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STUDY ON LYME BORRELIOSIS FOCUS IN THE LUBLIN REGION (EASTERN POLAND)

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Abstract: A suburban focus of Lyme borreliosis situated 11 km from the southern border of the city of Lublin (eastern Poland) was characterized. The focus covers an area of circa 100 km^2, surrounding 3 localities inhabited by circa 7,500 people engaged mostly in farming. It was demonstrated that on the area of focus the infection rate of *Ixodes ricinus* ticks with *Borrelia burgdorferi*, frequency of serological response of inhabitants to the antigen of *Borrelia burgdorferi*, and incidence of Lyme borreliosis were significantly ($p<0.001$) greater compared to the whole territory of Lublin province, and were respectively 13.1% *vs.* 4.7%, 33.0% *vs.* 13.7%, and 0.002% *vs.* 0.00075%.

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INTRODUCTION

Lyme borreliosis (LB) caused by the spirochete *Borrelia burgdorferi* is the commonest tick-transmitted disease in the world [3, 15, 32] manifested by a wide spectrum of clinical symptoms. Epidemiological data indicate that there are areas of particularly high risk of infection with *Borrelia burgdorferi*, called natural foci of LB, which may occur rapidly at previous non-endemic sites [36].

Establishing active natural foci of LB in selected localities is the first step in controlling and preventing the disease [5, 42]. To characterise the natural foci of LB the presence of infected vectors and reservoir hosts of different species of *Borrelia* as well as incidence of seropositive reactions and clinical cases in the exposed population should be taken into account [3, 21, 25, 36]. In Poland, where Lyme borreliosis is widespread with the increasing morbidity (11.6 per 100,000 in the year 2005, 17.5 in 2006, and 20.2 in 2007 [27]), the foci of LB were identified until recently in the northern part of the country (Podlasie, Masurian Lakeland and Eastern Pomerania) [37, 39, 41].

During recent years, we have focused our attention on a suburban area south of the city of Lublin where numerous clinical cases of LB were diagnosed by local practitioners among rural inhabitants. The aim of this study was to evaluate this area as a potential natural focus of LB by determination of the infection rate of *Ixodes ricinus* ticks with *Borrelia burgdorferi*, examination of serological response of inhabitants to *Borrelia burgdorferi* antigen, and characterization of clinical cases of LB. The results were compared with those obtained earlier for the whole territory of the Lublin province [10, 11].

MATERIALS AND METHODS

Study area. The study was conducted in an area of circa 100 km^2, surrounding 3 localities (Bychawka, Jabłonna, Piotrowice) situated in the southern part of the Lublin district, 11 km from the border of the city of Lublin. These localities are 5–8 km away from each other, forming a triangle, and are inhabited by circa 7,500 people. Farming is the main income source in this area, as cropland is 80% of

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the land and the rest of this territory is covered with mixed forest.

Collection of ticks. Unfed *Ixodes ricinus* ticks (adults and nymphs) were collected in the study area from April–June 2008 by flagging lower vegetation, mostly at the edge of forests, on the borderline between the wooded area and farmers' fields or glades, and on the forest paths. Collected ticks were placed in glass vials with 70% ethanol for further investigation.

Detection of *Borrelia burgdorferi* sensu lato DNA in ticks. 915 ticks collected from the Jabłonna, Bychawa and Piotrowice areas were checked first for the presence of *Borrelia burgdorferi* sensu lato (s.l.) by polymerase chain reaction (PCR), and next for the presence of 3 pathogenic *Borrelia* genospecies (*Borrelia burgdorferi* sensu stricto, *Borrelia afzelii* and *Borrelia garinii*) by nested PCR.

Bacterial DNA was isolated from ticks according to Rijpkema *et al.* [37] by crushing the tick with a pipette tip and then boiling in 0.7 M ammonium hydroxide at 90°C until evaporation of the ammonia. Thereafter, ticks were stored at -70°C. Adult ticks were investigated separately and nymphs in pools of 5 specimens. The tick isolates were examined for the presence of *Borrelia burgdorferi* s.l. DNA by PCR with oligonucleotide primer set Fla1/Fla2 (Eurogentec, Seraing, Belgium) specific for *fla* gene sequence, and PCR was performed as described previously [12].

Detection of *Borrelia burgdorferi* genospecies DNA. All tick lysates in which *Borrelia burgdorferi* s.l. DNA was detected were examined for the presence of 3 pathogenic *Borrelia* genospecies by nested PCR using species specific pairs of primers BB1/BB2, BA1/BA2 and BG1/BG3 (Eurogentec, Seraing, Belgium) to differentiate *Borrelia burgdorferi* s.l. into *Borrelia burgdorferi* sensu stricto, *Borrelia afzelii*, and *Borrelia garinii*. The conditions of nested PCR have been described earlier [12].

