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SEROLOGICAL RESEARCH TOWARDS LYME BORRELIOSIS IN HUNTERS AND FORESTRY WORKERS IN SELECTED AREAS OF POLAND AND UKRAINE

BADANIA SEROLOGICZNE W KIERUNKU BORELIOZY Z LYME WŚRÓD MYŚLIWYCH I PRACOWNIKÓW LEŚNICTWA W WYBRANYCH OBSZARACH POLSKI I UKRAINY

Małgorzata Tokarska-Rodak[1(A,B,C,D,E,F)], **Mariia Shkilna**[2(A,B,E,F)], **Dorota Plewik**[3(B,D)], **Anna Pańczuk**[4(B)], **Mykhailo Korda**[2(B)], **Ivan Klishch**[2(B)], **Justyna Paszkiewicz**[1(B)], **Mykhailo Andreychyn**[2(B)]

[1]Institute of Health Sciences, Pope John Paul II State School of Higher Education in Biala Podlaska, Poland
[2]I. Ya. Horbachevsky Ternopil State Medical University, Ukraine
[3]Innovation Research Centre, Pope John Paul II State School of Higher Education in Biala Podlaska, Poland
[4]Institute of Physical Education and Physiotherapy, Pope John Paul II State School of Higher Education in Biala Podlaska, Poland

Authors' contribution
Wkład autorów:
A. Study design/planning
zaplanowanie badań
B. Data collection/entry
zebranie danych
C. Data analysis/statistics
dane – analiza i statystyki
D. Data interpretation
interpretacja danych
E. Preparation of manuscript
przygotowanie artykułu
F. Literature analysis/search
wyszukiwanie i analiza literatury
G. Funds collection
zebranie funduszy

Summary

Background. The study aimed to analyse the prevalence of antibodies to specific *Borrelia burgdorferi* antigens in hunters from the area of Lublin Province (Poland) and forestry workers from the vicinity of Ternopil (Ukraine).
Material and methods. The study group included 240 hunters, members of the hunting clubs in Lublin Province (Poland), aged 23-80 and 339 forestry workers employed in the 5 forest districts subordinated to the Ternopil Regional Administration of Forestry and Hunting, aged 18-76. All persons were given anti-*B. burgdorferi* IgM/IgG antibodies (ELISA test and Western blot).
Results. Positive anti-*B. burgdorferi* IgM/IgG results were found in 21.7% of the hunters and 31.9% of the forestry workers. The occurrence of positive results in anti-*B. burgdorferi* antibodies depends significantly ($p<0.02$) on the area of the tested persons from Ukraine and was higher in the Berezhany district – 48.2% and the Buchach district – 47.5%.
Conclusions. Because of the high exposure to tick bites and the possibility of *B. burgdorferi* infection, it is justified and desirable to strive to implement a full two-step diagnostic approach to Lyme disease in forestry workers in Ukraine. Further, it is particularly vital to undertake extensive educational and diagnostic activities, especially in the Berezhany and Buchach districts. Finally, it would be beneficial to implement systemic solutions in this regard also in hunters due to the ever-increasing number of this professional group each year.

