SEROPREVALENCE OF *BORRELIA* IGG ANTIBODIES AMONG INDIVIDUALS FROM EASTERN SLOVAKIA

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SUMMARY

Objectives: Lyme borreliosis is a tick-borne disease of increasing incidence and public concern. Our cross-sectional study was aimed at evaluating seroprevalence of *Borrelia burgdorferi* in a group of respondents from Eastern Slovakia.

Methods: In total, 515 blood samples collected in 2013–2016 were analysed with NovaLisaTM, NovaTec – *Borrelia* IgG/IgM kit (Immunodiagnostica, Dietzenbach, Germany). Positive and equivocal IgG-antibody results were further examined with immunoblotting (LYMECHECK® OPTIMA IgG and IgM kits, BIOSYNEX, France). Data detected by serological methods were matched with those obtained from a questionnaire. Differences between groups by residence/seropositivity were tested by χ^2 test. The effect of socio-demographic and risk factors on seropositivity of IgG antibodies was assessed using binary logistic regression.

Results: IgG antibodies against *Borrelia burgdorferi* were detected in 67 cases (13.01%) and IgM antibodies in 40 cases (7.8%). Previous tick bite had been noted in 67.2% of these seropositive individuals. Higher seropositivity was observed in men and persons aged over 61 years. Rural residents had higher seropositivity (39%) than those living in urban (29%) areas. Very few of these seropositive persons reported prior symptoms.

Conclusion: The study reveals that IgG-seropositivity for *Borrelia burgdorferi* in Eastern Slovakia is predominant in men and occurs mainly in rural areas. The findings also suggest that exposure to *Borrelia burgdorferi* (with subsequent antibody response in serum) does occur, mostly without giving rise to clinical Lyme borreliosis.

Key words: Lyme borreliosis; seroprevalence, seropositivity, Borrelia burgdorferi sensu lato

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INTRODUCTION

Lyme borreliosis (LB) is a complex tick-borne zoonosis that poses an escalating public health threat in several parts of the world. The aetiological agent, *Borrelia burgdorferi sensu lato* (*s. l.*) is transmitted to humans by certain species of *Ixodes* ticks, which are found widely in temperate regions of the Northern hemisphere (1, 2). The western blacklegged tick, *I. pacificus* is the vector of LB on the Pacific Coast; the blacklegged tick, *Ixodes scapularis* is the vector of LB in the eastern and upper midwestern United States, and two of the most common tick species in Europe are the castor bean tick *Ixodes ricinus* and the meadow tick *Dermacentor reticulatus* (3, 4).

The risk of human infection is determined by the geographic distribution of vector tick species, ecologic factors that influence tick infection rates, and human behaviours that promote tick bite. Rates of infection are highest among children 5 to 15 years of age and adults older than 50 years (5).

Clinical features are diverse, but the most characteristic clinical manifestation of LB is erythema migrans (EM), which forms at the site of the tick bite in 70–80% of cases (1, 2). Human illness can cause also varied clinical manifestations including arthritis, facial palsy, lymphadenopathy, and carditis (4). The total burden of LB in Europe is estimated at 10.55 disability-adjusted life years (DALY) per 100,000 population. The majority of this burden is caused by symptoms, such as neuroborreliosis and Lyme arthritis. It is possible to only make approximate estimates of LB incidence in Europe, because few countries report LB as a compulsorily notifiable disease (6). The highest incidence of LB was reported in central Europe, particularly in Germany, Austria, Slovenia, Switzerland, and the coastal regions of Sweden (7, 8).

In Slovakia, this disease is mandatory notifiable. A total number of 582 (10.68/100,000) human LB cases were confirmed and reported in Slovakia in 2019. LB cases were recorded in each age group, with the highest age specific morbidity in the age group older than 45–54 years. Diseases were reported from all regions of the Slovak Republic, with the highest morbidity recorded in Žilina Region – 27.77, Trenčín Region – 24.07, and Banská Bystrica Region – 15.90 (9).

To correctly interpret the serological markers of LB, it is very important to determine the region's infection rate. The aim of this study was to ascertain the seroprevalence of specific IgG antibodies against *Borrelia burgdorferi s. l.* in Eastern Slovakia. Another aim was to evaluate prevalence of risk factors in seropositive individuals.