Examined population. A total of 94 rural inhabitants engaged in farming on the study area, comprising 28 males and 66 females were examined. The mean age of examined farmers amounted to 56.3 ± 14.3 years. All the examined population was interviewed regarding exposure to ticks and subjected to serological examination with *Borrelia burgdorferi* antigen. The reference group consisted of 50 healthy blood donors living in the city of Lublin, at the mean age of 29.7 ± 5.0 years.

Serological examination for the presence of anti-*Borrelia burgdorferi* antibodies. Sera of rural inhabitants from the study area and reference group were examined for the presence of specific anti-IgM and anti-IgG antibodies to *Borrelia burgdorferi* sensu lato (s.l.) according to European guidelines by two-step diagnostics [1, 16]. In the first step, the level of specific IgM and IgG antibodies was determined with the use of commercial ELISA test (*Borrelia* recombinant IgM and *Borrelia* recombinant IgG, Bellco Biomedica GmbH and Co. KG, Vienna, Austria). In the second step, positive and equivocal sera were examined by Western blot IgM and IgG tests (*Borrelia* recom Line IgM and *Borrelia* recom Line IgG, Mikrogen, Neuried, Germany). In all serological kits recombinant proteins of *Borrelia burgdorferi* s.l. were used as the antigen.

Clinical analysis. The medical staff of the local health service were interviewed for the prevalence of clinical cases of Lyme borreliosis among the population of the study area in the years 1998–2007, and the documentation was analysed to reveal specific features of the disease. Moreover, the case of occupational disease acquired on this terrain was taken into consideration in the present study.

Statistical analysis. Statistical analysis was carried out by chi-square and t-tests, using Statistica for Windows v. 5.0 package (Statsoft, Inc., Tulsa, Oklahoma, USA).

RESULTS

Infection of *Ixodes ricinus* ticks with *Borrelia burgdorferi* sensu lato. Altogether, 120 (13.1%) out of 915 ticks collected on the study area (individual and pooled samples) showed the presence of the *Borrelia burgdorferi* sensu lato DNA. The highest infection rate was observed in females (26.7%), followed by males (20.1%). Minimum infection rate in nymphs amounted to 7.5% (Tab. 1).

Further study of 120 tick samples positive for *Borrelia burgdorferi* s.l. showed that all 3 pathogenic genomic species of *Borrelia burgdorferi* sensu lato were noted in the study area. In 59 positive ticks (49.1%) single infection was observed, while in 51 specimens (42.5%) were found co-infections with 2 species and in 8 specimens (6.7%) co-infections with 3 species. In 2 ticks (1.7%), genospecies was not identified. The dominant genospecies in single infections was *Borrelia afzelii* while *Borrelia burgdorferi* s. stricto was noted most frequently in mixed infections (Tab. 1).

Prevalence of specific antibodies anti-*Borrelia burgdorferi*. In 31 out of 94 examined farmers inhabiting the study area (33.0%) the presence of specific IgG and/or IgM antibodies against *Borrelia burgdorferi* was found in ELISA test (26 positive and 5 borderline). Healthy urban dwellers from the reference group showed positive reactions to *B. burgdorferi* only in 6.0%. All the positive and borderline sera were confirmed as positive with Western blot test (Tab. 2). The difference between the prevalence of specific anti-*Borrelia burgdorferi* antibodies in both groups was highly significant ($p<0.001$). Ninety-one percent of examined farmers claimed tick bites during the last 15 years.

Clinical cases of Lyme borreliosis on study area. A total of 15 cases of Lyme borreliosis were diagnosed in

the years 1998-2007 on the territory of study area. Their characteristics are presented in Table 3. As seen in Table 3, the most common clinical forms observed among patients were Lyme arthritis and skin borreliosis. In one case, the occupational origin of Lyme borreliosis was documented in the scientist who collected wood samples for microbiological investigation.

Comparison of results obtained for study area with those recorded earlier for the whole territory of Lublin province. The comparison is presented in Table 4. The percentage of ticks infected with *Borrelia burgdorferi* on the study area was nearly 3 times higher compared to our earlier work [10, 11] comprising the whole Lublin province. Also, the percentage of seropositive results obtained with the *Borrelia burgdorferi* antigen among farmers from the study area was distinctly higher than in earlier own research [11] for whole province. Similarly, the incidence rate of Lyme borreliosis on study area was 2.7 times greater compared to official morbidity reports per 100,000 of the population compiled in the years 1998–2007 for Lublin province by the State Institute of Hygiene [27]. All these differences were highly statistically significant as measured with chi-square test (Tab. 4), thus indicating that the study area was a true natural focus of Lyme borreliosis.