Keywords: foresters, hunters, Borrelia burgdorferi, Poland, Ukraine

Streszczenie

Cel pracy. Celem pracy była analiza częstości występowania przeciwciał dla specyficznych antygenów *Borrelia burgdorferi* wśród myśliwych z terenu woj. lubelskiego (Polska) i pracowników leśnictwa z okolic Tarnopola (Ukraina).
Materiał i metody. Grupa badana obejmowała: 240 myśliwych zrzeszonych w kołach łowieckich na terenie województwa lubelskiego (Polska) w wieku 23-80 oraz 339 pracowników leśnictwa zatrudnionych w 5 nadleśnictwach podległych The Ternopil Regional Administration of Forestry and Hunting w wieku 18-76. U wszystkich osób wykonano oznaczenia przeciwciał IgM/IgG anty-*B. burgdorferi* (test ELISA i Western blot).
Wyniki. Dodatnie wyniki IgM/IgG anty-*B. burgdorferi* stwierdzono u 21.7% myśliwych i 31.9% pracowników leśnictwa. Przeciwciała IgG częściej ($p<0.0001$) stwierdzano u leśników z Ukrainy. Występowanie wyników dodatnich w zakresie przeciwciał anty-*B. burgdorferi* zależy istotnie ($p<0.02$) od rejonu z którego pochodzili badani z Ukrainy i był wyższy w okręgu Berezhany – 48.2% i okręgu Buchach – 47.5%.
Wnioski. Z racji na duże narażenie na pokłucia przez kleszcze i możliwość zakażenia *B. burgdorferi* uzasadnione i celowe jest dążenie do wdrożenia w Ukrainie obowiązku pełnej, dwuetapowej diagnostyki w kierunku boreliozy z Lyme u pracowników leśnictwa. Szczególnie ważne wydaje się podjęcie szeroko zakrojonych działań edukacyjnych i diagnostycznych szczególnie w rejonach Berezhany i Buchach. Korzystne byłoby wdrożenie systemowych rozwiązań w tym zakresie również wśród myśliwych z racji na z roku na rok rosnącą liczbę osób zajmujących się łowiectwem.

Słowa kluczowe: leśnicy, myśliwi, Borrelia burgdorferi, Polska, Ukraina

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Address for correspondence / Adres korespondencyjny: Małgorzata Tokarska-Rodak, Institute of Health Sciences, Pope John Paul II State School of Higher Education, Sidorska 102, 21-500 Biała Podlaska, Poland, e-mail: rodak.malgorzata@gmail.com, phone +48 83 344 99 00

Introduction

The primary vectors of *Borrelia burgdorferi* sensu lato spirochetes (*B. burgdorferi* s.l.) in Europe are larvae, nymphs and adult ticks *Ixodes ricinus* and *Dermacentor reticulatus* [1] feeding, depending on their developmental phase, mainly on various wildlife vertebrates [2]. The research did not show the relationship between B. *burgdorferi* s.l. and specific reservoir species [1]. The spirochetes are usually found in vertebrate organisms such as: small mammals (mice, goats, rabbits, rats, hamsters, rabbits, hedgehogs, squirrels, dormice, garden dormice), game (roe deer, deer, fallow deer), birds (blackbirds, trushes, guillemots, gulls, auks, wrens, pheasants, cormorants) as well as reptiles (lizards). Man is an incidental host for an infected nymph or adult tick [1,3,4,5].

The border area of Eastern Poland and Western Ukraine is similar in its natural surroundings. The area is characterised by mixed forests with rich lining, which provides a favourable habitat for ticks. The spread of infected ticks *B. burgdorferi* s.l. ranges from 6% - 15% in Poland and affects both rural and urban areas [1]. In western Ukraine, *B. burgdorferi* s.l spirochetes were identified in 14.2% - 17.2% of the adult ticks *Ixodes scapularis* [6,7]. Other authors suggest that the incidence of tick infections may be as high as 25% [8]. The Ternopil region, located in the western part of Ukraine, is an endemic area of Lyme disease. Habitats of the tick *Ixodes ricinus* were found in 57 settlements of the 15 districts in the vicinity of Ternopil [9].

Forestry workers, loggers and hunters working in these forest areas are prone to frequent tick bites and therefore ticks diseases, including Lyme borreliosis [10]. When that happens, the illness can significantly hinder or even prevent professional activity of the infected persons. Bacteria induce the degradation of extracellular matrix (ECM), activate enzymes (elastase, laminase), merge with fibronectin, integrins, proteoglycans (decorin), which as a result enables their survival in extracellular structures and generates diverse clinical manifestations of infection [11].

The study aimed to analyse the prevalence of antibodies for specific *Borrelia burgdorferi* antigens in hunters from the area of Lublin Province (Poland) and forestry workers from the vicinity of Ternopil (Ukraine). Because of their professional activity and hobbies, these persons are particularly prone to be bitten by ticks. The Polish hunters and the Ukrainian forestry workers (in contrast to the Polish foresters) are not covered by regular preventive and diagnostic measures that show be taken due to their exposure to *B. burgdorferi*.

