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Knowledge, protection behaviours and seroprevalence of Lyme borreliosis in inhabitants of Lublin Province, eastern Poland – evaluation of a prophylaxis programme

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Abstract

Introduction and Objective. Lyme borreliosis (LB) is the most frequent tick-borne disease with 17,338 cases reported in Poland in 2022. Since research on a LB vaccine is still ongoing, the promotion of individual behaviours and limiting of tick exposure is one of the most effective ways to prevent LB. The aim of the study was to evaluate the effectiveness of the LB prevention programme by assessing the knowledge, practice behaviours, seroprevalence of LB and satisfaction among the population of the Lublin Province in eastern Poland.

Materials and method. The prevention programme was carried out among 2,920 participants who were asked about their exposure to ticks, history of LB and prevention behaviours. Awareness of knowledge was evaluated before and after training. Seroprevalence of LB was rated by ELISA and immunoblot assays.

Results. Over 73% of participants reported tick bites in their lifetime, without significant differences between rural and urban area inhabitants. More than 80% of individuals declared that they use protective measures (PPM), such as proper clothes and body checking; repellents were the least frequently used by participants. The diagnosis of LB but not tick bites in a lifetime influenced the more frequent use of PPM. Increase in knowledge was observed in 86% of participants after education, and the highest knowledge was noted among respondents with higher education. The seroprevalence of anti-*B. burgdorferi* antibodies was 37% and was higher among men than women (40% vs. 36%).

Conclusions. The population of Lublin Province is highly exposed to tick bites and infection with *B. burgdorferi*. The high seroprevalence and increase in knowledge confirmed the effectiveness and need for preventive programmes. These results can be useful for optimizing and enhancing the effects of future prevention campaigns.

Key words

Lyme borreliosis, seroprevalence, tick bites risk, personal protective measures, prophylaxis

INTRODUCTION AND OBJECTIVE

Lyme borreliosis (LB), also known as Lyme disease, is the most common tick-borne disease (TBD) in Europe. The infection is caused by the spirochete *Borrelia (Borreliella) burgdorferi* sensu lato transmitted by infected *Ixodes* species ticks. In Europe, *Ixodes ricinus* is the predominant vector of *Borrelia*. Most cases of LB in Europe are caused by *B. garinii*, *B. afzelii* and *B. burgdorferi* sensu stricto genospecies [1, 2]. LB has a diverse clinical presentation, of which erythema migrans (EM) is the most characteristic symptom of an early stage of infection, and without treatment can disseminate into a systemic disease. The skin, nervous system, joints and heart are the most frequently affected organs [3, 4].

Lyme disease belongs to the constantly emerging and most common zoonotic diseases in Europe. In Western Europe, the incidence of LB has been estimated as 22.04 per 100,000 person-year [5], and more than 200,000 cases of LB per year are diagnosed and treated [2]. Generally, the number of TBD cases doubled between 2004–2016 and LB consisted 82% of total cases [4]. In 2022, 17,338 cases of LB were reported in Poland, and the incidence was estimated as 45.43 cases per 100,000 population [6].

The risk of *Borrelia* infection is associated with activity-related contact with ticks and depends on the density and infection rate of the tick population [7]. Outdoor workers, especially foresters and farmers, are occupationally exposed to infection with *B. burgdorferi*. Other activities (e.g. hunting or mushroom and berry collecting) are correlated with higher LB risk [8].

There is currently no vaccine available against LB and prevention strategies are based on the promotion of individual behaviours and taking action in the environment to reduce the density of ticks [9]. In Europe, a 2-tier algorithm for the laboratory diagnosis of Lyme borreliosis is recommended. In the initial step, a sensitive enzyme-linked immunosorbent assay (ELISA) should be used, and positive and equivocal results should be retested by immunoblot assay [2]. The diagnosis of LB requires clinical manifestations of infection. In some patients, uncritical interpretation of clinical signs may result in incorrect interpretation of the serological result,

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and misinterpretation of active LB in healthy individuals with positive results. In contrast, some patients with typical symptoms remain undiagnosed and untreated [10].

The aim of the study was to evaluate the effectiveness of a Lyme disease prevention programme carried out among the inhabitants of the Lublin Province in eastern Poland. Knowledge, practices behaviours and seroprevalence of LB among participants in the prophylaxis programme were assessed.

MATERIALS AND METHOD

Prophylaxis programme, data collection and analysis. The programme 'Prophylaxis of Lyme borreliosis among the inhabitants of the Lublin Province' was conducted between July 2020 – May 2022 and involved 2,920 participants. The project was implemented under the Regional Operational Programme for the Lublin Province 2014–2020. Inclusion criteria were: 1) age ≥ 15, 2) resident of the Lublin Province and 3) work active person. Recruitment for the programme took place through the distribution of educational materials (brochures and leaflets), advertising in the press, radio and television. The programme process was as follows: 1) initial visit to a care physician: checking the inclusion criteria, informed consent, completing the Qualification Questionnaire (QQ) and Evaluation Scale I (ESI), providing information on LB and the principles of tick bite prevention and blood collection for ELISA test; 2) second visit to the care physician: interpretation of the serological results; 2a) negative results – end of participation: completing the Evaluation Scale II (ESII) and the Satisfaction Questionnaire (SQ), or 2b) positive/borderline results: second blood sample collection for Immunoblot testing (the first 700 participants); 3) visit with infectious diseases specialists, complete ESII and SQ. For the rest of the participants with positive/borderline results in the ELISA test on the second visit (more than 700), the Evaluation Scale II and the Satisfaction Questionnaire were completed; consultation with infectious diseases specialists outside the programme was encouraged.

Informed consent was obtained from the participants before the start of the survey and blood sampling. The participants completed a Qualification Questionnaire inquiring about socio-demographic and work characteristics, exposure to ticks, and symptoms that may suggest Lyme borreliosis, history of laboratory tests and diagnosis of LB, and behaviours preventing tick-borne diseases. The study used a pre-test – post-test Evaluation Scale to estimate knowledge about Lyme disease and the prevention of tick-borne diseases. After completing the Evaluation Scale I (ESI), all enrolled participants were educated by a medical doctor about *Borrelia* infection and prophylaxis. On the last visit, the participants answered the same question in Evaluation Scale II. Participants who did not complete the procedures required in the programme (doctor visit with interpretation of laboratory tests) were excluded from evaluation of knowledge (10 participants). Assessment of the increase in knowledge (2,910 participants) was made by awarding points for answers in ESII versus ESI. One point was assigned for each correct answer in single-choice questions and 0.5 point for each correct answer in open-ended questions. An incorrect answer or 'I don't know' response was assigned 0 points. The difference in the sum of points on ESII versus ESI with scores greater than 0 meant an increase in knowledge, below 0 meant a worsening of knowledge, and scores equal to 0 indicated that the state of knowledge was unchanged. The state of knowledge was rated as 'poor' for the sum of points ≤ 4, 'average' between 4.5 and 8, and 'high' in the case of 8.5 points and above.

