticks in various European countries showed that, compared to the west, the infection rates of *Borrelia* in adult ticks in eastern Europe are higher (Rauter, Hartung, 2005).

Lyme disease in humans causes tiredness, fever, musculoskeletal pain, erythema migrans, and possible cardiac and neurological disorders (Chomel, 2015; Hussain et al., 2021). Different species of *B. burgdorferi* s. l. have been associated with different symptoms. Arthritis often occurs in the presence of *B. burgdorferi* s. s. infection, neurological symptoms are associated with *B. garinii* species, and skin lesions are associated with *B. afzelii* (Balmelli, Piffaretti, 1995; van Dam et al., 1993). Primary erythema migrans is the most common clinical symptom of *B. burgdorferi* infection and can be diagnosed in up to 89% of Lyme disease patients (Dandache, Nadelman, 2008; Es-

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posito et al., 2013). At a later stage of the disease, patients could often be diagnosed with Lyme arthritis, which is caused when *B. burgdorferi* bacteria enter the joint tissue and causes inflammation. The symptoms are joint swelling and constant pain in the joints, which starts within several months after infection. It occurs in 50–60% of patients who did not have treatment, and more often in children than in adults (Arvikar, Steere, 2015; Esposito et al., 2013; Feder et al., 2006).

In Europe, most cases of Lyme disease are registered in Germany, Austria, Slovenia, and also in Lithuania (Marques et al., 2021; Petrulionienė et al., 2020). Lyme disease has been observed in Lithuania since 1987, but its records officially started in 1991. One of the highest rates of Lyme disease observed in Lithuania was in 2019, and it was the highest number of cases in the last three decades. Although the data of the last few years show that Lyme disease cases have decreased, such changes could be caused by the number of unreported cases (NVSC, 2020; NVSC, 2021). As people cannot always recognize the symptoms of Lyme disease, they do not go to medical facilities on time. Therefore, the aim of this study was to determine the prevalence of IgG antibodies against *Borrelia burgdorferi* in adults who were bitten by a tick at least once and did not have symptoms of Lyme disease.

MATERIALS AND METHODS

This study was performed with 88 adults from various parts of Lithuania. The blood samples were taken between October 2022 and February 2023. Individuals whose serum samples were included in this study were bitten by a tick at least once, and they did not have the symptoms of Lyme disease.

The ELISA test was done based on the manufacturer's recommendations, using a kit for the determination of *B. burgdorferi* IgG in blood serum (Demeditec Diagnostics GmbH, Germany). Optical density at 450/620 nm was measured using a Multiskan FC plate reader (Thermo Scientific, China). The result of the ELISA test

was interpreted based on the calculated values of the samples in units and the provided recommendations of the manufacturer (Demeditec Diagnostics GmbH, Germany). The result was considered positive when $U > 11$.

An anonymous survey was conducted for data evaluation. Positive results were evaluated by age group (18–25 years, 26–45 years, 46–65 years, and over 65 years), sex (male and female), place of residence, outdoor activity, number of tick bites, and past diagnosis of Lyme disease. The data were analysed using MS Excel software. The categorical variables were analysed with a chi-squared test (χ^2) (Statistica for Windows, v. 7.0, Statsoft, Tulsa, OK, USA). The relationship between variables was statistically significant when $p < 0.05$.

RESULTS AND DISCUSSION

In this study, we report the importance of the use of serological tests in the Lyme disease diagnosis for patients with non-specific symptoms. Several previous studies also indicate the need for laboratory testing for the timely diagnosis of Lyme disease (Skogman et al., 2010; Zwerink et al., 2018; Petrulionienė et al., 2020; Häring et al., 2022).

This study estimated the presence of IgG antibodies against *B. burgdorferi* in adults in Lithuania. The overall prevalence of *B. burgdorferi* infection was 11.4% (10/88). Most of the participants (40.9%, 36/88) were 46 to 65 years old. There were six positive results from this age group, and four positive results came from the over-65 age group, which had the smallest number (12.5%, 11/88) of participants (Fig. 1). Other studies that evaluated the age-related prevalence revealed similar results: a higher prevalence of Lyme disease was observed in older people (Buczek et al., 2009; Hjetland et al., 2014; Wilking et al., 2015; Cora et al., 2017; Tulloch et al., 2019; Häring et al., 2022). The current study founded statistically insignificant correlation ($\chi^2(1, N = 57) = 1.17, p > 0.05$) when assessing the reliability between positive ELISA results and the age groups of the participants, excluding individuals younger than 46 years old.