Material and methods

Study group

The study group included:

- 240 hunters, members of the hunting clubs in Lublin Province (Poland), aged 23-80 (mean 52, SD 11.6), including 212 men (88.3%) and 28 women (11.7%);
- 339 forest workers employed in the 5 forest districts subordinated to the Ternopil Regional Administration of Forestry and Hunting: Berezhany (56), Ternopil (64), Chortkiv (65), Buchach (61), Kremenets (93) in the Ternopil region. The tested persons were aged 18-76 (mean 42, SD 11.2), including 337 men (99.4%) and 2 women (0.6%).

The blood samples were taken between 2014 and 2015.

Serological tests

In all persons, anti-*B. burgdorferi* IgM/IgG antibodies were marked using the ELISA test (anti-Borrelia ELISA IgM; anti-Borrelia plus VlsE ELISA IgG, Euroimmun, Germany) as the screening assay. The results obtained are interpreted following the manufacturer's instructions. The results above 22 relative units/ml (RU/ml) were interpreted as positive, below 16 RU/ml as negative, and those between 16 - 22 RU/ml as borderline.

The positive and borderline results obtained by the ELISA test were confirmed by Western blot (Wb) (anti-Borrelia Euroline-WB IgM/IgG, Euroimmun, Germany). Test strips showed *B. afzelii* antigens (p83, p41, p39/BmpA, p31/OspA, p30, p25/OspC, p21, p19, p17/DbpA) and a recombinant VlsE antigen. The test strips reading and the results interpretation were done following the manufacturer's instructions using the EuroLinescan software (Euroimmun, Germany).

The research was approved by the Bioethics Committee of the Medical University of Lublin (No. KE-0254/177/2014) and Bioethics Committee of I. Ya. Horbachevsky Ternopil State Medical University (No 30, dated 01/09/2015).

The results of the study were then statistically analysed using the Statistica v. 10 programme. In all examined cases, the significance level $p < 0.05$ was assumed.

Results

Positive anti-*B.* b*urgdorferi* IgM results were found in 2.1% of the hunters and 1.5% of the forestry workers. The presence of both anti-*B. burgdorferi* IgM/IgG was detected in 0.4% of the hunters and 3% of the forestry workers. The number of anti-*B. burgdorfer*i IgG was significantly higher in the Polish foresters ($p<0.0001$) (19.2% of the hunters; 27.4% of the foresters). The detailed results are given in Table 1.

The occurrence of positive results with regard to anti-*B. burgdorferi* antibodies depended significantly ($p<0.02$) on the area of the tested persons from Ukraine and was higher in the Berezhany district – 48.2% and the Buchach district – 47.5%. The detailed data are provided in Table 2.

The IgM and IgG antibodies for specific *B. burgdorferi* antigen proteins were detected by Western blot with different frequencies. The results are presented in Table 3.

IgG antibodies to some *B. burgdorferi* antigens were significantly more common in the Ukrainian forestry workers than in the Polish hunters: p21 ($p<0.0004$), p30 ($p<0.00001$), p31 ($p<0.05$), p39 ($p<0.00008$), VlsE ($p<0.0002$).

The patients in the Berezhany district (Ukraine) were more likely ($p<0.0004$) than those in the other districts to have anti-p19 *B. burgdorferi* IgG antibodies (10 persons, 17.9%), anti-p25 ($p<0.008$, 16 persons, 28.6%), anti-p39 ($p<0.004$, 12 persons, 21.4%), anti-VlsE ($p<0.05$) (18 persons, 32.1%).

In the Buchach district (Ukraine), anti-p25 *B. burgdorferi* IgG was encountered more often ($p<0.008$) in 22 persons (36.0%), anti-p39 ($p<0.004$) in 17 persons (27.9%), and anti-VlsE $p<0.05$ in 26 persons (42.6%).

