

Exposure to ticks and seroprevalence of *Borrelia burgdorferi* among a healthy young population living in the area of southern Podlasie, Poland

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Abstract

Objectives. The objective of the study was assessment of risk of infection with *Borrelia burgdorferi* in the area of southern Podlasie in Poland, near the border with Belarus, by analysis of post-exposure procedure, and evaluation of asymptomatic infection in adolescents bitten by a tick, confirmed by serologic tests.

Material and methods. The study was conducted among 128 healthy individuals aged 16–20 who declared being bitten by a tick. The level of IgM and IgG class antibodies was determined using the immunoenzymatic test (Borrelia 14 kD + OspC IgM ELISA and Borrelia IgG + VlsE ELISA, DRG Diagnostics). Positive and doubtful results were confirmed using the Western blot method (EUROLINE-WB, EUROIMMUN).

Results. In the study group, the largest number of respondents (59.4%) declared tick bite in the region of the lower extremities, most often in the knee pit. Among the methods for removing the tick the largest number of respondents indicated removing it with the use of tweezers, with a simple, swift steady movement (29.7%), and pulling it out with the fingers (22.7%). In the ELISA test, a positive or doubtful result in at least one class was observed in 25.0% of respondents (n=32/128): in IgM class – 23.4% (n=30/128), and in IgG class – 4.7% (n=6/128). After verification with the Western blot test, infection was confirmed in 5.5% of respondents (n=7/128): in IgM class – 1.6% (n=2/128), in IgG class – 3.9% (n=5/128). In IgM class antibodies, the Western blot test confirmed positive or doubtful results of the ELISA test in 6.7%, while in IgG class antibodies in 83.3%.

Conclusion. Evaluation of the actual infection with *Borrelia spp.* using serologic tests is difficult due to a certain non-specificity of the ELISA test, especially in IgM class antibodies, and difficulties with performance of a wide scope of specific Western blot tests. The variety of methods of tick removal declared by adolescents suggests that a wider education of society concerning appropriate methods of removing the tick should become an especially important element of prophylactic actions in the area of borreliosis.

Key words

tick bites, *Borrelia burgdorferi*, IgM, IgG

INTRODUCTION

Lyme borreliosis (Lyme disease) is among the most prevalent tick-borne diseases in the northern hemisphere. According to the estimation data and assessments performed by the World Health Organization, the whole of Europe satisfies the criterion of a Lyme borreliosis endemic area. The incidence of this disease is much varied and depends on the region. The disease more commonly occurs in Central and Eastern Europe than in its western part. The risk caused by this disease is clearly lower on the northern and southern outskirts of the continent [1, 2].

In the majority of European areas an increase is observed in the registered number of cases of borreliosis [1]. The causes for the increase in morbidity rates are multi-aspect, and concern the etiologic factor itself, the vector, as well as the reservoir of spirochetes. Another mentioned factor contributing to an increase in morbidity due to borreliosis is also increased popularity of active, open air leisure and tourism, often associated with the penetration of ecosystems

which have not been previously visited [2]. An increase in morbidity due to tick-borne diseases, including borreliosis, is also considered to be related with documented climatic changes, which may affect spatial and time distribution and dynamics of pathogenic agents and their vectors [1].

During the period 1999–2009 in Poland, Lyme disease morbidity rate increased annually from 2.3 – 27.1/100,000 inhabitants; and for a period of three years (2010–2012) maintained itself on a similar level, from 22.8 – 23.8/100,000 inhabitants [3]. The territorial scope of the occurrence of borreliosis covers the entire territory of Poland; however, the risk due to this disease varies according to the region. The highest risk is observed in north-eastern areas. A significantly higher incidence rate, in comparison with the whole of Poland, was observed for Podlaskie Province (Voivodeship), where it reached the value of 81.4/100,000 inhabitants in 2012 [3, 4].