MATERIALS AND METHODS

Data Collection and Demography

The cross-sectional study was conducted from 2013 to 2016. In this study we assessed a group of 515 participants in Eastern

Slovakia (people from areas with a higher incidence of LB and who voluntarily agreed to participate in the study and patients who were hospitalized with a disease unrelated to the infectious cause of LB). Individuals were included in the study, with an age range between 12 and 93 years (average age 49.3, SD 17.2 years), of whom 235 were women (mean age 54.4, SD 17.25 years) and 280 men (mean age 45.0, SD 16.04 years). Age was classified into groups of ≤ 45 years, 46–60 years and ≥ 61 years.

The studied population was classified into two groups: group 1– urban residents (267 people from Turňa nad Bodvou, Giraltovce, Košice, Prešov, Snina, Rožňava, Poprad, Bardejov, Humenné, Sačurov, Kráľovský Chlmec) and group 2 – inhabitants of villages (248 people from Zlatá Idka, Jánovce, Kostoľany nad Hornádom, Myslava, Dobšinská Maša, Kavečany, Poproč, Betliar, Rozhanovce, Veľký Ruskov, Veľká Ida, Krásna, Petrovce nad Laborcom, Čaňa, Košické Oľšany).

All participants filled in a questionnaire covering socio-demographic and epidemiological characteristics, exposure to tick bites during professional activities and leisure time, as well as details related to their potential clinical history of LB. Afterwards, a health worker took blood samples to assess the presence of anti-*Borrelia* IgG and IgM antibodies. All participants signed an informed consent.

Serum Samples

Blood samples were taken by venous puncture and processed according to the usual protocols. The serum was separated by centrifugation for 10 minutes at 2,500 rpm and stored at −70 °C until analysed.

Laboratory Analyses

Positive serology was identified using the recommended two-tiered algorithm. All serum samples (n = 515) were screened for IgG/IgM antibodies by ELISA test. Sera with a positive or borderline result for IgG antibodies were further analysed with immunoblot assay. The assay was performed with commercial ELISA assays for IgG/IgM (recombinant): IgG ELISA BORG0040 and IgM ELISA BORM0040 (NovaLisaTM, NovaTec, Immunodiagnostica, GmbH, Dietzenbach, Germany) according to the manufacturer's instructions. Manufacturers offered 98.8% sensitivity, 100% specificity for IgG-ELISA, and 93% sensitivity, 98.8% specificity for IgM-ELISA.

The presence of IgG antibodies against the specific antigen *Borrelia burgdorferi s. l.* was determined by immunoblot assay using commercial LYMECHECK® OPTIMA IgG and IgM kits (BIOSYNEX, France). The tests were carried out in accordance with the procedure recommended by the manufacturer. Positive or negative results were determined based on the sum of the point values assigned to the individual bands corresponding to the reaction of IgG antibodies with specific *B. burgdorferi* antigens. Results of ≤ 5 were considered negative, those of 6 were classified as borderline and those of ≥ 7 were considered positive.

Statistical Analyses

Descriptive statistics were used to describe demographic values: gender, age categories, tick bite, outdoor activities, frequent contact with animals, neurological/rheumatological/dermatological symptoms. Differences between groups by residence/seropositivity were tested by chi-squared test. The ELISA and immunoblot results and their association with the risk factors were statistically analysed using the logistic regression. All statistical calculations were made with the software SPSS package (Statistical Package for the Social Sciences IBM-SPSS, version 21.0). The level of significance was set at $p \leq 0.05$.

RESULTS

Samples were collected from 280 (54.4%) males and 235 (45.6%) females. The age range was 2–93 years. The mean age was 45.2 years. A total of 267 (51.8%) people lived in urban territories, and the rest 248 (48.2%) were village residents.

Differences between the groups of people by residence were tested using Pearson's chi-squared test, which highlighted differences between the groups ($p < 0.001$) with variables – age categories, frequent activities in nature, neurological and rheumatological symptoms. Frequent activities in nature were reported by 39.8% of respondents. There were significant statistical differences between the groups ($p < 0.001$). Out of all participants, 63.7% reported tick exposure with no significant differences between the groups. Differences between groups are also apparent in terms of neurological, rheumatological and dermatological symptoms and there were more reported in the group of urban residents (Table 1).