Table 1. Anti-*B. burgdorferi* IgM/IgG antibodies in the tested hunters from Lublin Province (Poland) and the forestry workers from the vicinity of Ternopil (Ukraine)

The tested group	Anti-B. burgdorferi antibodies							Total
	IgM- IgG-	IgM+	IgM+/-	IgM+ IgG+	IgM+/- IgG+	IgG+/-	IgG+	
Hunters (PL)	163 (67,9)	5 (2.1)	5 (2.1)	1 (0.4)	2 (0.8)	18 (7.5)	46 (19.2)	240 (100)
Forestry workers (UA)	219 (64.6)	5 (1.5)	5 (1.5)	10 (3.0)	7 (2.1)	-	93 (27.4)	339(100)

PL – Poland UA - Ukraine
the value in brackets is given in percent (%)
- a negative result
-/+ a borderline result
+ a positive result

Table 2. Anti-*B. burgdorferi* IgM/IgG antibodies in the forestry workers from Ukraine by the areas from which the tested persons came

Forestry workers (UA)	Anti-B. *burgdorferi* antibodies							Total
	IgM- IgG-	IgM+	IgM+/-	IgM+ IgG+	IgM+/- IgG+	IgG+/-	IgG+	
1	28(50.0)	0(0)	1(1.8)	5(8.9)	1(1.8)	-	21(37.5)	56(16.5)
2	44(68.7)	2(3.1)	1(1.6)	0(0)	1(1.6)	-	16(25.0)	64(18.9)
3	48(73.8)	0(0)	0(0)	1(1.5)	1(1.5)	-	15(23.1)	65(19.2)
4	31(50.8)	1(1.6)	1(1.6)	3(4.9)	0(0)	-	25(41.0)	61(18.0)
5	68(73.1)	2(2.1)	2(2.1)	1(1.1)	4(4.3)	-	16(17.2)	93(27.4)
Overall	219 (64.6)	5 (1.5)	5 (1.5)	10 (3.0)	7 (2.1)	-	93 (27.4)	339(100)

UA – Ukraine, 1-Berezhany, 2-Ternopil, 3-Chortkiv, 4-Buchach, 5- Kremenets
the value in brackets is given in percent (%)
- a negative result
-/+ a borderline result
+ a positive result

Table 3. IgM and IgG antibodies identified by Western blot for specific *B. burgdorferi* antigenic proteins in the group of hunters from Poland and forestry workers from Ukraine

Antibody class	Country	*B. burgdorferi* antigenic proteins								
		p17	p19	p21	p25	p30	p31	p39	p83	VlsE
IgM	PL (hunters)	-	-	-	6 (2.5)	-	-	-	-	-
	UA (forestry workers)	2 (0.6)	-	-	10 (2.9)	-	4 (1.2)	-	-	-
IgG	PL (hunters)	36 (15.0)	25 (10.4)	5 (2.1)	13 (5.4)	13 (5.4)	16 (6.7)	19 (7.9)	24 (10.0)	31 (13.0)
	UA (forestry workers)	52 (15.3)	26 (7.7)	34 (10.0)	22 (6.5)	67 (19.8)	44 (13.0)	62 (18.3)	48 (14.2)	93 (27.4)

PL – Poland, UA - Ukraine
the value in brackets is given in percent (%)