At the end of the project, participants completed a Satisfaction Questionnaire on their self-assessment of participation in the programme and its organization. Satisfaction with participation in the programme was assessed using a 5 point scale, from 5 (very satisfied) to 1 (very dissatisfied).

Serological examination. A blood sample (up to 5 ml) was collected from all 2,920 enrolled participants by using the vacuum technique. Serum samples were prepared by centrifugation of blood samples for 10 min at 2,000 × g. All samples were analysed at the Medical Diagnostic Laboratory of the Department of Health Biohazards and Parasitology at the Institute of Rural Health in Lublin.

The presence of IgM and IgG antibodies anti-*Borrelia burgdorferi* was detected with a commercial ELISA tests with recombinant antigen (Biomedica Medizinprodukte GmbH, Austria), according to the manufacturer's instructions. The results were expressed in BBU/ml (Biomedica Borrelia Units); results between 9–10 BBU/ml were considered borderline, and results equal to 11 BBU/ml and above were considered positive. According to the information provided by the tests manufacturer, the specificity and sensitivity of the both kits were 96% and 100%, respectively.

The first 700 participants with positive or borderline results (IgM and/or IgG) in ELISA methods were also tested with the use of an immunoblot assay to detect IgM and IgG antibodies against *B. burgdorferi* sensu stricto*, B. garinii, B. afzelii, B. spielmanii and B. bavariensis* (*recom*Line Borrelia IgM and IgG (MIKROGEN GmbH, Germany). The results were determined by summing the point values of the individual bands reactive ≥ cut-off. The sum of points ≤ 5 was considered negative in both classes of immunoglobulin, 6 or 6–7 points were borderline for IgM or IgG, respectively. Results were classified as positive when points were ≥ 7 (IgM) or ≥ 8 (IgG). The specificity was 100% for IgM test and 99.4% for IgG. In patients with Lyme arthritis, acrodermatitis chronica atrophicans and neuroborreliosis, the diagnostic sensitivity of IgM and/or IgG was assessed as 96.4%, 100%, and 97.1%, respectively (test manufacturer's data). The serological results were interpreted by a primary care physician or infectious diseases specialists within the context of clinical symptoms and history of tick bites.

Statistical analysis. Statistical analysis was performed using the STATISTICA® v. 13 (StatSoft, Tulsa, USA). Associations between the answers in the Evaluation Scales and level of education, comparison between the serological reactions and socio-demographic data, work characteristics and tick bite prevention behaviours were analysed by the $\chi 2$ test. Significance of the associations between seroprevalence and questionnaire data was assessed by odds' ratio calculation using MedCalc® v. 22.009 [11]. A P-value<0.05 was considered significant.

Ethical considerations. According to Polish law, a prophylaxis programme does not require the approval of the an Ethics Committee. All medical procedures were performed in the health centre by qualified medical staff. All the participants gave their informed consent to participate in the study. Parental consent was obtained for 15–17-year-old individuals. The confidentiality of personal information and data was guaranteed.

RESULTS

Participants information and tick preventive behaviours. A total of 3,220 individuals declared their willingness to participate in the project, and from among them, 2,920 (90.7%) were qualified for the programme. However, the last visit with the interpretation of laboratory tests was missing for 10 participants, and 2,910 (99.7%) of the total qualified individuals completed all procedures required in the programme. Consequently, 2,920 participants were included in the analyses of serological tests, risk factors, and the use of prevention methods, and 2,910 individuals were included in the knowledge analyses. The mean age of the 2,920 participants was 48.7±13.9 years. The majority of participants were women (63.4%) with a mean age of 49.2±13.7 years; the mean age of males (36.6%) was 47.8±14.0 years. Most of the individuals were in the age range of 40–49 years. The majority of participants live in cities (61.0%) and were exposed to tick bites only during recreational activity (73.4%), compared to 8.2% of respondents with occupational risk. A high proportion of participants (73.2%) declared that they had been bitten by a tick in their lifetime, and 46.3% reported several tick bites. The proportion of individuals with a history of tick bites was greater in the occupational risk group than in the group at risk during recreational activities (75.6% and 72.4%, respectively, χ^2=9.672; p=0.002) and among men (77.7%) than women (70.6%) (χ^2=16.434; p=0.0001). No significant differences were found between rural and urban areas, but respondents living in rural areas more often reported multiple tick bites, compared to urban areas (8.4% and 7.1%, respectively; the difference not statistical significant). In cases of behaviours after tick bites, 2,185 (74.8%) individuals used tweezers or tick tweezers, and 557 (19.1%) declared that they used their fingers to remove a tick. According to behaviours, most participants reported that they always check themselves after outdoor activity and wear clothing covering the body (92.2% and 80.9%, respectively). Seventy-six percent of participants used a repellent in tick habitat areas. Significantly more respondents with diagnosed or suspected LD compared to those without diagnosis before the project inspected their bodies after returning from the forest, 95.5% and 92.1%, respectively (χ^2=7.998; p=0.005). Also, the group with diagnosed or suspected LB more often used repellent (77.6% vs. 76%) and protective clothes (98.1% vs. 97.4%), but no statistically significant differences were found. Participants who had experienced tick bites in the past, compared to not-bitten people, more often inspected their bodies (94.5% vs. 91.3%) but used repellent (76.6% vs. 81.4%) and protective clothes (97.5% and 97.9%, respectively) less often.

Females (98.2%) more often than males (96.6%) used protective clothes (χ^2=6.665; p=0.010) and repellents (77.6% and 74.2%, respectively; χ^2=4.207; p=0.040). In turn, body inspection activity was the same (92.6%) among males and females. The use of protective measures was associated with the education level of participants. Respondents with a high education level (75.6%) were more likely to use a repellent than those with secondary (74.6%) and primary school education (68.2%) (χ^2=10.157; p=0.006) and protective clothes (97.9%, 97.4% and 96.4%, respectively). No significant differences were found in the group with knowledge improvement compared to the group with unchanged or worsening knowledge in the case of checking the body and using repellent. Only wearing protective clothes covering the body was significantly higher among participants with higher knowledge (97.9%) versus unchanged/worsening in knowledge (95.3%; χ^2=10.283; p=0.001).

Among respondents who applied repellent to their skin, the tick bite was slightly rarer (78.9%) compared to non-using participants (83.4%, χ^2=5.669; p=0.017). A similar correlation was found for wearing covering clothes (79.9% versus 82.5%, but the difference was insignificant). Tick bites occurred more frequently when participants checked their bodies after exposure than among respondents who do not check their bodies (80.6% vs. 72%; χ^2=7.138; p=0.008).