Infection with *Borrelia burgdorferii* spirochetes in humans takes place at least 24 hours after tick bite; therefore, its early removal prevents infection [5, 6]. In order to correctly remove a tick stuck in the skin, most often it is recommended to grasp it with tweezers as close to the skin as possible, and pull it straight out with a swift steady movement. The removal of the tick by manipulations which may cause the regurgitation

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of intestine contents into the wound (e.g. squeezing with fingers, irritation with toxic chemical, or tearing in a way that the guts come into contact with the wound after the bite) increase the risk of infection [6].

The diagnosis of borreliosis should be a clinical diagnosis confirmed by immunoserologic test. Only erythema migrans does not require serologic confirmation. The two-stage diagnostics recommended in Europe consists in the application of a test of lower specificity and high sensitivity by the ELISA method, and then the performance of Western blot test [6]. The presence of antibodies specific to *B. burgdorferi* in healthy individuals evidences a contact with this microorganism and the possibility of an asymptomatic course of infection [7].

Objectives. The objective of the study was assessment of the risk of infection with *Borrelia burgdorferi* in the area of the southern Podlasie Region through analysis of post-exposure procedure, and evaluation of asymptomatic infection in adolescents bitten by a tick, confirmed by serologic tests.

MATERIALS AND METHOD

In order that the results reflect the situation concerning the contact with ticks (vectors of *Borrelia spp.*) within the last several years, the study was conducted among young people who declared a tick bite, and covered 128 adolescents attending secondary and post-secondary schools aged 16 – 20, mean age 17. In the study group, females (72.7%), and rural inhabitants (70.3%) dominated. As many as 55.5% of respondents reported that they lived in the vicinity of forest areas, more than a half (54.7%) mentioned staying in green areas often, and 14.9% very often.

The study was carried out in eastern Poland, near the border with Belarus, in southern Podlasie (a northern part of the Lublin Province). Economy in this area is of an agricultural character, and due to the high forestation, forestry also plays an important role. A considerable percentage of the working population are occupationally exposed to contact with ticks, and large areas of cultivated lands and forests increase the probability of the contact with ticks among inhabitants of this province.

All study participants completed a questionnaire designed by the author of the presented study, and had serologic tests performed using immunoenzymatic test ELISA, by which the level of *Borrelia burgdorferi* IgM and IgG class antibodies were determined in blood serum. For this purpose, tests were used by DRG Diagnostics: Borrelia IgG + VlsE ELISA and Borrelia 14 kD + OspC IgM ELISA. According to the producer's recommendations; result above 11 U/ml was considered positive, whereas a result within 9–11 U/ml as doubtful. The positive and doubtful results obtained were confirmed using the Western blot method. The test EUROLINE-WB was applied to determine IgM and IgG class antibodies against *Borrelia burgdorferi* by EUROIMMUN. A precise reading of the intensity of staining of the bands was obtained by using computer software EUROLineScan (EUROIMMUN). Consent for the study was obtained from the Bioethical Committee at the Medical University in Lublin. Under-age respondents participated in the study with the written consent of their parents. The results of the study were subjected to statistical analysis. In order to verify

the hypotheses concerning independence of variables χ^2 Pearson test was applied. The p value $p < 0.05$ was considered statistically significant. Statistical analyses were performed using software STATISTICA v. 7.1 (StatSoft, Poland).

RESULTS

Exposure to ticks. In the study group, 34.4% of respondents admitted that they had been bitten by a tick more than three times in their lives. A single episode of this arthropod bite was declared by 27.3%, three times – 20.3%, and twice – 18.0% of respondents (Tab. 1).

Table 1. Characteristics of tick bite episodes among adolescents in the study (N=128)

