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Assessment of the frequency of IgM and IgG antibodies against *Borrelia burgdorferi* sensu lato in the serum of inhabitants the Poprad Landscape Park in southern Poland

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Abstract

Introduction and Objective. Borreliosis, also known as Lyme disease, is a chronic, multi-organ illness that is very difficult to diagnose. It is caused by the spirochete *Borrelia burgdorferi* sensu lato and transmitted to humans as a consequence of being bitten by a tick, mostly of the Ixodes genus, infected with the pathogen. The aim of the study is to assess the frequency of *B. burgdorferi* s.l. infections among a randomly selected human population living in the Poprad Landscape Park in southern Poland.

Materials and Method. Serum for the study was obtained from 99 randomly selected patients who reported for routine testing at the medical diagnostic laboratory in Krynica-Zdrój. The presence of IgM and IgG antibodies against *B. burgdorferi* s.l. spirochetes in the sera were defined using the ELISA method. Western Blot test verified positive and doubtful results.

Results. In total, positive or borderline results for at least one class of anti-Borrelia antibodies were found in 22.2% of human sera. Only in two samples were the positive results in anti-Borrelia IgM and IgG shown. Antibodies against the spirochete *B. burgdorferi* s.l. were detected both in people who had found a tick on their body, and in people who claimed they never had.

Conclusions. Studies have shown a high percentage of people with antibodies against detected *B. burgdorferi* s.l. This may indicate frequent bites of the inhabitants of the Poprad Landscape Park by ticks, during which transmission of the *B. burgdorferi* s.l. spirochete occurs.

Key words

serum, *Borrelia burgdorferi* sensu lato, Elisa, Western Blot, Poprad Landscape Park

INTRODUCTION

The Poprad Landscape Park, located along the Poprad River in the southern Beskid Sądecki mountains, is one of Poland's largest landscape parks. It features diverse forest ecosystems and rich biodiversity, valuable for both conservation and tourism. Within its boundaries lies Krynica-Zdrój, a well-known spa town with mineral springs and a developed tourist infrastructure that attracts visitors all year round.

Lyme disease is the most prevalent vector-borne disease in Poland and in Europe, caused by *Borrelia burgdorferi* sensu lato transmitted by *Ixodes ticks*. The spirochete circulates in nature via complex cycles involving birds and rodents as key reservoir hosts. Of the 11 known genospecies in Europe, six are commonly detected in *Ixodes ricinus* ticks, with *B. afzelii*, *B. garinii*, and *B. valaisiana* most frequently isolated in Central Europe [1]. Three genospecies of this spirochete are responsible for developing Lyme borreliosis in humans: *B. burgdorferii* s.s., *B. afzelii,* and *B. garinii*. The spread of spirochetes in the body takes place through the blood, lymph and peripheral nerves [2, 3]. Lyme disease is a chronic, multi-system infection that progresses through distinct stages. Early localized disease often presents with erythema migrans and flu-like symptoms. In the early disseminated stage, complications such as arthritis, neuroborreliosis, and carditis may occur. Without treatment, the disease can advance to chronic forms, leading to cognitive impairment, joint damage, and persistent neurological symptoms. Some patients experience post-treatment Lyme disease syndrome (PTLDS), marked by fatigue, joint pain, and cognitive issues. Early diagnosis and antibiotic therapy are essential to prevent severe outcomes [4, 5]. In Poland in 2024, nearly 30,000 borreliosis cases were detected in humans [6].

The diagnosis of the early stage of Lyme disease by the appearance of erythema migrans, together with confirmed contact with a tick, can be established based on the clinical picture. The remaining stages of Lyme disease require careful

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differential diagnosis and are proven using laboratory tests [7, 8, 9]. The broad spectrum of clinical and serological manifestations of Lyme disease poses significant diagnostic and therapeutic challenges. Limited knowledge of disease progression, insufficient diagnostic tools, and lack of access to specialized methods, often lead to under-diagnosis. The multi-stage nature of the disease and potential coinfections with other tick-borne pathogens complicate both diagnosis and treatment. Therapy can be prolonged and costly, particularly in advanced cases [2, 5].