Knowledge about Lyme borreliosis and prevention methods. Table 1 shows the results regarding knowledge of

Table 1. Proportion of participants who gave correct answers in Evaluation Scale I and Evaluation Scale II and increase of knowledge about Lyme borreliosis and prevention methods

Total No. of respondents N – 2,910		Correct answer to the question				Increased knowledge		State of knowledge unchanged	
		Evaluation Scale I		Evaluation Scale II					
No.	Question	N	%	N	%	N	%	N	%
1	LB is an infectious disease	1,420	48.8	2,672	91.8	1,312	45.1	1,538	52.9
2	LB is a disease caused by bacteria	2,609	89.7	2,736	94.0	289	9.9	2,459	84.5
3	Awareness the ways of infection with LB[1]	2,609	89.7	2,844	97.7	292	10.0	2,553	87.7
4	LB is a multi-organ disease	2,690	92.4	2,889	99.3	213	7.3	2,683	92.2
5	Being aware of LB symptoms[1]	2,411	82.9	2,856	98.1	1,959	67.3	602	20.7
6	Methods of preventing tick-borne diseases[1]	2,323	79.8	2,797	96.1	1,714	58.9	816	28.0
7	LB is treatable disease	1,611	55.4	2,286	78.6	864	29.7	1,857	63.8
8	A vaccine for LB is not currently available	1,417	48.7	2,703	92.9	1,339	46.0	1,518	52.2
9	Knowledge of occupational groups exposed to LB[1]	2,670	91.8	2,892	99.4	1,133	38.9	1,441	49.5
10	LB is an occupational disease	2,068	71.1	2,831	97.3	790	27.1	2,093	71.9
	Total					2,503	86.0	151	5.2

LB – Lyme borreliosis; [1]open-ended question

Lyme borreliosis and prevention methods. Education was effective in improving the knowledge of 2,503 participants (86.0%), which included cases where people knew the correct answers to single-choice questions or gave more correct answers to open-ended questions on the second scale. The range of overall scores was between 0–12.5 in Evaluation Scale I and II, and above 10 points were assigned to 84 of the participants in ESI and 937 in ESII (2.9% and 32.2%, respectively). Knowledge improvement was slightly higher in the group of women (87%) than among men (84.4%).

The highest increase of knowledge after training was observed in the field of LB symptoms and methods to prevent tick-borne disease (67.3% and 58.9% respondents, respectively). Over 21% of individuals after education did not know that LB is a treatable disease, and improving awareness was observed in 864 (29.7%) participants. The two most common protective measures against tick-borne disease reported by participants were using a tick repellent (54.6% before and 78.7% after education) and wearing protective clothing (46.8% in ESI and 65.3% in ESII) in tick habitat. Answers 'avoiding areas that may have ticks' (ESI: 8.3% and ESII: 5.0%) and 'treat with a preventive antibiotic after a tick bite' (5.0% in ESI and 1.1% in ESII) were less frequent after education. Participants most commonly identified joint pain (ESI: 55.1%, ESII: 75.1%) and erythema migrans (51.1% in ESI and 80.6% in ESII) as symptoms of *Borrelia* infection. A forestry worker (86.8% and 96.6%) and a farmer (58.6% and 85.2%) were most often mentioned by respondents as professions at high risk of *Borrelia* infection in ESI and ESII, respectively.

The highest state of knowledge before and after education during the programme was observed among participants with higher education (30.7% in ESI and 84.3% in ESII) and the lowest among respondents with primary education (12.7% and 74.5%, respectively) (Tab. 2). Higher levels of education were associated with higher knowledge on both evaluation scales (χ^2=100.006; p<0.00001 in ESI). On the other hand, progress in acquiring knowledge decreased along with the increase in the level of education, from 89.1% among participants with primary school through 87.2% with secondary school, and 84.7% with respondents with high education, but the relationship was not significant.

Seroprevalence of Lyme borreliosis. Table 3 presents the prevalence of antibodies against *Borrelia* in the study group, with the use of the ELISA method depending on the gender and age of participants. An overall seroprevalence of 37.3% was reported. A significant difference was found between the groups of females and males (35.8% vs. 40.0%; χ^2=5.151; p=0.023). An increase in the frequency of positive/borderline reactions was observed in the oldest age ranges, from 25.0%

Table 2. Knowledge of participants according to level of education

Level of education	Total No. of participants (ESI or ESII)	State of knowledge [N(%)]					
		ESI			ESII		
		Poor	Average	High	Poor	Average	High
Primary	110	23 (20.9)	73 (66.4)	14 (12.7)	0	28 (25.5)	82 (74.5)
Secondary	1362	185 (13.6)	916 (67.3)	261(19.2)	8 (0.6)	297 (21.8)	1057 (77.6)
Higher	1438	85 (5.9)	912 (63.4)	441 (30.7)	0	226 (15.7)	1212 (84.3)
Total	2910	293 (10.1)	1901 (65.3)	716 (24.6)	8 (0.3)	551 (18.9)	2351 (80.8)

ESI – Evaluation Scale I (before training); ESII – Evaluation Scale II (after training);
ESI – χ^2 = 100.006; p < 0.00001

Table 3. Prevalence of anti-*Borrelia burgdorferi* antibodies detected by ELISA method in residents of the Lublin Province (Poland) depending on gender and age

	No. of examined participants	Results in IgM class				Results in IgG class				Positive or borderline in one or both classes	
		Positive		Borderline		Positive		Borderline			
		N	%	N	%	N	%	N	%	N	%
Gender											
Females	1,850	417	22.5	104	5.6	232	12.5	33	1.8	662	35.8
Males	1,070	242	22.6	55	5.1	217	20.3	19	1.8	428	40.0
Age group (years)											
15–19	68	12	17.6	4	5.9	5	7.4	1	1.5	17	25.0
20–29	217	51	23.5	10	4.6	15	6.9	3	1.4	68	31.3
30–39	432	114	26.4	37	8.6	41	9.5	7	1.6	171	39.6
40–49	772	198	25.6	51	6.6	94	12.2	8	1.0	300	38.9
50–59	760	160	21.1	31	4.1	118	15.5	17	2.2	256	33.7
60–69	493	98	19.9	22	4.5	122	24.7	12	2.4	203	41.2
70–79	167	24	14.4	4	2.4	49	29.3	3	1.8	68	40.7
80–91	11	2	18.2	0	0.0	5	45.5	1	9.1	7	63.6
Total	2,920	695	23.8	159	5.4	449	15.4	52	1.8	1,090	37.3

Difference between positive results in females and males, assessed by χ^2 test: χ^2 = 5.151, p = 0.023, difference significant,
Variation depending on age, assessed by χ^2 test: χ^2 = 20.971, p = 0.0038, variation significant

Table 4. Seroprevalence of Lyme borreliosis (ELISA methods) according to demographic data reported by participants