	n	%
No. of tick bite episodes	once	35 27.3
	twice	23 18.0
	three times	26 20.3
	more than three times	44 34.4
Site of bite*	lower extremities	76 59.4
	upper extremities	49 38.3
	abdomen	44 34.4
	chest	2 1.6
	back	19 14.8
	neck	28 21.9
Methods of removing tick *	head	28 21.9
	removed by physician or nurse	23 18.0
	removed by other person	75 58.6
	application of a disinfectant	7 5.5
	spread with fatty substance	5 3.9
	twisted out with tweezers	19 14.8
	removed with tweezers with a swift steady movement	38 29.7
	grasped in fingers and pulled out	29 22.7
	scratched with nail	4 3.1
	bite site was disinfected after tick removal	40 31.3
Date of last bite	other methods	1 0.8
	1–2 months	7 5.5
	3–5 months	35 28.9
	approx. half a year	10 7.8
	approx. a year	15 11.7
	2–5 years	43 33.6
	more than 5 years	12 9.4
I do not remember	4 3.1	
Duration of tick attachment to the skin during last bite	several hours	77 60.2
	approx. 1 day	16 12.5
	approx. 2 days	16 12.5
	more than 2 days	11 8.6
	I do not know	8 6.3
Occurrence of dermal symptoms after last bite	yes	19 14.8
	no	69 53.9
	I do not remember	40 31.3
Occurrence of flu-like symptoms after last bite	yes	2 1.6
	no	87 68.0
	I do not remember	39 30.5

* – results do not add up to 100% due to the possibility of multiple choice of reply.

The largest number of respondents (59.4%) were bitten by a tick in the region of the lower extremities, including the groin. They most often reported a bite in the knee pit – 24.2%. A similar percentage of respondents declared being bitten on the upper extremities, including the skin in the armpit, and on the abdomen (38.3% and 34.4%, respectively). A bite in the skin of the head, neck and back was more rarely mentioned, while the smallest group were respondents who had a tick bite on the skin of the chest (Tab. 1).

Among the methods for removing the tick the largest number of respondents indicated removing it with the use of tweezers, with a swift steady movement (29.7%). The second method of removing a tick was pulling it out with the fingers (22.7%). Use of professional assistance from the medical staff was indicated by 18.0% of adolescents, and 31.3% of respondents mentioned disinfection of the site of the bite after tick removal (Tab. 1).

More than a half of respondents (53.9%) reported that they were last bitten by a tick during the previous year. In the majority of cases (60.2%), during the last tick bite it remained attached to the skin for several hours. The presence of dermal symptoms at the site of the tick bite was declared by 14.8% of respondents. Reddening of the site where the tick was attached was indicated by 14.1% of respondents, the occurrence of oedema – by 1.6%, similarly an itching sensation. Only 1.6% of the adolescents in the study reported flu-like symptoms after tick bite. Detailed data concerning the characteristics of tick bite episodes are shown in Table 1.

Serologic tests using ELISA. In all schoolchildren, serologic examinations were performed by the ELISA test for IgM and IgG class antibodies. A positive or doubtful result in at least one of the classes was observed in 25.0% of respondents (32). Among the remaining 75.0% samples (96 respondents) negative results were obtained in both classes of antibodies (Tab. 2).

In the IgM class, positive results were obtained among 15.6% of respondents (20), whereas doubtful results in 7.8% (10). In the IgG class no doubtful results were observed, while positive results were obtained in 4.7% of respondents (6) (Fig. 1; Tab. 2).

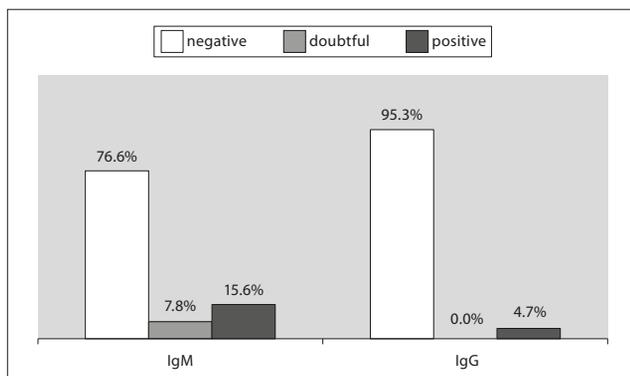


Figure 1. Results of ELISA test with division into IgM and IgG class antibodies

In IgM class, the titres obtained remained within the range from 2.37 – 66.41; mean result 7.58 (standard deviation SD=8.10), while in the IgG class – from 2.27 – 165.64; mean result 6.56 (standard deviation SD=17.37).