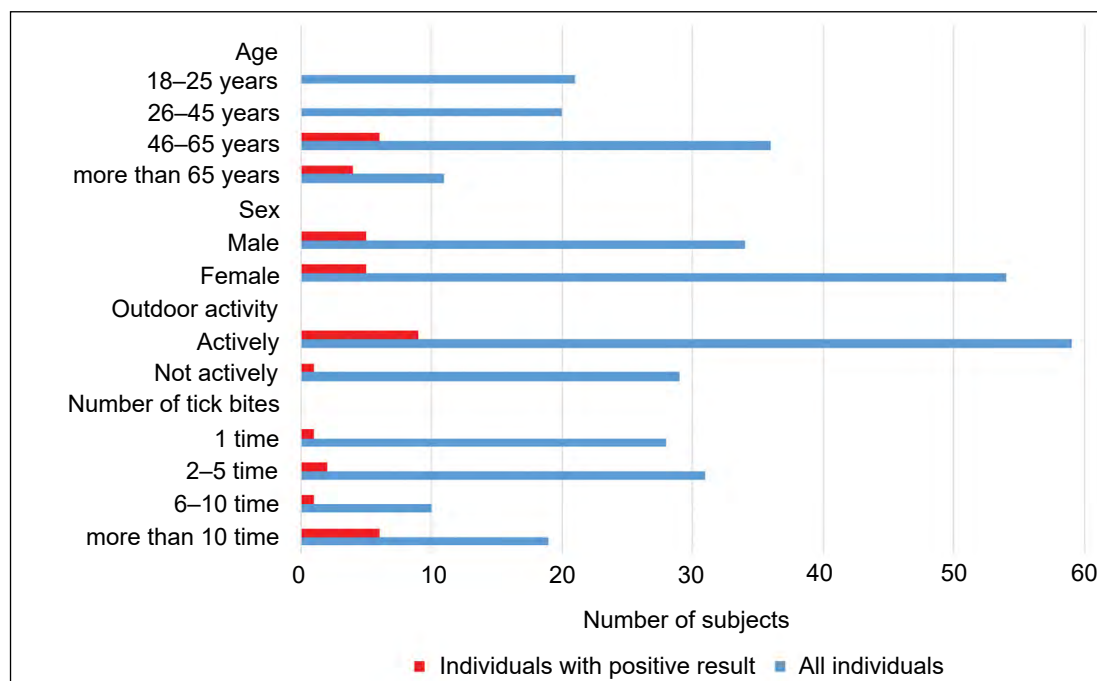


Fig. 1. Lyme disease incidence rate between subjects by age group, sex, outdoor activity, and number of tick bites

Analysis of the data by place of residence showed that all people who tested positive (11.4%) were from Kaunas. Such results are not surprising, because according to NVSC data, the highest number of Lyme disease cases in 2021 were registered in the districts of Vilnius, Kaunas, Tauragė, and Telšiai, and the lowest rates in Klaipėda and Marijampolė districts (NVSC, 2021).

The prevalence of Lyme disease by sex varies in different countries. Females tend to be affected more often than males in Sweden, Spain, and Finland (Bennet et al., 2007; Lledó et al., 2014; Sajanti et al., 2017). However, in Norway, Germany, and United Kingdom more frequent infection of males is observed (Hjetland et al., 2014; Wilking et al., 2015; Tulloch et al., 2019; Häring et al., 2022). In this study, IgG antibodies against *B. burgdorferi* were detected in 14.7% males and 9.3% females (Fig. 1). A chi-square test of independence showed that there was no significant association between sex and prevalence of Lyme disease ($\chi^2 (1, N = 88) = 0.61, p > 0.05$). Evaluation of the prevalence of Lyme disease by sex among those who received a posi-

tive result is inappropriate, as the study included a higher proportion of females than males. However, other studies previously conducted in Lithuania showed that Lyme disease was more common in females (Petruilionienė et al., 2020; Petruilionienė et al., 2021).

In the current study, the data were analysed to evaluate the dependence between prevalence of Lyme disease and time spent outside. Those who spent at least four hours a day outside were considered as spending time actively, just as those who work outside, who like spending their time in the parks and forest, do outdoor sports, or often go on hikes. A total of 15.3% (9/59) of the participants who received positive results claimed that they actively spend time outdoors (Fig. 1). The correlation between actively spending time outside and a positive ELISA test result was statistically insignificant ($\chi^2 (1, N = 88) = 2.69, p > 0.05$). Previous studies also found that the primary risk factor for *Borrelia* infection is leisure activities or behaviours (Wilking et al., 2015; Younger et al., 2016; Petruilionienė et al., 2021). Moreover, the reduced prevalence of Lyme disease among

individuals with a higher educational level was observed and could be linked to their increased awareness and taking of necessary precautions during outdoor activities (Cora et al., 2017).