Question	No. of participants with positive or borderline results in at least one of the classes of *B. burgdorferi* antibodies	No. of examined participants	%
1. Living in			
City	654	1780	36.7
Country	436	1140	38.2
2. Exposure to tick bite #			
Occupational	97	238	40.8
Recreational	786	2144	36.7
3. Risk group #			
Farmer	175	466	37.6
Forestry worker	7	18	38.9
Hunter	8	22	36.4
4. Tick bites			
Yes	816	2138	38.2
No	194	534	36.3
Does not remember	80	248	32.3
5. Number of tick bites # #			
Once	190	542	35.1
Several	509	1352	37.6
Dozen	113	223	50.7
Does not remember	4	17	23.5
6. Erythema migrans			
Yes	290	587	49.4
No	551	1636	33.7
Other skin lesion	158	450	35.1
Does not know	86	228	37.7
7. Diagnosed or suspected borreliosis			
Yes	356	578	61.6
No	721	2314	31.2
Does not remember	8	16	50.0
8. Performed tests for borreliosis			
Yes	456	826	55.2
No	619	2061	30.0
Does not remember	14	23	60.9
9. Result of borreliosis test # #			
Positive	317	406	78.1
Negative	75	289	26.0
Borderline	39	81	48.1
Does not remember	25	47	53.2
10. Inspection of the body after return from the forest			
Yes	1000	2692	37.1
No	26	77	33.8
Rarely	58	138	42.0
11. Method to remove a tick #			
Use of tweezer, tick tweezer	791	2102	37.6
Use of fingers	180	475	37.9
Others	72	218	33.0
12. Use of repellents			
Yes	840	2215	37.9
No	120	362	33.1
Accidental	124	325	38.2
13. Use of protective clothes			
Always	886	2361	37.5
Sometimes	171	471	36.3
Never	30	70	42.9
14. Current occupation with a high risk of tick bite			
Yes	200	487	41.1
No	689	1896	36.3

participants who answered only one question; not including questions with multiple answers; # # participants who answered 'Yes' to questions 4 and 8 were tested

in the age range 15–19 years to 63.6% in the range 80–91 years, with a slight decrease in the 40–59 and 70–79 age groups (χ^2=20.971; p=0.0038).

A higher prevalence (positive or borderline results) of anti-*Borrelia* antibodies was found in the IgM class (29.2%) than in the IgG class (17.2%). Positive results of IgM antibodies and negative results of IgG were found in 457 samples (15.7%). Among participants with such results, only 69.4% recalled a tick bite, and 19.5% reported erythema migrants in the past. In the case of 192 (6.6%) participants, positive results in both classes of antibodies were obtained. A slight higher seroprevalence was found in participants from rural environments (38.2%) relative to those from cities (36.7%) but the results were not significant (Tab. 4). A greater proportion of positive and borderline results was observed among those at occupational risk (40.8%) than at recreational risk (36.7%); however, the differences were not significant. Among groups with a higher risk of tick bite, the seroprevalence was similar, the highest in forestry workers (38.9%), lowest in farmers (37.6%) and in hunters (36.4%).

Among the participants bitten by ticks, a higher proportion of positive or equivocal results was observed for reporting tick bites repeatedly (50.7%) than only once (35.1%), compared to the group without tick bites in their lifetime (36.3%). However, significant differences were found only between individuals with no history of tick bites compared to a group with repeated tick bites in the past (χ^2=45.071; p<0.00001). The seropositivity rate was higher among participants with erythema migrans in the past (49.4%) than in people without this symptom (33.7%) and with diagnosed or suspected LB (61.6%) compared to patients without diagnosis (31.2%). Also, the percentage of participants with positive or borderline results was higher for those with a history of such results (73.1%) than for those with negative results (26.0%). Surprisingly, the ratio of seropositivity was greater among those who checked themselves for ticks after being outdoors (37.1%) compared to those who did not check (33.8%), and among participants using tick repellent (37.9%) than not using repellent (33.1%), but differences were not statistically significant. Only lower seropositivity was observed among those using protective

Table 5. Associations between questionnaire data and serological reactions to *B. burgdorferi* (ELISA methods)

Question	Seropositive to B. burgdorferi*	Seronegative to B. burgdorferi	Odds Ratio (OR)	95% CI	p_{OR}	Significance
1. Living in						
City	654/1,090 (60.0%)	1,126/1,830 (61.5%)	0.938	0.804-1.093	*p* = 0.412	NS
Country	436/1,090 (40.0%)	704/1,830 (38.5%)	1.066	0.915-1.243	*p* = 0.412	NS
2. Exposure to tick bite #						
Occupational	304/1,090 (27.9%)	472/1,830 (25.8%)	1.113	0.940-1.317	*p* = 0.215	NS
Recreational	993/1,090 (91.1%)	1,689/1,830 (92.3%)	0.855	0.652-1.120	*p* = 0.254	NS
3. Risk group #						
Farmer	185/1,090 (17.0%)	303/1,830 (16.6%)	1.030	0.843-1.259	*p* = 0.771	NS
Forestry worker	9/1,090 (0.8%)	16/1,830 (0.9%)	0.944	0.416-2.143	*p* = 0.890	NS
Hunter	17/1,090 (1.6%)	21/1,830 (1.1%)	1.365	0.717-2.598	*p* = 0.342	NS
4. Tick bites						
Yes	816/1,090 (74.9%)	1322/1,830 (72.2%)	1.144	0.965-1.358	*p* = 0.122	NS
No	194/1,090 (17.8%)	340/1,830 (18.6%)	0.949	0.781-1.153	*p* = 0.597	NS
Does not remember	80/1,090 (7.3%)	168/1,830 (9.2%)	NC	NC	NC	NC
5. No. of tick bites # #						
Once	190/816 (23.3%)	352/1,318 (26.7%)	0.833	0.680-1.021	*p* = 0.078	NS
Several	509/816 (62.4%)	843/1,318 (64.0%)	0.934	0.780-1.119	*p* = 0.461	NS
Dozen	113/816 (13.8%)	110/1,318 (8.3%)	1.765	1.336-2.332	*p* < 0.0001	+++
Does not remember	4/816 (0.5%)	13/1318 (1.0%)	NC	NC	NC	NC
6. Symptoms #						
Fatigue, sleeping problems	626/1,090 (57.4%)	1,069/1,830 (58.4%)	0.960	0.825-1.118	*p* = 0.602	NS
Muscle pain	521/1,090 (47.8%)	830/1,830 (45.4%)	1.103	0.949-1.282	*p* = 0.200	NS
Joint pain	706/1,090 (64.8%)	1,131/1,830 (61.8%)	1.136	0.972-1.328	*p* = 0.108	NS
Headache	467/1,090 (42.8%)	825/1,830 (45.1%)	0.913	0.785-1.062	*p* = 0.239	NS
Pain in other parts of the body	125/1,090 (11.5%)	231/1,830 (12.6%)	0.897	0.711-1.131	*p* = 0.356	NS
Depression, depressed mood	365/1,090 (33.5%)	589/1,830 (32.2%)	1.061	0.904-1.244	*p* = 0.469	NS
Cognitive complaints (memory, attention)	443/1,090 (40.6%)	690/1,830 (37.7%)	1.131	0.970-1.319	*p* = 0.115	NS
Peripheral neuropathy	588/1,090 (53.9%)	972/1,830 (53.1%)	1.034	0.890-1.202	*p* = 0.664	NS
7. Erythema migrans						
Yes	290/1,085 (26.7%)	297/1,816 (16.4%)	1.866	1.553-2.241	*p* < 0.0001	+++
No	551/1,085 (50.8%)	1,085/1,816 (59.7%)	0.695	0.597-0.809	*p* < 0.0001	+++
Other skin lesion	158/1,085 (14.6%)	292/1,816 (16.1%)	0.890	0.721-1.098	*p* = 0.275	NS
Does not know	86/1,085 (7.9%)	142/1,816 (7.8%)	NC	NC	NC	NC
8. Diagnosed or suspected borreliosis						
Yes	356/1,085 (32.8%)	222/1,823 (12.2%)	3.522	2.915-4.255	*p* < 0.0001	+++
No	721/1,085 (66.5%)	1,593/1,823 (87.4%)	0.286	0.237-0.345	*p* < 0.0001	+++
Does not remember	8/1,085 (0.7%)	8/1823 (0.4%)	NC	NC	NC	NC
9. Performed tests for borreliosis						
Yes	456/1,089 (41.9%)	370/1,821 (20.3%)	2.825	2.393-3.335	*p* < 0.0001	+++
No	619/1,089 (56.8%)	1,442/1,821 (79.2%)	0.346	0.294-0.408	*p* < 0.0001	+++
Does not remember	14/1,089 (1.3%)	9/1,821 (0.5%)	NC	NC	NC	NC
10. Result of borreliosis test # #						
Positive	317/446 (71.1%)	89/363 (24.5%)	7.565	5.522-10.364	*p* < 0.0001	+++
Negative	75/446 (16.8%)	214/363 (59.0%)	0.141	0.102-0.195	*p* < 0.0001	+++
Borderline	39/446 (8.7%)	43/363 (11.8%)	0.713	0.451-1.127	*p* = 0.146	NS
Does not remember	25/446 (5.6%)	22/363 (6.1%)	NC	NC	NC	NC
11. Inspection of the body after return from the forest						
Yes	1,000/1,084 (92.3%)	1,692/1,823 (92.8%)	0.922	0.693-1.226	*p* = 0.575	NS
No	26/1,084 (2.4%)	51/1,823 (2.8%)	0.854	0.529-1.378	*p* = 0.517	NS
Rarely	58/1,084 (5.4%)	80/1,823 (4.4%)	1.232	0.871-1.742	*p* = 0.238	NS