Standard Lyme disease diagnosis relies on indirect serological tests detecting IgM and IgG antibodies. ELISA is the most commonly used screening method due to its simplicity and low cost. According to European Union (EU) guidelines, positive or equivocal ELISA results should be confirmed by Western Blot to distinguish false positives from true infections. Serological findings must always be interpreted in conjunction with clinical symptoms and a thorough medical history [9, 10, 11].

Recent studies by Koczanowicz et al. [12, 13] conducted in selected recreational areas of the Poprad Landscape Park showed a high potential risk of exposure of residents and tourists to tick-borne infection with these spirochetes. Therefore, the aim of this study was to assess the frequency of *B. burgdorferi* s.l., infections among a randomly selected human population living in the Poprad Landscape Park

MATERIALS AND METHOD

The sera for this study were collected in August 2021 from 99 randomly selected patients reporting for routine tests to the medical diagnostic laboratory of the J. Dietl Hospital in Krynica-Zdrój, located near the Poprad Landscape Park. The serum was obtained in accordance with the opinion of the Bioethics Committee at the District Medical Chamber in Kraków which acknowledged and approved the conduct of the study and the publication of its results, including statistical data (Approval No. OIL/KBLT/74/2021, dated 16 June 2021). The serum was transported to the Department of Parasitology, Faculty of Pharmaceutical Sciences, in Sosnowiec, Medical University of Silesia in Katowice, and stored at -80 °C for further serological testing to detect the presence of IgM and IgG antibodies directed against *B. burgdorferi* s.l. The patients from whom serum was collected were asked to complete a short original questionnaire concerning, among others, age, gender, and through the survey, basic information on contact with ticks and Lyme disease. The ELISA test was conducted with the use of the ready-made tests NovaLisa™ *Borrelia burgdorferi* IgM – ELISA (recombinant) and NovaLisa™ *Borrelia burgdorferi* IgG – ELISA (recombinant) (NovaTec Immunodiagnostica GmbH, Dietzenbach, Germany), using the MINDRAY MR-96A device (High-tech Industrial Park, Nanshan, Shenzhen, PR,China) according to the manufacturer's protocol. For the Western Blot analysis, the ready-made sets Anti-Borrelia EUROLINE-RN-AT-adv (IgM) and Anti-Borrelia EUROLINE-RN-AT-adv (IgG) (Euroimmun Medizinische Labordiagnostica AG, Lűbeck, Germany) were used according to the manufacturer's protocol. The strips were scored manually using a ready-made control template included with the protocol. Western Blot analysis for IgM concerned the antigens: OspC-adv Bsp, OspC-adv Bg, OspC-adv Bb, OspC-adv Ba, p39, p41, and VlsE Bb. In turn, analysis for IgG included the following antigens: p18, p19, p20, p21, p58, OspC (p25), p39, p83, p41, LBb, LBa, VIsE Bg, VIsE Bb, and VIsE Ba.

Results from ELISA, Western Blot, and survey information were tested with a chi-square test. The level of significance varied in survey questions because of multiple testing when there were more options in answers, and multiple variants of IgG and IgM status, in which case the Bonferroni correction was used. For questions regarding gender and diagnosis of Lyme disease – $p<0.017$; for question about the age of patients – $p<0.002$, and for questions about place of residence, contact with a tick, and contact with nature – $p<0.005$ was considered significant. Doubtful results of antibody detection were treated as positive for survey analysis. All analyses were performed in Statistica software version 13 (TIBCO Software Inc., Palo Alto, CA, USA).

RESULTS

Most of the collected serum samples (66%; n=65) came from women. The majority of respondents were in the age group of 40–70 years and 71%'; n=70 of respondents confirmed that the tick was found on their body at least once, and 12%; n=12 noticed that the tick was moving around in their clothes or on their body to look for a place to bite, but it was removed before it could do so.

Of the 99 samples tested with the ELISA, a positive or borderline result in at least one class of anti-*Borrelia* antibodies was found in 22.2%; n=22 of patients.