A total of eight (9.1%) infected people have never had Lyme disease before, had no symptoms, and did not know about the disease until this test. Two people (2.3%) who had previously been diagnosed with Lyme disease tested positive for *B. burgdorferi* IgG antibodies again this time (Fig. 2). The correlation between previous Lyme disease and a positive ELISA test result was statistically insignificant ($\chi^2 (1, N = 88) = 1.62, p > 0.05$).

In humans, Lyme disease can be caused by an infected tick bite (Stanek et al., 2012; Chomel, 2015; Wilking et al., 2015). In the present work, participants were divided into groups by the number of tick bites: one bite (31.8%, 28/88), 2–5 bites (35.2%, 31/88), 6–10 bites (11.4%, 10/88), and more than ten bites (21.6%, 19/88) (Fig. 1). Two persons who had positive results had been bitten by ticks from two to five times. One person had a tick bite only once and one participant had from six to ten bites. A total of six out of ten participants who received positive results had more than ten tick bites.

The correlation between the number of tick bites and a positive ELISA test result was found statistically significant ($\chi^2 (1, N = 88) = 10.16, p < 0.05$). In previous studies, similar results were revealed: the presence of Lyme disease was higher in people who had a bigger number of tick bites (Bennet et al., 2007; Hjetland et al., 2014; Petrulionienė et al., 2020). Furthermore, according to some authors, sometimes more than one tick bite is needed before antibodies to *B. burgdorferi* can be detected (Lledó et al., 2014).

In Lithuania, the highest number of Lyme disease cases was registered in 2019. A few years later, in 2021, a decrease in cases was observed (NVSC, 2021). The results of this study show that half of participants who tested positive had high (>30 V) unit values (Fig. 3). This shows that Lyme disease may have become chronic as people do not always recognize the symptoms of the disease and do not go for medical help in time, and therefore the number of cases may be higher than published in the official data. Furthermore, a recent study conducted in Lithuania showed that the mean prevalence of *B. burgdorferi* s. l. in *I. ricinus* ticks is 13.4% and can vary between 1% and 35% depending

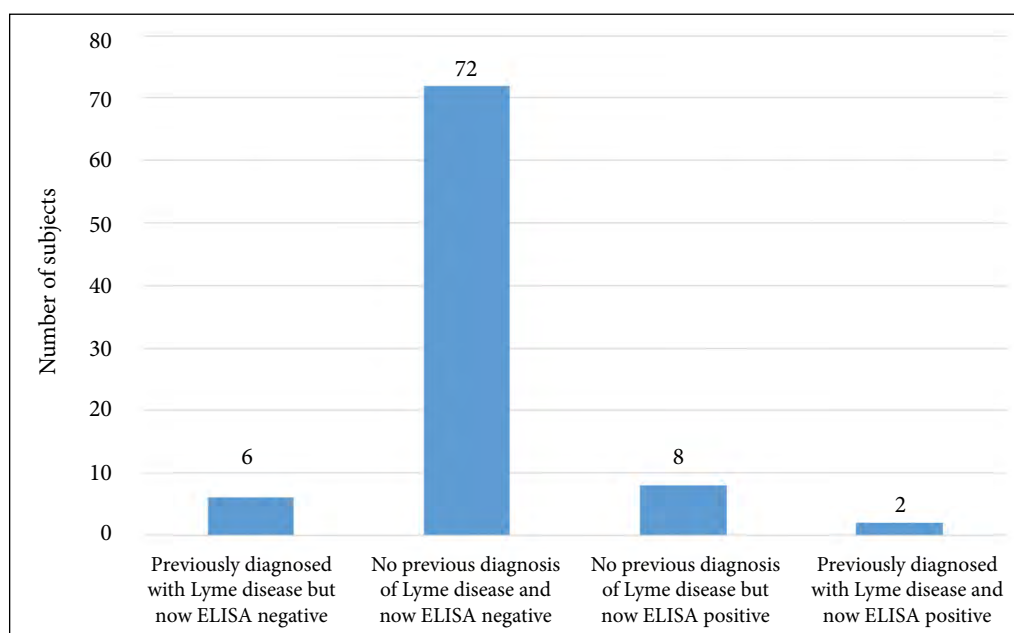


Fig. 2. Incidence rate of Lyme disease by past and present diagnosis of Lyme disease