Table 5. Associations between questionnaire data and serological reactions to *B. burgdorferi* (ELISA methods) – continuation

Question	Seropositive to B. burgdorferi*	Seronegative to B. burgdorferi	Odds Ratio (OR)	95% CI	p_{OR}	Significance
12. Ability to remove a tick from the body						
Yes	748/1,086 (68.9%)	1,161/1,820 (63.8%)	1.256	1.070-1.474	*p* = 0.005	++
No	196/1,086 (18.0%)	381/1,820 (20.9%)	0.832	0.687-1.007	*p* = 0.059	NS
Does not know	142/1,086 (13.1%)	278/1,820 (15.3%)	NC	NC	NC	NC
13. Removed a tick from the body in the past						
Yes	721/1,086 (66.4%)	1,113/1,820 (61.2%)	1.255	1.072-1.469	*p* = 0.005	++
No	365/1,086 (33.6%)	707/1,820 (38.8%)	0.797	0.681-0.933	*p* = 0.005	++
14. Method used to remove a tick #						
Use of tweezer, tick tweezer	826/1,081 (76.4%)	1,359/1,804 (75.3%)	1.061	0.889-1.265	*p* = 0.513	NS
Use of fingers	213/1,081 (19.7%)	344/1,804 (19.1%)	1.041	0.861-1.260	*p* = 0.676	NS
Others	80/1,081 (7.4%)	154/1,804 (8.5%)	0.856	0.646-1.134	*p* = 0.279	NS
15. Use of repellents						
Yes	840/1,084 (77.5%)	1,375/1,818 (75.6%)	1.109	0.928-1.326	*p* = 0.255	NS
No	120/1,084 (11.1%)	242/1,818 (13.3%)	0.811	0.642-1.023	*p* = 0.077	NS
Accidental	124/1,084 (11.4%)	201/1,818 (11.1%)	1.039	0.819-1.318	*p* = 0.752	NS
16. Use of protective clothes						
Always	886/1,087 (81.5%)	1,475/1,815 (81.3%)	1.016	0.838-1.233	*p* = 0.872	NS
Sometimes	171/1,087 (15.7%)	300/1,815 (16.5%)	0.943	0.768-1.157	*p* = 0.573	NS
Never	30/1,087 (2.8%)	40/1,815 (2.2%)	1.259	0.780-2.034	*p* = 0.345	NS
17. Level of education						
Primary education	39/1,090 (3.6%)	71/1,830 (3.9%)	0.919	0.617-1.369	*p* = 0.679	NS
Secondary education	495/1,090 (45.4%)	873/1,830 (47.7%)	0.912	0.785-1.060	*p* = 0.230	NS
Higher education	556/1,090 (51.0%)	886/1,830 (48.4%)	1.109	0.955-1.289	*p* = 0.175	NS
18. Current occupation with a high risk of tick bite						
Yes	200/889 (22.5%)	287/1,494 (19.2%)	1.221	0.996-1.496	*p* = 0.054	NS
No	689/889 (77.5%)	1,207/1,494 (80.8%)	0.819	0.668-1.004	*p* = 0.054	NS

In the fields 'Seropositive to *B. burgdorferi*' and 'Seronegative to *B. burgdorferi*' are given: Total positive to particular question/total examined (percent of seropositive or seronegative respondents to this question).
OR – Odds Ratio; 95% CI – 95% Confidence Interval; p_{OR} – probability calculated for OR; NS – Not Significant; +++ – *p* <0.0001; ++ – *p* <0.01; NC – Not Calculated; * including borderline results; # some respondents indicated more than one answer; # # persons who answered 'Yes' to question # 4 and # 9 were tested

Table 6. Prevalence of anti-*Borrelia burgdorferi* antibodies detected by immunoblot assay in residents of the Lublin Province (Poland) depending on gender and age