Discussion

The ticks infestation in a given area can be estimated from the prevalence of *B. burgdorferi* antibodies in the blood of the residents or workers in a given region [12]. In European countries, anti-*B. burgdorferi* IgM/IgG antibodies were reported in forestry workers (woodcutters, foresters, hunters) with different frequencies depending on the country and geographic region: in France 14.1 – 20.2%; in Italy 5.4 – 23.2; in Germany 8 – 43%; in the Netherlands 19.3%; in Slovenia 23.8%; in Romania 9.4%; in Turkey 10.9; in Hungary 37% [13] and in Austria 7% – 42% [10]. In Poland, antibodies for specific antigens of *B. burgdorferi* s.l. were found in forestry workers with deferent frequencies depending on the region of the country: 32.7% (in Kujawsko-Pomorskie Province) [14], 47.9% [15] – 55% (Lublin Province) [16], whereas in the area of Polesie and Forest-Steppe of Ukraine 30.4 – 50.0% [17]. While forestry workers in Poland are well-monitored for the risk of *B. burgdorferi* infection and Lyme disease, there are no such procedures provided for this occupational group in Ukraine. There are also no uniform Lyme diagnostic recommendations. However, according to The Order of the Minister of Health of Ukraine, N133 of 19.07.1995, Lyme disease was classified into the group of particularly dangerous infections.

In the conducted two-stage studies (ELISA tests, Western blot), the anti-B. *burgdorferi* IgM/IgG antibodies were found in 31.9% of the forestry workers from the vicinity of Ternopil (Ukraine). The occurrence of positive anti-*B. burgdorferi* results depends significantly on the Ukrainian region from which the tested persons came. Higher positive results were in Berezhany district (48.2%) and Buchach district (47.5%).

The group of the Polish people who are not adequately protected against tick-borne diseases are hunters, who are mainly exposed to frequent tick-bites and the risk of Lyme borreliosis when undertaking activities related to hunting and wildlife conservation. In the conducted studies, the anti *B. burgdorferi* IgM/IgG were present in 21.7% of the hunters from Lublin Province (Poland). The results show that seroprevalence in this group is significantly higher than in those who are not exposed to ticks (7.1% – 12.5% of the blood donors) [13, 18], and similar to that obtained for farmers from Lublin Province (27.3% [19], and 28% [16]) of those professionally exposed to Lyme disease. The obtained data indicate that these particular groups of people from both countries should be provided with meticulous care for the diagnosis and prevention of Lyme disease.

The diagnosis of Lyme borreliosis is based on three criteria: the individual's ticks bite history, clinical manifestations of the disease and serological tests (ELISA tests and Western blot) indicating the presence of the specific anti-*B. burgdorferi* IgM/IgG antibodies [20]. While evaluating and interpreting the results of the serological tests, both the antibody class for the specific *B. burgdorferi* antigenic proteins and the type of bacterial antigen for which these antibodies are produced are significant. Outer surface proteins (Osp) play an important role in the immune response to the infection because they are highly immunogenic, and anti-OspC (p25), OspA (p31), and OspB antibodies can destroy *B. burgdorferi* spirochetes [21].

The EUCALB (European Union Concerted Action on Lyme Borreliosis) programme demonstrated that the following *B. burgdorfer*i s.l. antigenic proteins: OspC and p41 for IgM and p83/100, p58, p41, p39, OspC, DbpA (p17) are useful in Western blot testing for IgG. It is essential that the diagnostic criteria take into account the immune response to the antigens of the most common strains in the area. In Europe, it is Western blot tests with *B. afzelii* antigens (the PKo strain) that are recommended [22]. In these tests, recombinant *B. burgdorferi*

antigens are most useful: p100, p58, p41i, VlsE, OspC, DbpA [23]. The diagnostic significance of OspC decreases with the progress of the immune response and an increased production of IgG antibodies to other spirochete antigen proteins [24, 25].

In forest workers, B. *burgdorferi* anti-DbpA IgG (65%) and anti-OspC (35%) were identified [16]. The studies have confirmed the diagnostic role of p17 (DbpA), p19, p25, p39, VlsE antigens in the assessment of late manifestation of *B. burgdorferi* infection.

Conclusions

Because of the high exposure to tick bites and the possibility of *B. burgdorfer*i infection, it is justified and desirable to strive for the implementation of a full two-step diagnostic approach to Lyme disease in forestry workers in Ukraine. It is particularly vital to undertake wide-ranging educational and diagnostic activities among Ukrainian foresters, especially in the Berezhany and Buchach districts. Further, it would also be beneficial to implement systemic solutions for hunters in this area due to the ever-increasing number of this professional group each year.

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