Out of 515 persons tested for *B. burgdorferi s. l.* IgG with ELISA testing, 407 (79%) were seronegative, 16 (3.1%) had borderline result and 92 (17.9%) were positive. All positive or equivocal results (n = 108) were automatically reflex tested for a immunoblot assay. We found that 67 (62.1%) individuals tested positive, 9 (8.3%) tested borderline and 32 (29.6%) were negative for IgG antibodies (Fig. 1).

The *B. burgdorferi* screening ELISA test in urban residents revealed 43 (16.1%) positive samples; whereas 7 (2.6%) samples were borderline and 217 (81.3%) were negative. Further testing of the borderline and positive samples with immunoblot resulted in 29 (58%) positive specimens. The detailed results are presented in Figure 2.

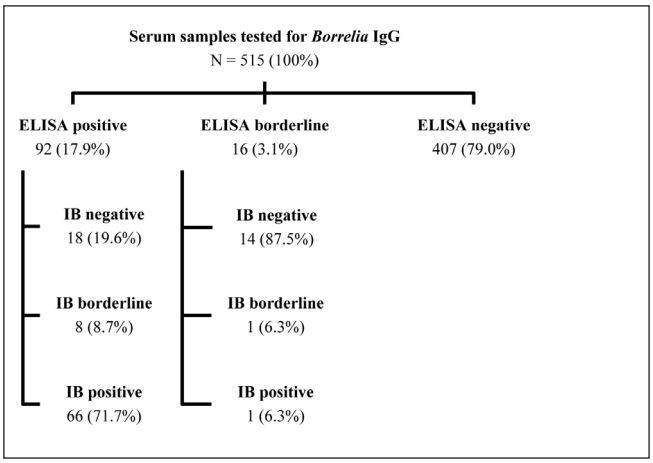

Fig. 1. *Flow chart of samples tested, according to ELISA and immunoblotting results in study group.*

Table 1. *Group differences in demographic characteristics, risk factors and symptoms*

	Urban residents N = 267 n (%)	Rural inhabitants N = 248 n (%)	Total N = 515 n (%)	χ^2 test (p-value)
Gender				
Men	140 (52.4)	140 (56.5)	280 (54.4)	0.36 (ns)
Women	127 (47.6)	108 (43.5)	235 (45.6)	
Age categories				
≤45	99 (37.1)	131 (52.8)	230 (44.7)	< 0.001
46–60	74 (27.7)	73 (29.4)	147 (28.5)	
≥61	94 (35.2)	44 (17.7)	138 (26.8)	
Tick bite				
No	95 (35.6)	92 (37.1)	187 (36.3)	0.72 (ns)
Yes	172 (64.4)	156 (62.7)	328 (63.7)	
Frequent activities in nature				
No	198 (74.2)	112 (45.2)	310 (60.2)	< 0.001
Yes	69 (25.8)	136 (54.8)	205 (39.8)	
Frequent contact with animals				
No	147 (55.1)	115 (46.4)	262 (50.9)	0.05
Yes	120 (44.9)	133 (53.6)	253 (49.1)	
Neurological symptoms				
No	181 (67.8)	211 (85.1)	392 (76.1)	< 0.001
Yes	86 (32.2)	37 (14.9)	123 (23.9)	
Rheumatological symptoms				
No	168 (62.9)	196 (79.0)	364 (70.7)	< 0.001
Yes	99 (37.1)	52 (21.0)	151 (29.3)	
Dermatological symptoms				
No	186 (69.7)	194 (78.2)	380 (73.8)	0.02
Yes	81 (30.3)	54 (21.8)	135 (26.2)	

χ^2 test – chi-squared test; ns – not significant

Furthermore, 319 participants from group of rural inhabitants were tested for ELISA: 49 (19.8%) were positive, 9 (3.6%) borderline and 190 (76.6%) were negative. The immunoblot was positive in 38 subjects (77.6%). The detailed results are presented in Figure 3.

In our sample of 515 serologic tests, we identified 40 (7.8%) instances of a positive IgM ELISA, 17 (3.3%) borderline and 458 (88.9%) a negative IgM ELISA test result. In urban residents, IgM were detected in 17 participants (6.4%); in rural inhabitants the seropositivity was 9.3% (n = 23) (Table 2).