No relationship was found between the positive or doubtful results obtained, and the respondents' place of residence,

Table 2. Results of ELISA test for IgM and IgG class antibodies

Antibodies against <i>B. Burgdorferi</i>		Study group N=128	
IgM	IgG	n	%
positive	positive	2	1.6
positive	negative	18	14.1
doubtful	positive	2	1.6
negative	positive	2	1.6
doubtful	negative	8	6.3
negative	negative	96	75.0
Total		128	100.0

frequency of staying in green areas and the number of tick bites. A relationship close to significance ($\chi^2=3.81$; $p=0.0511$) was noted in relation to the distance from the place of residence to the forest, where doubtful or positive results were more frequently found among respondents who declared that they live far from forest areas (33.3%), compared to those living in the vicinity of the forest (18.3%).

Verification of the results of ELISA test by Western blot method. After verification of the results of ELISA test by the Western blot method, positive or doubtful results were obtained in 5.5% of respondents (7). After verification, all doubtful results of ELISA test proved to be negative. Among samples with a positive result of ELISA test, 19.2% of the results were confirmed by Western blot test, 7.7% provided a doubtful result, while the remaining 73.1% – negative (Fig. 2).

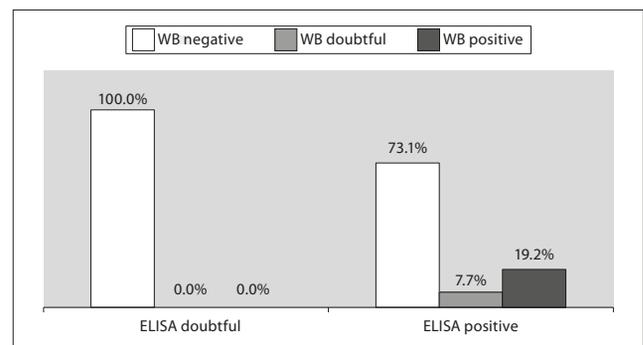


Figure 2. Results of Western blot test for confirmation of positive or doubtful results of ELISA test

In the IgM class antibodies, after verification by Western blot method, all doubtful results of ELISA test (10 sera) proved to be negative, and from among 20 positive results, a doubtful result was obtained in only 2 of the cases analyzed (Tab. 3). Thus, in IgM class, the Western blot test confirmed positive or doubtful results of ELISA test in 6.7%.

In the IgG class using ELISA test no doubtful results were obtained, hence, only the samples with positive results (6 sera) were verified by Western blot test. In this group, the test was confirmed in 5 adolescents (Tab. 3). Thus, in the IgG class antibodies, Western blot test confirmed the results of ELISA test in 83.3%.

In 2 doubtful results obtained in the Western blot test in the IgM class, the presence of a poorly-stained band was observed for the OspC antigen (p25). Among 5 positive results in the Western blot test in the IgG class, all results were characterized by the presence of the band for VlsE antigen.

Table 3. Results of ELISA and Western blot tests

Antibodies against <i>B. burgdorferi</i>				Study group N=128	
ELISA		Western blot			
IgM	IgG	IgM	IgG		
positive	positive	negative	positive	1	0.8
		doubtful	negative	1	0.8
positive	negative	doubtful	*	1	0.8
		negative	*	17	13.3
doubtful	positive	negative	positive	2	1.6
negative	positive	*	positive	2	1.6
doubtful	negative	negative	*	8	6.3
negative	negative	*	*	96	75.0
total				128	100.0

* – test not performed (the result of ELISA test was negative)

In 1 of the sera, a poor staining of this band was observed, while in the remaining 4 sera the staining was strong. Among the remaining antigens the presence of bands was noted for:

- p86 (2 sera with poor staining of the band);
- p39 (BmpA) (2 sera with strong staining of the band);
- p31 (OspA) (1 serum with poor staining of the band);
- p30 (2 sera with strong staining of the band);
- p21 (2 sera with strong staining of the band);
- p19 (1 serum with strong staining of the band);
- p17 (3 sera with strong staining of the band).

In 2 sera with doubtful results of Western blot test in the IgM class, in previously performed ELISA test, similar titre values were obtained (32.16 and 31.72). In 5 sera with positive results in the Western blot test in IgG class, titre values obtained in ELISA test ranged from 21.63 – 165.64; mean result 73.60 (standard deviation SD=54.22).