Table 1. Distribution of results between ELISA results and Western Blot tests detecting antibodies specific to *Borrelia burgdorferi* sensu lato

ELISA test	Western blot test			Total
	IgM (+) /IgG (+)	IgM (+) /IgG (-)	IgM (-) /IgG (+)	
IgM (+) /IgG (+)	2	0	0	2
IgM (+) /IgG (-)	4	2	1	7
IgM (-) /IgG (+)	4	1	0	5
IgM (+/-) /IgG (+)	1	0	0	1
IgM (+) /IgG (+/-)	0	1	0	1
IgM (+/-) /IgG (-)	2	3	0	5
IgM (-) /IgG (+/-)	0	1	0	1
Total	13	8	1	22

IgM (+) – positive result, IgM (+/-) – doubtful result, IgM (-) – negative result, IgG (+) – positive result, IgG (+/-) – doubtful result, IgG (-) – negative result.

In the IgM class, there were 16 people, of whom 10 had a positive result, and 6 had a doubtful result. In the IgG class, antibodies were detected in 10 people, of whom 8 had a positive result, and 2 had a doubtful result. Additionally, 4 people from the tested group showed antibodies in both the IgM and IgG classes, of which 1 person had a positive IgM result, but a doubtful IgG result, 1 person had a positive IgG result but a doubtful IgM result, and 2 people had both a positive IgM result and a positive IgG result (Tab. 1).

A positive or borderline result in at least one class of antibodies appeared in 24.2% of women (16/66) and in 18.2% of men (6/33). The largest number of antibodies against *B. burgdorferi* s.l., was detected in the age group 30–39 years, 40% among women and 50% among men. In both genders,

no antibodies were detected in people aged over 70 years. Moreover, in men, there are no positive and borderline results in the ranges below 18 years of age and between 18–29 years of age.

In total, the Western Blot tests were conducted on 22 samples that had positive or doubtful results from the ELISA tests (Tab. 1). All 22 tested samples showed a positive result in at least one class of antibodies. IgM was confirmed in 21 samples (95.4%), including 9 samples that were positive in the ELISA test, 6 that were previously doubtful, and 6 that were negative. One sample, in which the Western Blot was not confirmed, was positive in the ELISA test. IgG antibodies were confirmed in 14 samples (63.6%), including 7 that were positive in the ELISA test, 3 that were doubtful, and 4 negative. Of the 8 Western Blot samples that were negative in the ELISA tests, 3 were positive, 3 doubtful, and 2 were negative.

The majority of the samples confirmed as positive by Western Blot correlate with the positive or doubtful results of the ELISA test, indicating consistency between the tests. Eight people confirmed that they had been diagnosed with Lyme disease in the past, but only 3 of them people had antibodies detected – 2 in the IgG class, 1 positive in the IgM class, and doubtful in the IgG class. In all 3 patients, IgM and IgG antibodies were confirmed by the Western Blot test.

Statistical analysis of the survey results did not reveal any significant statistical correlation between detected antibodies and gender, age, place of residence, contact with ticks, Borrelia disease, and spending time in nature. The results are presented as Tables in Supplement 1.

DISCUSSION

Among the 22 patients in whom antibodies against *B. burgdorferi* s.l. were detected, as many as 5 had never noticed a tick feeding on their body. Three of these individuals had IgM antibodies, 1 had IgG antibodies, and 1 had both IgM and IgG. This indicates that ticks, due to their small size and painless bite, often go unnoticed by the host, which may lead to *B. burgdorferi* s.l., infection without awareness of tick exposure. Additionally, 8 individuals confirmed that they had been diagnosed with Lyme disease in the past; however, only 3 of them tested positive for antibodies – 2 in the IgG class and 1 in both the IgG and IgM classes. Similarly, in the study by Zalewska-Ziob et al. [14], antibodies were detected both in individuals with a prior diagnosis of early-stage *B. burgdorferi* s.l., infection, and in those in whom the infection had been ruled out. In these cases, this may indicate that the disease has resolved or that the test result was a false negative. The highest incidence of antibodies against *B. burgdorferi* s.l. was detected in the age group of 30–39 years. Individuals in this age range often actively spend time outdoors in recreational areas, which increases their potential exposure to tick infestation and potential infection with tick-borne diseases. Moreover, in all age groups, as many as 98% of the respondents confirmed that they often or very often spend time in recreational, green and forest areas, which significantly increases the potential risk of exposure to ticks and tick-borne diseases. This was also confirmed by Zając et al. [15], who in their studies conducted in central and eastern Poland showed that people who often spent time in the forest had a significantly higher rate of seropositive reactions.