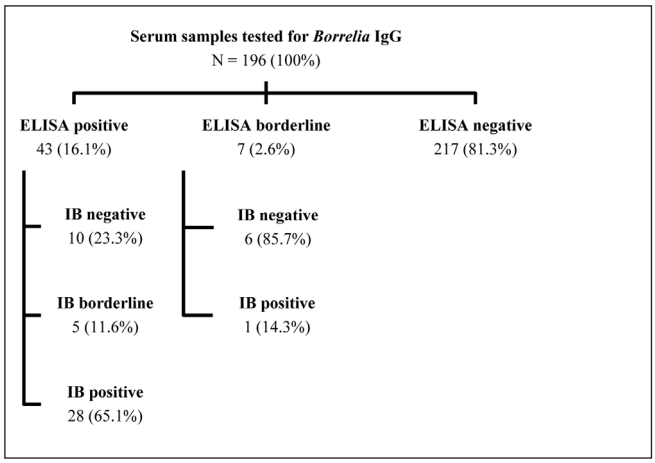

Fig. 2. *Flow chart of samples tested, according to ELISA and immunoblotting results in urban residents.*

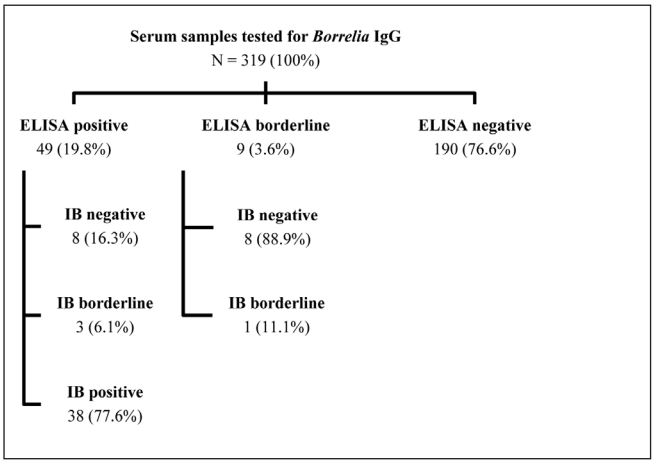

Fig. 3. *Flow chart of samples tested, according to ELISA and immunoblotting results in rural inhabitants.*

Table 2. *Results of serology of IgM antibodies against B. burgdorferi s. l. by ELISA*

	Serum samples tested for Borrelia IgM		
	Negative n (%)	**Borderline n (%)**	**Positive n (%)**
Urban N = 267 (100%)	242 (90.6)	8 (3.0)	17 (6.4)
Rural N = 248 (100%)	216 (87.1)	9 (3.6)	23 (9.3)
Total N = 515 (100%)	458 (88.9)	17 (3.3)	40 (7.8)

Higher seroprevalence was observed in men (n = 44, 65.7%) than in women (n = 23, 34.3%), and in individuals living in rural (56.7%) than in urban (43.3%) areas (Table 3). Using logistic regression, we found a statistically significant relationship between seropositivity and gender (OR = 0.42, 95% CI: 0.24–0.75, p < 0.001) and residence (OR = 2.11, 95% CI: 1.19–3.75, p < 0.01) in the whole sample (Table 4).

Our studied population was divided into three groups. We confirmed higher seropositivity in individuals older than 61 years of age (n = 30, 44.8%) than younger people (p = 0.001) (Table 3). Using logistic regression, we found a statistically significant relationship between seropositivity and age (OR = 1.03, 95% CI: 1.02–1.05, p < 0.001) in the whole sample and in urban inhabitants (OR = 1.04, 95% CI: 1.02–1.07, p < 0.002) (Table 4).

A total of 45 (67.2%) seropositive respondents reported tick exposure, 30 (44.8%) reported frequent contact with animals (ownership of pets or contact with animals) and 25 (37.3%) reported frequent outdoors activities. However, these differences were not statistically significant (Table 3, 4). Among those who reported neurological and rheumatological symptoms were seropositive for IgG antibodies against *Borrelia burgdorferi* only 25.4 and 22.4% of individuals, respectively. Dermatological symptoms were reported in 17.9% of seropositive participants. Also, no statistically significant differences in seropositivity were observed (Table 3, 4).