	No. of examined participants	Results in IgM class				Results in IgG class				Positive or borderline in one or both classes	
		Positive		Borderline		Positive		Borderline			
		N	%	N	%	N	%	N	%	N	%
Gender											
Females	419	147	35.1	1	0.2	66	15.8	35	8.4	216	51.6
Males	281	98	34.9	0	0.0	73	26.0	39	13.9	165	58.7
Age group (years)											
15–19	11	4	36.4	0	0.0	3	27.3	1	9.1	5	45.5
20–29	44	20	45.5	0	0.0	5	11.4	2	4.5	23	52.3
30–39	109	36	33.0	1	0.9	9	8.3	11	10.1	48	44.0
40–49	201	81	40.3	0	0.0	31	15.4	17	8.5	106	52.7
50–59	160	60	37.5	0	0.0	32	20.0	19	11.9	89	55.6
60–69	127	35	27.6	0	0.0	37	29.1	19	15.0	77	60.6
70–79	45	9	20	0	0.0	20	44.4	4	8.9	30	66.7
80–91	3	0	0.0	0	0.0	2	66.7	1	33.3	3	100.0
Total	700	245	35.0	1	0.1	139	19.9	74	10.6	381	54.4

Difference between positive results in females and males, assessed by χ^2 test: $\chi^2 = 3.484$; p = 0.062, difference not significant.
Variation depending on age, assessed by χ^2 test: $\chi^2 = 12.708$; p = 0.080, variation not significant

Table 7. Correlation of serological detection of IgM and IgG antibodies against *Borrelia burgdorferi* with ELISA and immunoblot tests in the group of 700 participants of the Lyme borreliosis prevention programme

		Immunoblot IgM			
		Positive	Borderline	Negative	Total
ELISA IgM	Positive	222 (31.7%)	1 (0.1%)	215 (30.7%)	438 (62.6%)
	Borderline	13 (1.9%)	0	81 (11.6%)	94 (13.4%)
	Negative	10 (1.4%)	0	158 (22.6%)	168 (24.0%)
	Total	245 (35.0%)	1 (0.1%)	454 (64.9%)	700 (100.0%)
Conformance = 54.3%					
		Immunoblot IgG			
		Positive	Borderline	Negative	Total
ELISA IgG	Positive	135 (19.3%)	61 (8.7%)	96 (13.7%)	292 (41.7%)
	Borderline	2 (0.3%)	4 (0.6%)	23 (3.3%)	29 (4.1%)
	Negative	2 (0.3%)	9 (1.3%)	368 (52.6%)	379 (54.1%)
	Total	139 (19.9%)	74 (10.6%)	487 (69.6%)	700 (100.0%)
Conformance = 72.4%					

clothes (37.5%) compared to those who never use them (42.9%). The seroprevalence was similar in the group of participants who removed ticks using a tweezer/tick tweezer and their fingers (37.6% and 37.9%, respectively) and among those with a current occupation with a high risk of tick bite compared to individuals without a work risk (41.1% and 36.3%, respectively). The differences were not statistically significant.

Positive and significant relationships were found between a seropositive reaction to *B. burgdorferi* and the following questionnaire data: recognized erythema migrans, diagnosed or suspected borreliosis, positive results of a serological test, and ability to remove an attached tick. Relationships were also found for removing a tick in the past and a dozen tick bites, but not for experiencing a tick bite only once or several times. No association was found between seroprevalence and tick bite prevention behaviours (Tab. 5).

Among 700 participants with positive/borderline results in ELISA (IgM and/or IgG), the seroprevalence with immunoblot (positive and borderline results) was 54.4%; 51.6% in women, and 58.7% in men (Tab. 6). The conformance of ELISA and immunoblot results was higher for the IgG class (72.4%) than for the IgM (54.3%) (Tab. 7). Overall, the immunoblot confirmed the results of the ELISA in 381 individuals (54.4%). Among 301 patients with positive results of IgM antibodies and negative results of IgG (ELISA), the immunoblot showed positive results in 40.5%.

Satisfaction of participants. Analysis of the Satisfaction Questionnaire showed that 98.6% of total respondents were very satisfied (85.5%) and satisfied (12.8%) with their participation in the prophylaxis programme, 381 (1.3%) individuals were neutral, and 38 (0.1%) participants rated as dissatisfied or very dissatisfied (Tab. 8). Respondents were the most satisfied with the interview with the doctor, the level of service under the programme, and the knowledge and competence of personnel implementing the programme, and the least satisfied with expanding information about LB after training and access to programme information.

DISCUSSION

The prevention of tick-borne diseases is based on tick biology, ecology and control measures. Reducing tick abundance with the use of biological and chemical methods could be inefficient [12]. However, practices for tick bite prevention, knowledge and attitudes can reduce TBD risk for individuals, especially since research on a LB vaccine has still not been finalized. The promotion of individual behaviours and limiting tick exposure are the most effective ways to prevent LB [13]. The use of personal protective measures (PPMs) – applying repellent and checking the body – has been found to be effective in preventing TBD diagnosis. In addition, the greater the knowledge about LB and the higher perception of TBD and their implications translated to the use of tick prevention methods, such as the application of acaricides in the environment. Also, awareness of LB

Table 8. Satisfaction with being a participant in the Lyme borreliosis prevention programme.

Satisfaction	Total No. of answers	Rating given by participants [N (%)]				
		Very satisfied	Satisfied	Neutral	Dissatisfied	Very dissatisfied
Knowledge and competence of personnel implementing the programme (M±SD=4.96±0.21)	2,890	2,771 (95.9%)	114 (3.9%)	5 (0.2%)	0	0
Access to programme information (M±SD=469±0.58)	2,891	2,151 (74.4%)	595 (20.6%)	133 (4.6%)	9 (0.3%)	3 (0.1%)
Content of the educational brochure (comprehension, relevance, completeness and quality) (M±SD=4.83±0.39)	2,885	2,405 (83.4%)	467 (16.2%)	13 (0.5%)	0	0
State of knowledge - expanding information regarding Lyme borreliosis after completing participation in the programme (M±SD=4.51±0.61)	2,890	1,637 (56.6%)	1,091 (37.8%)	155 (5.4%)	6 (0.2%)	1 (0.03%)
Quality of service during blood collection (M±SD=4.88±0.40)	2,891	2,606 (90.1%)	236 (8.2%)	38 (1.3%)	8 (0.3%)	3 (0.1%)
Quality of service in laboratory tests (delivery time and form of the result) (M±SD=4.89±0.35)	2,888	2,599 (90.0%)	266 (9.2%)	20 (0.7%)	2 (0.07%)	1 (0.03%)
Quality of the interview with the doctor (M±SD=4.96±0.20)	2,888	2775 (96.1%)	110 (3.8%)	3 (0.1%)	0.0	0
Possibility of conducting further tick-borne diseases health programmes in the future (M±SD=4.84±0.38)	2,804	2,363 (84.3%)	433 (15.4%)	6 (0.2%)	2 (0.07%)	0
Level of service under the programme (courtesy, availability, willingness to help) (M±SD=4.95±0.23)	2,819	2,684 (95.2%)	131 (4.6%)	3 (0.1%)	1 (0.04%)	0
Overall assessment of the programme (M±SD=4.92±0.29)	2,820	2,601 (92.2%)	212 (7.5%)	5 (0.2%)	2 (0.07%)	0
Total	28,666	24,592 (85.8%)	3,655 (12.8%)	381 (1.3%)	30 (0.1%)	8 (0.03%)

symptoms, early consultation with a physician and treatment, are essential for the prevention of disseminated borreliosis [14, 15].