In Poland, the ELISA test revealed the lowest percentage of *B. burgdorferi* s.l. antibodies among healthy individuals in the Mazowieckie Province of eastern-central Poland – 10% for IgM and 2.2% for IgG [16]. In contrast, the highest level of antibodies was detected among forestry workers in the West Pomeranian region, with 19.2% for IgM and 26.9% for IgG [17]. In the current study, a positive or borderline result in at least one antibody class was detected in 22% of patients, with 16% in the IgM class and 10% in the IgG class. A similar percentage of positive or borderline IgM antibodies was found among farmers in the Lublin Province in eastern Poland (16.8%) and the Masovian Province (15.3%) [15]. Similarly, a slightly higher percentage of positive or borderline IgG antibodies compared to our study was found among farmers in the Lublin Province (13.6%) and among office workers in southern Poland (13.7%) [18]. Comparable levels of IgM and IgG antibodies detected among farmers, office workers, and the randomly selected group of patients in the current study, suggest that infection with *B. burgdorferi* s.l. is not limited to individuals working in high-risk tick-exposure environments, but also affects those who spend their leisure time in recreational areas. This may also indicate the widespread presence of infected ticks in the region, which has been confirmed in tick surveillance studies conducted between 2018 – 2021 in the Poprad Landscape Park. A high prevalence of *B. burgdorferi* s.l. was found, along with the presence of other tick-borne pathogens, including *Anaplasma phagocytophilum* and *Babesia microti* [12, 13].

Table S1. Sex of participants

	IgM+/IgG+ Western blot	IgM+/IgG- Western blot	IgM-/IgG+ Western blot	IgM+/IgG+ ELISA	IgM+/IgG- ELISA	IgM-/IgG+ ELISA	IgM+ any method	IgM-/IgG-	*p*-value
Female	10	6	0	2	9	5	16	50	Western blot: IgM+/IgG+ vs. rest: 1.785 (0.456 - 6.987); p=0.405 IgM+/IgG- vs. rest: 1.55 (0.295 - 8.136); p=0.604 IgM-/IgG+ vs. rest: NaN (1 case) ELISA: IgM+/IgG+ vs. rest: 0.484 (0.065 - 3.602); p=0.479 IgM+/IgG- vs. rest: 1.579 (0.398 - 6.273); p=0.516 IgM-/IgG+ vs. rest: 2.623 (0.294 - 23.420); p=0.388 IgM+ any method vs. IgM-: 1.440 (0.505 - 4.109); p=0.496
Male	3	2	1	2	3	1	6	27	