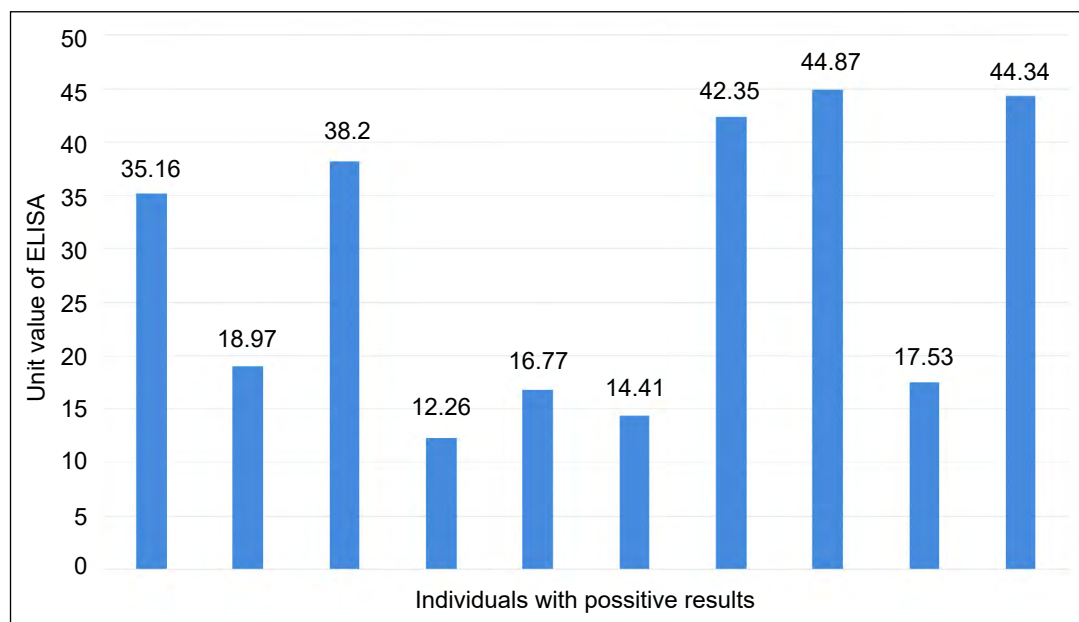


Fig. 3. Unit values of all individuals with a positive result

on different locations (Radzišauskienė et al., 2023). The highest prevalence of *B. burgdorferi* s. l. in Lithuania (19.4%) was found in deciduous and mixed forests and a lower (8.6%) in pine forests (Paulauskas et al., 2008). This may be explained by environmental factors that have a big influence on the distribution of ticks: areas with low humidity can reduce tick activity, survival, and distribution (Wallace et al., 2019; Hussain et al., 2021).

CONCLUSIONS

This study showed that Lyme disease caused by *Borrelia burgdorferi* is still widespread in Lithuania, despite the decreased number of cases over the past few years. Many individuals who tested positive were unaware of the infection and felt no symptoms show that the number of cases in Lithuania may be higher than officially announced. The results showed that a higher number of tick bites can affect the risk of infection with Lyme disease. Also, a higher number of cases is observed among older people.

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IgG ANTIKŪNŲ PRIEŠ *BORRELIA BURG- DORFERI* APTIKIMAS TARP SUAUGUSIŲJŲ

Santrauka

Šio tyrimo tikslas buvo nustatyti IgG antikūnų prieš *Borrelia burgdorferi* paplitimą tarp suaugusių žmonių, kuriems bent kartą buvo įsisiurbusi erkė ir kurie neturėjo klasikinių Laimo ligos požymių. Iš viso buvo ištirti 88 žmonės iš skirtingų Lietuvos miestų. Tyrimo duomenimis, infekcijos paplitimas tarp dalyvių sudarė 11,4 %. Vieneto vertės svyravo nuo 12,26 U iki 44,78 U. Daugiau Laimo ligos atvejų buvo užfiksuota tarp vyresnio amžiaus ir aktyviai laiką lauke leidžiančių žmonių. Ši analizė rodo serologinių tyrimų svarbą diagnozuojant Laimo ligą pacientams, kuriems nepasireiškia specifiniai simptomai.

Raktažodžiai: Laimo liga, IgG antikūnai, aktyvumas lauke, erkės įkandimas