Table 4 presents the characteristics of respondents who obtained a positive or doubtful result in the Western blot test.

Seven respondents who obtained a positive or doubtful result in Western blot test were females living in rural areas; 5 of them declared that they lived far from forest areas, while 2 lived in the vicinity of a forest. Six respondents reported that they were bitten by a tick 3 or more times, while 1 – only once. Analysis of the frequency of staying in green areas showed that frequent and rare staying in these areas was mentioned by 3 respondents each. None of the 7 respondents reported flu-like symptoms after tick bite, whereas 1 respondent indicated the occurrence of dermal symptoms in the form of redness at the site of tick bite.

DISCUSSION

After landing on the body of a potential host (most often an extremity), ticks wander, seeking a suitable place on the skin. Most frequently, this is the skin of the lower extremities, buttocks, groins and abdomen, and in children the head and its region [8]. In own studies, the largest group of respondents (59.4%) declared tick bite in the region of the lower extremities, including the groin. The most frequent location indicated on the lower extremities were knee pits, followed by the skin of the upper extremities and abdomen (38.3% and 34.4%, respectively). In the studies by Bartosik et al. conducted among patients reporting to health care

Table 4. Characteristics of respondents who obtained a positive or doubtful result in Western blot test

		Result of Western blot test						
		doubtful (IgM class)		positive (IgG class)				
		2 individuals		5 individuals				
	No. of respondents	16	17	16	16	18	17	17
	Age (years)							
Gender	Female	+	+	+	+	+	+	+
Place of residence	rural area	+	+	+	+	+	+	+
Vicinity of forest areas in the place of residence	in vicinity of forest					+		+
	far from forest	+	+	+	+			+
Frequency of staying in green areas	rarely			+	+			+
	frequently	+	+			+		
	very frequently							+
No. of tick bite episodes	once							+
	three times			+	+	+		
	more than three times	+	+					+
Site of tick bite	lower extremities	+	+		+	+		
	upper extremities	+	+					+
	abdomen	+				+		
	back	+		+	+			
	neck					+		+
Methods of removing a tick	removed by someone else	+	+		+			+
	spread with fatty substance					+		
	twisted with tweezers				+			+
	removed with tweezers with a swift steady movement	+		+				+
	grasped in fingers and pulled out				+	+		
	tick bite site disinfected after tick removal							+
Date of last tick bite	3–5 months					+		
	approximately half a year	+	+		+			
	approximately a year			+				+
	more than 5 years							+
Duration of tick remaining attached to the skin during last tick bite	several hours	+	+		+	+		
	approximately 2 days							+
	I do not know			+				+
Occurrence of dermal symptoms after last tick bite	yes			+				
	no	+		+	+	+		+
	I do not remember							+
Occurrence of flu-like symptoms after last tick bite	no	+	+			+	+	+
	I do not remember			+	+			

centres for the removal of a tick and treatment of complaints occurring after tick bite, the most frequent sites of bites were the upper (28.8%) and lower (27.1%) extremities, lower percentages of tick bites occurred on the skin of the abdomen,

head, and back (16%, 11% and 10%, respectively) [9]. In the above-mentioned studies, only single episodes of tick bites were analyzed, whereas in the presented study – all the episodes which occurred. For this reason, the percentage data obtained cannot be compiled; nevertheless, in our study the domination of tick bites in the region of the lower extremities is noteworthy.

A very important element of the prophylaxis of borreliosis is to remove the tick feeding in the skin as quickly as possible, as well as the method of its removal. In humans, infection with spirochetes *Borrelia burgdorferi* takes place at least 24 hours after tick bite; therefore, its early removal prevents potential infection [5,6,10]. In the presented study, the majority of respondents (60.2%) estimated that during the last tick bite this parasite remained attached to the skin for several hours; thus, the time of feeding was sufficiently short that infection with the spirochetes *Borrelia burgdorferi* should not occur.