Significant results with p<0.017 with Bonferroni correction

Table S2. Age of participants

	IgM+/IgG+ Western blot	IgM+/IgG- Western blot	IgM-/IgG+ Western blot	IgM+/ IgG+ ELISA	IgM+/ IgG- ELISA	IgM-/ IgG+ ELISA	IgM+ any method	IgM-/IgG-	*p*-value
Under 18	0	1	0	0	1	0	1	5	Under 18: Western blot: IgM+/IgG+ vs. rest: 0.459 (0.024 - 8.622); p=0.603 IgM+/IgG- vs. rest: 2.457 (0.251 - 24.049); p=0.440 IgM-/IgG+ vs. rest: NaN (1 case) ELISA: IgM+/IgG+ vs. rest: 1.530 (0.074 - 31.612); p=0.783 IgM+/IgG- vs. rest: 1.491 (0.159 - 13.968); p=0.726 IgM-/IgG+ vs. rest: 1.036 (0.052 - 20.485); p=0.982 IgM+ any method vs. IgM-: 0.686 (0.076 - 6.197); p=0.737
18-29	1	0	0	0	1	0	1	11	18-29: Western blot: IgM+/IgG+ vs. rest: 0.568 (0.067 - 4.810); p=0.604 IgM+/IgG- vs. rest: 0.374 (0.020 - 6.894); p=0.508 IgM-/IgG+ vs. rest: NaN (1 case) ELISA: IgM+/IgG+ vs. rest: 0.742 (0.038 - 14.637); p=0.845 IgM+/IgG- vs. rest: 0.628 (0.074 - 5.352); p=0.671 IgM-/IgG+ vs. rest: 0.502 (0.027 - 9.463); p=0.645 IgM+ any method vs. IgM-: 0.286 (0.035 - 2.345); p=0.243
30-39	1	2	0	0	2	1	3	4	30-39: Western blot: IgM+/IgG+ vs. rest: 1.111 (0.123 - 10.051); p=0.925 IgM+/IgG- vs. rest: 5.733 (0.913 - 35.987); p=0.062 IgM-/IgG+ vs. rest: NaN (1 case) ELISA: IgM+/IgG+ vs. rest: 1.311 (0.064 - 26.743); p=0.860 IgM+/IgG- vs. rest: 3.280 (0.561 - 19.187); p=0.188 IgM-/IgG+ vs. rest: 2.900 (0.291 - 28.952); p=0.364 IgM+ any method vs. IgM-: 2.882 (0.594 - 13.987); p=0.189
40-55	4	4	1	1	6	2	9	31	40-55: Western blot: IgM+/IgG+ vs. rest: 0.617 (0.176 - 2.162); p=0.451 IgM+/IgG- vs. rest: 1.528 (0.359 - 6.502); p=0.566 IgM-/IgG+ vs. rest: NaN (1 case) ELISA: IgM+/IgG+ vs. rest: 0.479 (0.048 - 4.773); p=0.530 IgM+/IgG- vs. rest: 1.559 (0.465 - 5.232); p=0.472 IgM-/IgG+ vs. rest: 0.724 (0.126 - 4.152); p=0.717 IgM+ any method vs. IgM-: 1.027 (0.392 - 2.694); p=0.956
56-70	7	1	0	3	2	3	8	23	56-70: Western blot: IgM+/IgG+ vs. rest: 3.014 (0.919 - 9.886); p=0.069 IgM+/IgG- vs. rest: 0.291 (0.034 - 2.470); p=0.258 IgM-/IgG+ vs. rest: NaN (1 case) ELISA: IgM+/IgG+ vs. rest: 7.179 (0.716 - 72.016); p=0.094 IgM+/IgG- vs. rest: 0.400 (0.082 - 1.946); p=0.256 IgM-/IgG+ vs. rest: 2.321 (0.441 - 12.216); p=0.320 IgM+ any method vs. IgM-: 1.342 (0.495 - 3.634); p=0.563
Above 70	0	0	0	0	0	0	0	3	Above 70: Western blot: IgM+/IgG+ vs. rest: 0.884 (0.043 - 18.079); p=0.936 IgM+/IgG- vs. rest: 1.487 (0.071 - 31.271); p=0.798 IgM-/IgG+ vs. rest: NaN (1 case) ELISA: IgM+/IgG+ vs. rest: 2.937 (0.131 - 65.881); p=0.497 IgM+/IgG- vs. rest: 0.966 (0.047 - 19.832); p=0.982 IgM-/IgG+ vs. rest: 1.989 (0.093 - 42.773); p=0.661 IgM+ any method vs. IgM-: 0.473 (0.024 - 9.506); p=0.625