Overall, education during the programme brought positive results for most participants. Unfortunately, knowledge about the curability of LB was low even after education. In another survey in the endemic area of Poland, a minority of participants were convinced that LB is a curable disease [16]. In the other study, a higher proportion of respondents answered that LB can be treated, but more than half of participants incorrectly chose long-term antibiotic treatment instead of short-term in the question about treating LB [17]. Most patients with LB responded well to antibiotics recommended by guidelines, and doxycycline treatment for 10 days showed the same effectiveness as treatment for 15 or 21 days in cases of erythema migrans. Guidelines also recommend a single 200 mg dose of doxycycline within 72 hours after a tick bite in cases where the tick has been attached for a long time, and in highly endemic regions. In patients with persistent symptoms after antibiotic treatment, many studies showed no evidence for active *B. burgdorferi* infection or any benefit of prolonged treatment with antibiotics [18, 19]. This indicated that awareness about antibiotic treatment and chronic forms of LB is still insufficient, and education should focus on this point. The knowledge about vaccines for LB, the infectious character of the disease and the curability of LB was the lowest among participants, and this field should be taken into consideration in future education programmes. It was observed that before education, the highest knowledge was associated with the highest education level, and in this regard the programme had not contributed significantly to any increase. Greater emphasis should be placed on training people with primary and secondary education. Training should be repeated, as studies by Crist et al. [20] have shown significantly higher knowledge among veterinary practitioners with training in TBD within the last five years, compared to participants whose training was more than five years ago.

The use of repellent was the least practiced prevention method among participants in this study, while inspection of the body and wearing protective clothes were the most often used, which is in accordance with other studies [12, 15, 16, 21]. The high cost of frequent use of repellent and concerns about its toxicity probably resulted in the rare use of these products [15].

In the current study, women and respondents with a high education level were more likely to use protective measures (covered clothes and repellent), but no differences were found in terms of body inspection after exposure. The use of protective measures was higher among respondents with diagnosed or suspected Lyme borreliosis before participation in the prophylaxis programme. These findings are in accordance with other studies confirming that women, persons with a high education level and better knowledge and awareness about TBD, were more likely able to prevent tick bites [15, 16]. Numerous studies have confirmed that increased risk perceptions positively affect the use of prevention measures [16]. The current study only partially confirmed that high awareness and personal experience influence tick protection behaviours. The respondents in this study with diagnosed or suspected LB and participants with increased knowledge after education were more likely to use protective measures, but for those with a history of tick bites, it was observed that prevention behaviours were rarer. This may indicate that a history of TBD and experiences with tick bites partially influence behavioural change in individuals. Other factors influencing attitudes in the population, such as motivation and social norms, should also be taken into consideration in planning effective prevention programmes [17]. Respondents of the survey in LB endemic areas of the United States indicated forgetting to check the body or not checking thoroughly, forgetting to use repellent, anxiety about safety, or general dislike of bug repellent as the main barriers to prevention behaviours [22].

Based on a literature review, Richardson et al. [23] confirmed the effectiveness of personal protection strategies, such as the use of tick repellents and protective clothes in reducing tick bites and the incidence of LB. Also, a study on Indiana residents in the USA showed that inspection of the body and using a repellent were protective against self-reported diagnoses of tick-borne disease [24]. However, Eisen [25] indicated that the results of reviewed studies varied and depended on each personal protection measure, and some of these studies found no protective effect. According to behaviours reported by participants in the current study, a slightly positive effect was found in using repellent and covering clothes on tick bite risk. While respondents who declared inspecting their bodies more often reported tick bite incidents in their lifetime. In this case, persons checking the body after activities in a tick habitat may contribute to the more frequent finding of ticks attached to skin, compared to individuals who had ticks but did not notice them. The results obtained in the current study showed that the seroprevalence of LB was also high among respondents without a history of tick bites, which may indicate that these persons may not have noticed the attached tick. In many cases, patients with diagnosed LB did not recall a tick bite [4]. The chance of finding the attached tick depends on various factors, such as location on the body and size of the tick, the duration of feeding time, and the individual's sensitivity to tick bites [25]. In the current study, respondents were not asked when they used protective measures and whether they used them before tick bites, thus, the results should be interpreted with caution. A tick bite could induce higher awareness and a change in behaviours, or participants may have used personal protection less often than they declared. Moreover, the respondents in the current study were not asked what type of repellent was used, because the botanical oils often used could be less effective [25], and respondents may have preferred to use natural over synthetic repellent [22]. Also, information about re-applying repellent during long-term activities in tick habitats is missing from this study.

The participants in the presented study more often used tweezers or tick tweezers to remove a tick than respondents in the studies of Kopsco and Mather [14] and Bayles et al. [26]. Unfortunately, nearly 20% of respondents in the current study confirmed that they use their fingers to remove the attached tick, and frequent inappropriate behaviours, e.g. using fingers or applying oils or other substances to facilitate tick removal. Such behaviours were reported by other authors [14, 15, 26]. A study by De Keukeleire et al. [27] among Belgian forest workers demonstrated that using fingers to remove an attached tick was a significant risk factor. Prompt and proper removal of attached ticks is essential in preventing *B. burgdorferi* and other tick pathogen infections because *Borrelia* transmission needs time after attachment of an infected tick [28]. In the presented study, only 65% of respondents confirmed that they would be able to remove the attached tick from their skin. More campaigns that include education material in the form of videos should focus on detailed instructions on how to remove an attached tick from the skin. The implementation of practical classes on tick removal requires verification.

The risk of tick bite among the population of the Lublin Province is very high, almost twice as high as in the LB endemic regions of France [15], and much higher than reported in other studies from Poland [12, 16]. Also, as stated, men were more often exposed to tick bites than women, and occupational exposure was not significantly higher than during recreational activities. In turn, the risk of tick bites for those living in urban and rural areas was similar in the presented study, although rural inhabitants more often reported multiple tick bites.