The use of inappropriate methods of tick removal increases the risk of transmission of pathogens from the body of the tick to the skin. The most common recommendation is to grasp the tick with tweezers, as close to the skin as possible, and pull it straight out with a swift steady movement, vertical to the skin, and then disinfect the site of the tick bite [6]. In the presented study, various methods of removing the tick have been mentioned; 29.7% of adolescents declared the removal of this parasite using tweezers, with a swift steady movement, and 31.3% mentioned disinfection of the site of the bite after tick removal. Among the methods of tick removal, pulling the tick out with the fingers occupied second place (22.7%), followed by using professional assistance, indicated by 18.0% of the respondents. In the studies carried out by Bartosik et al., the most frequently reported method of tick removal was pulling it out with the fingers (44%). Only 17% of respondents mentioned the use of the safest and recommended method of tick removal with tweezers [8]. The use of improper methods of tick removal increase the potential risk of infection with spirochetes [6,8], and the reported methods of its removal are often inappropriate; therefore, education of the proper method of removal of this parasite should become an important element of prophylactic actions in the area of borreliosis.

In Poland, the majority of studies evaluating the occurrence of antibodies specific for *Borrelia burgdorferi* concern patients ill with borreliosis and the population classified into the groups at risk (forestry workers, farmers). First studies in healthy individuals (blood donors) in Poland were published by Chmielewski and Tylewska-Wierzbanowska, who investigated 1,000 samples of serum. The presence of antibodies specific for *B. burgdorferi* was confirmed by the ELISA method in 11–13% of the healthy Polish population [7]. In the studies of soldiers newly enlisted into basic military service who were quartered in endemic areas of Lyme disease (north-eastern Poland), the occurrence of antibodies against *B. burgdorferi* of IgM class was found using ELISA test in 9.6% of the soldiers examined, while antibodies of IgG class – in 8.7%. The highest percentage of seropositive test results was noted among soldiers stationed in the Biała Podlaska region, i.e., the region covered by the presented study; however, attention should be paid to the small size of the group examined (17). In this group, IgM class antibodies were found in 35.3% of soldiers (6), and IgG class in 23.5% (4). Among soldiers from military units from non-endemic areas,

the presence of specific IgM class antibodies was observed in 3.4% of the soldiers examined, while IgG class – in 5.5% [11]. Walory et al. analyzed the occurrence of antibodies against *B. burgdorferi* (IgG) among healthy, adult inhabitants of the north-eastern regions of Poland. The study group were young individuals (aged 19–22), who had been bitten by the tick within the period of 2 years preceding the study. The presence of IgG class antibodies was confirmed in 16.9% of the population examined. In the control group, this percentage was 4.0%. The remainder were inhabitants of central Poland (non-endemic region) who, in the survey, did not report a tick bite. In all cases, the samples of sera were collected from the same individuals 5–6 months after the first study. Positive results in IgG class were obtained in 9.8% of population from endemic areas, and 2.0% of the control group [12].

The occurrence of antibodies among healthy individuals is most often presented in the form of a control group, who are blood donors, compared to those from groups at risk (forestry workers, farmers). In the studies by Cisak et al., where the control group were 50 blood donors from Lublin, the presence of specific antibodies was found in 4.0%. Among the blood donors in the study the presence of antibodies was confirmed only in IgG class [13]. In the studies by Zwoliński et al., in the control group, which covered sera from 56 blood donors, positive reactions were observed in 7.1% [14]. Investigations carried out in Tuscany, Italy, by ELISA test showed the presence of specific IgM and IgG class antibodies among 4.9% of the control group, and confirmed by Western blot test – in 3.6% of the population examined [15]. In studies conducted in Romania, seroprevalence was found among 4.3% of blood donors [16].