Table 3. *Differences between seropositive and seronegative individuals in demographic characteristics, risk factors and symptoms*

	Seronegativity* ELISA + IB n = 448	**Seropositivity ELISA + IB n = 67**	**χ^2 test (p-value)**
Gender			
Men	236 (52.7)	44 (65.7)	0.03
Women	212 (47.3)	23 (34.3)	
Age categories			
≤ 45	205 (45.8)	25 (37.3)	0.001
46–60	135 (30.1)	12 (17.9)	
≥ 61	108 (24.1)	30 (44.8)	
Residence			
Urban	238 (53.1)	29 (43.3)	0.13 (ns)
Rural	210 (46.9)	38 (56.7)	
Tick bite			
No	165 (36.8)	22 (32.8)	0.52 (ns)
Yes	283 (63.2)	45 (67.2)	
Frequent activities in nature			
No	268 (59.8)	42 (62.7)	0.65 (ns)
Yes	180 (40.2)	25 (37.3)	
Frequent contact with animals			
No	225 (50.2)	37 (55.2)	0.45 (ns)
Yes	223 (49.8)	30 (44.8)	
Neurological symptoms			
No	342 (76.3)	50 (74.6)	0.76 (ns)
Yes	106 (23.7)	17 (25.4)	
Rheumatological symptoms			
No	312 (69.6)	52 (77.6)	0.18 (ns)
Yes	136 (30.4)	15 (22.4)	
Dermatological symptoms			
No	325 (72.5)	55 (82.1)	0.33 (ns)
Yes	123 (27.5)	12 (17.9)	

*number of seropositive and border samples; χ^2 test – chi-squared test; ns – not significant

DISCUSSION

Lyme borreliosis is a zoonosis caused by infections with *Borrelia*. People infected with this aetiological agent can be asymptomatic or can develop disseminated disease. Diagnosis and recognition of groups at risk of infection with *Borrelia burgdorferi* is very important. Serologic testing is the standard for laboratory diagnosis and confirmation of LB.

Table 4*. Association of socio-demographic characteristics and risk factors with IgG seropositivity*

Variable	**Group**	**Odds ratio**	**95% CI**	**p-value**
Gender (Ref. men)	All[1]	0.42	0.24–0.75	< 0.001
	Urban	0.38	0.16–0.89	0.03
	Rural	0.82	0.41–1.66	0.58 (ns)
Age	All[1]	1.03	1.02–1.05	< 0.001
	Urban	1.04	1.02–1.07	0.002
	Rural	1.01	0.99–1.03	0.25 (ns)
Residence (Ref. urban)	All	2.11	1.19–3.75	< 0.001
Activities in nature	All[1]	0.8	0.44–1.46	0.47 (ns)
	Urban	0.9	0.37–2.22	0.82 (ns)
	Rural	0.7	0.35–1.40	0.32 (ns)
Frequent contact with animals	All[1]	0.71	0.41–1.22	0.22 (ns)
	Urban	1.16	0.54–2.52	0.71 (ns)
	Rural	0.58	0.29–1.16	0.12 (ns)
Tick exposure	All[1]	1.47	0.82–2.63	0.21 (ns)
	Urban	0.89	0.40–1.98	0.78 (ns)
	Rural	1.54	0.73–3.28	0.26 (ns)

Logistic regression models; [1]residence adjusted; ns – not significant

In 2008, 2009 and 2016, there was a significant increase in the annual incidence of laboratory-confirmed LB cases in Slovakia (9). The reasons of increasing incidence of LB are likely to be multifactorial and include geographical, environmental and climate factors. Seroprevalence data provide information on the incidence of exposure to *B. burgdorferi s. l.* in a certain geographical area. In Eastern Slovakia, seropositivity for *B. burgdorferi s. l.* of 13.01% (n = 67) was observed after two-tier testing. Our results are in line with that of a study in a rural district in northern Spain. In that study, 13.2% of the individuals presented a positive result for *B. burgdorferi* IgG antibodies (2), compared with our seropositivity of 13.01%. There have been also a number of studies in the USA and Europe that have looked at the seroprevalence of *B. burgdorferi* antibodies. Our results are also comparable to 13.9% seropositivity for *B. burgdorferi*, which was reported in a serosurvey of 230 persons in Maine, USA (10). Studies in other countries report different percentages: 4.2% in Scotland (11), 5.1% in Beijing (12), 9.4% in Germany (13), and 4.3% in Romania (14).