In Europe, an increase in tick activity and the number of LB cases has been noticed in recent years. The growth is due to ecological factors, including climate change, host tick migration, landscape management, and anthropogenic factors, mainly high outdoor activities and rearrangement of land exploitation [28]. In the Lublin Province, the percentage of *I. ricinus* ticks infected with *B. burgdorferi* s.l. increased twice, from 6% in 2008–2009 to 15.3% in 2013–2014 [29]. The Lublin Province has one of the highest numbers of LB cases per year. The incidence rate of recognized LB increased in the Lublin Province from 34.3/100,000 (739 cases) in 2010 to 87.3 (1,843 cases) in 2019. The overall incidence rate in Poland was estimated as 53.7 in 2019 [6]. In the current study, the seroprevalence was higher among men compared to women and increased with the age of participants, which is in accordance with studies of the general population of The Netherlands [30] and most of the studies in European countries, as demonstrated in the review paper by Burn et al. [31]. In turn, LB cases were more common among women (55.7%) than men during 2015–2019 in Poland [32]. The seroprevalence of LB reported in population-based studies was estimated to range from 2.7% in Norway to 20% in Finland, and differences were observed between studies according to design, study population, serological methods used, and sampling sizes [31]. The seroprevalence among inhabitants of the Lublin Province in this study was evaluated as 37.3%, which was much higher compared to most European countries. The high seroprevalence among participants in presented study could be explained by the inclusion criteria for the project, which enrolled only people with an occupational or recreational risk of tick bite. Acke et al. [33], in their review paper identified farmers, forestry workers, animal breeders, livestock and dairy producers, livestock farm laborers and hunting dog caretakers as non-healthcare workers with a risk of *B. burgdorferi* infection related to work. In current study, the high seroprevalence was determined among forestry workers and farmers and was much higher than among these professions in Italy (6.5% and 0%, respectively). In those studies, the authors indicated that clinical cases of LB were not recognized in the study area, but occupational exposure to tick bites and experience of tick bites in the past were very high (up to 100%) compared to other outdoor workers [34]. According to a previous study among farmers from the Lublin Province, the prevalence of LB varied between localities and ranged from 20% – 50% [35]. No significant differences were found in the risk of tick bite between occupational and recreational risk groups, which indicates that the risk of *B. burgdorferi* infection is associated with all activities, professional and recreational, that are associated with tick habitats.

The two-tiered serological procedure recommends the use of ELISA methods, and in cases of positive or borderline results, an immunoblot should be performed. The limitation of this serological methods is low sensitivity in the early stage of *B. burgdorferi* infection during the first weeks, and among patients with erythema migrants, the results are mostly negative. The first symptoms of infection are non-specific and can overlap with other health conditions, leading to a missed or delayed LB clinical diagnosis or inadequate treatment [4]. Nevertheless, both classes of antibodies can persist after antibiotic treatment of LB infection and in healthy individuals exposed to ticks. In the current study, 15.7% of the participants received positive results only for IgM antibodies without IgG positive or doubtful results (ELISA). The persistent IgM could be explained by a cross-reaction with other than *Borrelia* antigens, or stimulation of B cells without evidence of ongoing infection [36].

On the basis of research among patients with persistent IgM, Markowicz et al. [36] speculate that anty-*B. burgdorferi* antibodies are sustained by continuous stimulation with cross-reactive autoantigens, other pathogen antigens, or environmental factors. Other studies among blood donors with continuously positive results of IgM antibodies showed no seroconversion of IgG, and no correlation between antibody detection and tick bites [37]. Unfortunately, despite the lack of evidence of an active infection, many patients receive antibiotic therapy. In the diagnosis of LB, it is very important to combine the serological results with clinical symptoms. In the current study, the positive/borderline results in the ELISA method were confirmed by immunoblot only in 54% of participants, and the detection of anti-*B. burgdorferi* antibodies had to be confronted with clinical signs to confirm the LB diagnosis. Additionally, only 41.9% of positive results of IgM antibodies assessed by ELISA were confirmed by immunoblot methods. The recombinant antigens used in this study confirmed the effectiveness and purpose of two-tiered serological tests, as this newer generation of tests showed higher sensitivity and specificity compared to the previous generation with whole-cell lysate and purified antigens [38].

The presented study showed no differences in risk of tick bite between rural and urban inhabitants, with a slightly higher seroprevalence of LB among participants living in the countryside. In recent years, increasing importance has been attributed to urban areas at high risk of tick bites, and a similar risk of *B. burgdorferi* infection was found in urban areas compared to natural areas [39]. In urban areas in particular, where human activity is usually much higher than in rural areas, there is a high risk of tick bite [40]. In Poland, the number of LB incidences was similar among urban and rural residents in 2008–2016 [41]. The considerable risk of tick bite and TBD in green urban areas should be popularized to increase population awareness and promote protection measures in these tick habitats.

This study has demonstrated that men are at higher risk of *B. burgdorferi* infection than women, and were significantly more exposed to tick bite and a higher seroprevalence of Lyme borreliosis was noticed. Unfortunately, men less frequently use protective measures against tick bite. Men also applied for the programme in a smaller percentage (less than 40%) than women, which may indicate that they are less interested in their health. More effort should be made to engage this group in preventive measures.

Previous studies suggested a positive influence of the national prevention plan by the information campaigns, because an increase in knowledge and preventive behaviours was observed in the French population after implementation of a national plan [15]. The participants of the presented *Borrelia* prevention programme rated their participation as very satisfying, and assessed their increase in knowledge as moderate; however, this is a subjective assessment because the analysis showed an increase in knowledge in 86% of participants.

Other forms of education should also be considered during future prevention programmes, such as a smartphone app and a social media education campaigns instead of brochures and training during doctor visits. Buczek et al. [12] showed that television and electronic media, in contrast to magazines, family, friends and medical staff, play a more important role in raising awareness among young people in Poland. To increase the awareness and effective use of tick prevention measures, more efforts should be made to recognize such barriers as cost and individual presumptions and to popularize the efficacy of such activities. The high seroprevalence of LB among inhabitants of the Lublin Province and the confirmed increase in knowledge about risk and tick prevention behaviours as a result of education in the project confirmed the effectiveness and the need for future preventive programmes in the area of tick-borne diseases.

CONCLUSIONS

The presented study showed that the population of the Lublin Province is highly exposed to tick bite and infection with *B. burgdorferi*. Education about Lyme borreliosis and prevention of tick bite during the prophylaxis programme was successful, which confirmed the effectiveness of preventive programmes. Nevertheless, the seroprevalence of LB and tick bite risk among residents of the Lublin Province was very high, although widespread use of prevention measures was reported by participants in the study. Improvement of knowledge about the risk and protective measures against tick bite induces practice behaviours, but is only one of the factors that affects accurate implementation. Further studies should focus on understanding the causes of such behaviours. Overall, the results of this study provide critical information about the risk of tick bite, the seroprevalence of Lyme borreliosis and risk factors, the use of preventive measures and satisfaction from participating in the proramme among residents of the Lublin Province. The results can be useful for optimizing and enhancing the effects of future prevention campaigns.

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Compliance with Ethical Standards. All procedures performed involving human participants were in accordance with ethical standards and with the 1964 Helsinki Declaration and its later amendments, or comparable ethical standards. According to Polish law, the approval of a Bioethics Committee for preventive programmes is not required. The confidentiality of participants' data was guaranteed. Informed consent was obtained from all individual participants included in the study.

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