In Sweden, using ELISA test, 2,000 sera from 5-year-old children were examined for the presence of IgG antibodies. Positive results were obtained among 3.2% of the children; in the group of seropositive children, the majority (94%) had not been previously treated for borreliosis. Tick bite occurred in 66% of children with positive results [17]. In Germany during the period 2003–2006, cross-sectional studies were carried out among 12,614 children and adolescents aged from 1 – 17. In the study group, 35 had been diagnosed with borreliosis. The general frequency of occurrence of IgG antibodies in ELISA test was 4.8%. After confirmation of positive and doubtful results of ELISA test by the immunoblot method, the result was obtained on the level of 4.0%. It was also observed that seroprevalence confirmed using ELISA test increased with age, from 1.3% in the group aged 1–2, up to 7.1% in the age group 14–17 [18]. Studies for the presence of IgM and IgG specific antibodies among 14-year-olds from northern Sardinia, Italy, in ELISA test showed their presence among 6.1% of the teenagers examined [19]. In the studies for the presence of IgM and IgG antibodies among a healthy Turkish population, in ELISA test, the presence of IgG antibodies was noted among 4% of the examined individuals, and Western blot test confirmed the presence of these antibodies in 3.3% [20].

In the presented study, using ELISA test, a positive or doubtful result in at least 1 class was observed in 25.0% of the adolescents examined (IgM 23.4%, IgG 4.7%). After verification with Western blot test, positive or doubtful results were obtained in 5.5% of all respondents. Among positive or doubtful results in the IgM class, Western blot test confirmed results in 6.7%, while in the IgG class – in 83.3%. ELISA test in IgG class occurred to be much more reliable

than in the IgM class. Many positive or doubtful results in the immunoenzymatic test in the IgM class, not confirmed by Western blot test, suggest its low specificity and high sensitivity, and are probably related with a low specificity of the first-mentioned antibodies, especially in individuals with no pathological symptoms. Also, among patients with the diagnosis of borreliosis, Badora-Chmielewska et al, and Oldak et al. showed that the IgG class antibodies are a better diagnostic marker in laboratory studies of borreliosis in humans [21, 22]. In the previously mentioned studies of soldiers who served military service in north-eastern Poland, a part of the results obtained in immunoenzymatic ELISA tests was verified with Western blot tests. In the IgM class, Western blot test confirmed the results in 50%, while in the IgG class – in 70% [13]. In studies conducted in Turkey in a group of farmers and forestry workers, ELISA test in the IgG class antibodies showed seroprevalence among 10.9% of the individuals examined, while Western blot test confirmed this among only 1.1% [23]. In a group of 202 forestry workers from the region of Białystok, north-eastern Poland, investigations were carried out concerning the frequency of occurrence of false positive reactions in screening tests. The presence of antibodies against *B. burgdorferi* of IgG and IgM class was evaluated using ELISA screening tests by 3 producers, and all positive and doubtful results were confirmed by Western blot tests. In the case of IgM class antibodies, false positive results of ELISA tests were obtained in 20 – 74 individuals (according to the producer), while in the IgG class in 3 – 27 individuals. These data reflect how important is the performance of the Western blot tests which, due to their high specificity, eliminate false positive results [24]. False positive results of serologic tests may be caused by infections with *Herpes* viruses (especially *Epstein-Barr*), or with other spirochetes, as well as by autoimmune diseases. This problem concerns primarily all antibodies detected in the IgM class. The cause of cross-reactions are selected *Borrelia* antigens which are also present in other microorganisms [22, 24]. A false positive response may also be due to the bacteria *B. burgdorferi*, which do not possess pathogenic features [25]. In the studies conducted among healthy individuals, many false positive results may also suggest that the ELISA test indicates contact with the spirochetes *Borrelia*, whereas Western blot test serves for identification of the disease.

CONCLUSIONS

1. The ELISA test showed the presence of antibodies among 25.0% of healthy adolescents, while Western blot test confirmed the infection in 5.5% of respondents, which indicates the necessity for two-stage diagnostics.
2. Evaluation of the actual infection with *Borrelia* spp. using serologic tests is difficult due to a certain non-specificity of the ELISA test, especially in IgM class antibodies, and difficulties with the performance of the wide scope of specific Western blot tests.
3. The variety of the methods of tick removal declared by adolescents, considering that the duration this parasite remaining on the skin and the method used for its removal decides about infection, suggests that a wider education in society concerning appropriate methods of removing the tick should become an especially important element of prophylactic actions in the area of borreliosis.

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