Significant results with p<0.002 with Bonferroni correction

Table S3. Participants' contact with a tick

	IgM+/IgG+ Western blot	IgM+/IgG- Western blot	IgM-/IgG+ Western blot	IgM+/ IgG+ ELISA	IgM+/ IgG- ELISA	IgM-/ IgG+ ELISA	IgM+ any method	IgM-/IgG-	*p*-value
Yes – infestation	9	8	0	3	9	5	17	54	Infestation vs. rest: Western blot: IgM+/IgG+ vs. rest: 0.871 (0.245 - 3.097); p=0.831 IgM+/IgG- vs. rest: 7.630 (0.426 - 136.774); p= 0.168 IgM-/IgG+ vs. rest: NaN (1 case) ELISA: IgM+/IgG+ vs. rest: 1.191 (0.119 - 11.961); p=0.882 IgM+/IgG- vs. rest: 1.210 (0.302 - 4.841); p=0.788 IgM-/IgG+ vs. rest: 2.046 (0.228 - 18.336); p=0.522 IgM+ any method vs. IgM-: 1.448 (0.477 - 4.395); p= 0.513
Yes – only superficial	1	0	0	0	1	0	1	11	Superficial vs. rest Western blot: IgM+/IgG+ vs. rest: 0.568 (0.067 - 4.810); p=0.604 IgM+/IgG- vs. rest: 0.374 (0.020 - 6.894); p= 0.508 IgM-/IgG+ vs. rest: NaN (1 case) ELISA: IgM+/IgG+ vs. rest: 0.742 (0.038 - 14.637); p=0.845 IgM+/IgG- vs. rest: 0.628 (0.074 - 5.352); p=0.671 IgM-/IgG+ vs. rest: 0.502 (0.027 - 9.463); p=0.645 IgM+ any method vs. IgM-: 0.286 (0.035 - 2.345); p=0.244
No	3	0	1	1	2	1	4	12	No vs. Yes Western blot: IgM+/IgG+ vs. rest: 1.685 (0.408 - 6.961); p=0.471 IgM+/IgG- vs. rest: 0.269 (0.015 - 4.899); p=0.375 IgM-/IgG+ vs. rest: NaN (1 case) ELISA: IgM+/IgG+ vs. rest: 1.778 (0.173 - 18.262); p=0.628 IgM+/IgG- vs. rest: 1.043 (0.206 - 5.282); p=0.960 IgM-/IgG+ vs. rest: 1.040 (0.113 - 9.547); p=0.972 IgM+ any method vs. IgM-: 1.204 (0.346 - 4.186); p=0.771

Significant results with p<0.005 with Bonferroni correction

Table S4. Diagnosis of Lyme disease

	IgM+/IgG+ Western blot	IgM+/IgG- Western blot	IgM-/IgG+ Western blot	IgM+/IgG+ ELISA	IgM+/IgG- ELISA	IgM-/IgG+ ELISA	IgM+ any method	IgM-/IgG-	*p*-value
Yes	3	0	0	1	0	2	3	5	Western blot: IgM+/IgG+ vs. rest: 4.86 (1.006 - 23.476); p= 0.049 IgM+/IgG- vs. rest: 0.578 (0.031 to 10.911); p= 0.715 IgM-/IgG+ vs. rest: NaN (1 case)
No	10	8	1	3	12	4	19	72	ELISA: IgM+/IgG+ vs. rest: 4.333 (0.397 - 47.302); p= 0.229 IgM+/IgG- vs. rest: 0.374 (0.020 - 6.894); p= 0.508 IgM-/IgG+ vs. rest: 7.250 (1.097 - 47.908); p= 0.040 IgM+ any method vs. IgM-: 2.274 (0.498 - 10.375); p= 0.289

Significant results with p<0.017 with Bonferroni correction

Similar serological studies have also been conducted accross the border in Slovakia, not far from Krynica-Zdrój, where serum samples were collected from gardeners and soldiers occupationally exposed to tick bites. The proportion of positive and borderline results reached 9.9% for IgM and 19.1% for IgG [19]. In the current study, the high percentage of positive and borderline IgM antibody results, which may indicate recent infection, could be associated with the season during which the serum samples were collected. Blood was drawn in summer when tick exposure is more frequent. Study participants also reported that they often spend time outdoors, which may be related to the attractive landscape of the region and the high number of tourist destinations. Additionally, Western Blot was performed on samples with positive and borderline ELISA results, and confirmed a positive result in at least one antibody class. IgM was confirmed in 95.4% of samples and IgG confirmed in 63.6%. The majority of the samples were confirmed as