The immune response to infection with *Borrelia* depends on several factors, such as the quantity and characteristics of the infectious agent, duration of the infection and the ability of the host to respond to the infection. Also, although the humoral immune response to an infection with *B. burgdorferi s. l.* is often long lasting, the persistence of antibodies can vary widely, going from several months to many years (15). A seroprevalence of 13% is therefore not an exhaustive estimation of exposure to the bacteria.

The difference in seropositivity observed by sex was statistically significant ($p < 0.001$) in our study, a higher seroprevalence in men is also observed in the studies from other countries (13, 16). However, the predominance of men in the current study population does not concur with other European studies, where women had higher seropositivity (17, 18). In the USA, LB is more prevalent in men compared with women less than 60 years old, and equal or higher in women above 60 than among men (19). This is likely due to a higher exposure to ticks during their professional and leisure outdoor activities (15).

LB primarily affects those in middle age (51–60 years of age) and adult urban residents (20). There was significant difference in IgG seropositivity between the following age groups: ≤ 45, 46–60 and ≥ 61. In some studies, seropositivity for IgG antibodies was assessed according to the socio-demographic characteristics of persons. In our study, there were more seropositive people from rural (38%) than urban (29%) areas. This may be due to the fact that rural inhabitants carry out more frequent outdoor activities and have been more exposed to risk from ticks. It is logical to think that people with frequent activities in nature and with occupations in this field should have a higher infection rate. In Belgium, there are no differences between sera in rural and urban areas, with prevalence of 2.6 and 2.9%, respectively (21). The seropositivity according to the place of residence was analysed also in Germany, revealing higher rates in rural areas (13). Similarly, the seroprevalence was higher outside the city centre in the study of Cora et al. (22).

Among individuals with a history of tick bites, the percentage that was seropositive for *B. burgdorferi* was two times greater than that for seropositive individuals without a history of tick bites. Tick bites were observed in more than half of all LB cases 328 (63.7%). In our study, animals' ownership as a risk factor for Lyme disease is not statistically significant. In their study, Curran and Fish described association between cat ownership and increase of LB risk and in other study only 12% of the seropositive hunters had hunting dogs which were also seropositive (23, 24).

Neurological symptoms occurred in 17 (25.4%) IgG-seropositive cases, while rheumatological symptoms did not exceed a quarter of all IgG-seropositive cases (22.4%). There was statistically significant difference for frequency of neurological/rheumatological symptoms between urban and rural respondents. Fifty persons with antibodies to Borrelia by IgG immunoblot were without the above symptoms. Dermatological symptoms were reported in 17.9% of seropositive individuals. Previous studies from other countries demonstrated that individuals often exposed to ticks had a high proportion of asymptomatic, positive serologic responses, while the number of clinical cases was relatively low (25, 26).

There are no data on the real seroprevalence of antibodies to *B. burgdorferi* in asymptomatic or symptomatic people in Slovakia. The study by Ekerfelt et al. showed that asymptomatic seropositive individuals and patients with clinical LB have a similar *Borrelia*-specific interferon γ response in peripheral blood mononuclear cells (27). This finding cannot explain why some people have an antibody response when being exposed to the *B. burgdorferi* without developing symptoms of LB and why some persons get clinical LB.

One of the largest limitations of this study is the absence of knowledge about the clinical diagnoses associated with LB. The mentioned symptoms are the result of a questionnaire survey of respondents and are based only on their subjective assessment of health status. Consequently, we have not described our data as LB prevalence or incidence figures.

Although the sensitivity and specificity of the 2-tier antibody assay for *B. burgdorferi* is good validated (10), nonetheless, our findings might represent overestimates or underestimates of actual exposure to *B. burgdorferi s. l.* because of false-positive or false-negative results. These data provide evidence that humans are exposed to *B. burgdorferi* in Eastern Slovakia and help define the seroprevalence of human infection caused by that tick-borne pathogen.

CONCLUSION

In summary, we can highlight that the global climate change has expanded the range of tick vectors, suggesting that LB will remain an important health issue in the forthcoming decades. In this study it has been shown that IgG-seropositivity against *B. burgdorferi* in Eastern Slovakia have male predisposition and its occurrence is mainly in rural areas. Our results only provide information on historical exposure to *B. burgdorferi s. l.* but not on the incidence of disease as asymptomatic infections do occur. A good knowledge of the seroprevalence in a specific population is crucial for understanding the risk factors associated with seropositivity and we believe our results can be useful when testing other groups of people in Slovakia.

Acknowledgements

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