Table S5 Participants contact with nature

	IgM+/IgG+ Western blot	IgM+/IgG- Western blot	IgM-/IgG+ Western blot	IgM+/ IgG+ ELISA	IgM+/ IgG- ELISA	IgM-/ IgG+ ELISA	IgM+ any method	IgM-/IgG	*p*-value
Rarely	1	1	0	0	1	1	2	8	Rarely vs. rest Western blot: IgM+/IgG+ vs. rest: 0.713 (0.083 - 6.143); p=0.758 IgM+/IgG- vs. rest: 1.302 (0.143 - 11.811); p=0.815 IgM-/IgG+ vs. rest: NaN (1 case) ELISA: IgM+/IgG+ vs. rest: 0.905 (0.045 - 18.015); p= 0.948 IgM+/IgG- vs. rest: 0.788 (0.091 - 6.834); p= 0.829 IgM-/IgG+ vs. rest: 1.867 (0.196 - 17.789); p=0.587 IgM+ any method vs. IgM-: 0.863 (0.169 - 4.391); p=0.859
Often	3	6	0	3	4	2	9	36	Often vs. rest Western blot: IgM+/IgG+ vs. rest: 0.314 (0.081 - 1.222); p= 0.095 IgM+/IgG- vs. rest: 4.000 (0.766 - 20.897); p=0.100 IgM-/IgG+ vs. rest: NaN (1 case) ELISA: IgM+/IgG+ vs. rest: 3.786 (0.380 - 37.727); p=0.256 IgM+/IgG- vs. rest: 0.561 (0.157 - 2.001); p=0.373 IgM-/IgG+ vs. rest: 0.581 (0.102 - 3.331); p=0.543 IgM+ any method vs. IgM-: 0.789 (0.302 - 2.061); p= 0.628
Very often	9	1	1	1	7	3	11	33	Very often vs. rest Western blot: IgM+/IgG+ vs. rest: 3.279 (0.936 - 11.488); p=0.063 IgM+/IgG- vs. rest: 0.160 (0.019 - 1.349); p=0.092 IgM-/IgG+ vs. rest: NaN (1 case) ELISA: IgM+/IgG+ vs. rest: 0.403 (0.041 - 4.017); p= 0.439 IgM+/IgG- vs. rest: 1.892 (0.556 - 6.433); p=0.307 IgM-/IgG+ vs. rest: 1.268 (0.243 - 6.616); p= 0.778 IgM+ any method vs. IgM-: 1.333 (0.516 - 3.447); p=0.553

Significant results with p<0.005 with Bonferroni correction

positive by Western Blot and correlated with the positive or doubtful results of the ELISA test, indicating consistency between the tests.

Studies on the prevalence of antibodies against *B. burgdorferi* s.l. conducted using a two-step diagnostic approach (ELISA followed by confirmatory Western Blot), have been carried out in various regions of Poland. In the Warmian-Masurian Province in the northern part of the country, forestry workers were examined and the percentage of positive results reached 63.1% [20]. In turn, in the Lublin Province in eastern Poland, the study population included hunters and individuals who regularly spent time in forested areas, with antibodies detected in 38% of cases [21]. In the same region, farmers and a control group of healthy individuals were also tested. The results revealed a significant difference: antibodies were found in 33% of farmers, while only 6% of healthy controls tested positive [22]. A similar comparison was conducted in the Lublin Province and Podlaskie Province (north-eastern Poland), where both forestry and agricultural workers were examined. The findings showed that forestry workers were at higher risk of infection than farmers – antibodies were detected in 55% of foresters compared to 28% of farmers [23]. In western Poland, where foresters were studied, antibodies were found in 37.5% of samples [24]. In the Łódź Province in central Poland, the percentage of positive results was 21%, one of the lowest rates among occupational groups at high risk of tick exposure [25].

The above data confirm that forestry workers and individuals who frequently visit forested areas are at a high potential risk of exposure to *B. burgdorferi* s.l. compared to farmers and the general population. At the same time, regional differences suggest that the risk of infection may depend on local environmental conditions and the prevalence of infected ticks.

Currently, no active prophylaxis in the form of vaccination is available against *Borrelia burgdorferi*; therefore, the primary prevention of Lyme disease involves protecting the body from ticks by wearing appropriate clothing and avoiding tall, uncut vegetation. The use of repellents and the prompt mechanical removal of a feeding tick are also helpful. Additionally, removing leaves, tall grass, and shrubs from workplaces or residential areas can reduce tick habitats.

CONCLUSIONS

The study showed that in a high percentage of residents of the Poprad Landscape Park and tourists to the area, their serum showed the presence of antibodies against *B. burgdorferi*

s.l. These antibodies were present both in people who had been diagnosed with Lyme disease in the past and in those who had not yet developed symptoms of the disease, and had not had or did not remember contact with a tick. This high percentage of positive results may confirm the previous results of field studies that showed a high percentage of ticks infected with *B. burgdorferi* s.l. in this area. Unfortunately, the obtained results of screening tests also indicate their questionable effectiveness if specific disease symptoms do not occur. Hence, it seems that clinical assessment and carefully conducted differential diagnosis remain the best tools for making a decision on Lyme disease treatment and assessing its effectiveness.

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