Table 3. Characterization of the Lyme borreliosis clinical cases diagnosed in the years 1998-2007 on the territory of study area.

No.	Patient's initials	Patient's profession	Year of diagnosis	Clinical form of disease
1	M. M.	Farmer	1998	Skin borreliosis
2	H. N.	Farmer	1998	Lyme arthritis
3	M. S.	Pupil	1999	Neuroborreliosis
4	C. S.*	Scientist	2000	Lyme arthritis
5	J. Z.	Teacher	2002	Lyme arthritis
6	B. C.	Farmer	2004	Lyme arthritis
7	M. S.	Farmer	2005	Lyme arthritis
8	K. K.	Clerk	2005	Lyme arthritis
9	B. A.	Farmer	2006	Lyme arthritis
10	C. R.	Farmer	2007	Skin borreliosis
11	K. T.	Farmer	2007	Lyme arthritis
12	S. K.	Teacher	2007	Skin borreliosis
13	K. L.	Farmer	2007	Lyme arthritis
14	A. M.	Farmer	2007	Lyme arthritis
15	A. W.	Farmer	2007	Lyme arthritis

* case of occupational borreliosis

DISCUSSION

At least 3 conditions are needed to define a particular area as a natural focus of Lyme borreliosis (LB): • increased infection rate of tick vectors and/or vertebrate animal hosts with *Borrelia burgdorferi*; • increased immunological response to *Borrelia burgdorferi* antigen among human inhabitants; • increased incidence of clinical cases of LB. All these conditions were fulfilled in study area investigated in the present work, which could be recognized as the first well defined LB focus in mid-eastern Poland.

The results of the present study could be compared with earlier studies describing LB natural foci mainly with regard to the infection rate of ticks which was highlighted by most authors. Compared to the LB natural foci described by Stańczak *et al.* [39, 41] in northern Poland, the total

Table 1. Prevalence of *Borrelia burgdorferi* DNA in ticks collected from vegetation in the study area.

Tick stage	Number of specimens examined	Positive (percent)								
		B.a.	B.g.	B.b. s.s.	B.a. + B.g.	B.a. + B.b.s.s.	B.g. + B.b.s.s.	B.a. + B.g. + B.b.s.s.	Unidentified genospecies	Total – B.b. s.l.
Females	161	7 (4.4%)	3 (1.9%)	10 (6.2%)	2 (1.2%)	4 (2.5%)	15 (9.3%)	2 (1.2%)	0 (0)	43 (26.7%)
Males	164	10 (6.1%)	1 (0.6%)	2 (1.2%)	0 (0)	2 (1.2%)	16 (9.8%)	0 (0)	2 (1.2%)	33 (20.1%)
Nymphs*	590	20 (3.4%)	1 (0.2%)	5 (0.9%)	2 (0.3%)	2 (0.3%)	8 (1.4%)	6 (1.0%)	0 (0)	44 (7.5%)**
Total	915	37 (4.0%)	5 (0.6%)	17 (1.8%)	4 (0.4%)	8 (0.9%)	39 (4.3%)	8 (0.9%)	2 (0.2%)	120 (13.1%)

* examined in pools of 5 specimens; ** minimum infection rate; B.a. – *Borrelia afzelii*, B.g. – *Borrelia garinii*, B.b.s.s. – *Borrelia burgdorferi* sensu stricto, B.b. s.l. – *Borrelia burgdorferi* sensu lato.

Table 2. Presence of antibodies to *Borrelia burgdorferi* s.l. in rural inhabitants of the study area and reference group of healthy urban dwellers.

Group	Number of examined people	Positive and borderline serological reactions confirmed with Western blot (number, percent)		
		ELISA		Western blot
		Positive	Borderline	Confirmed as positive
Rural inhabitants of study area	94	26 (27.7%)	5 (5.3%)	31 (33.0%)
Healthy urban dwellers	50	3 (6.0%)	0	3 (6.0%)

Table 4. Comparison of epidemiological results obtained in the present work for the study area with the analogical data obtained earlier for the whole Lublin province.

	Positive/examined (percent)		Significance of difference between data for study area and whole Lublin province
	Study area	Whole Lublin province	
Infection of ticks with *Borrelia burgdorferi*	120/915 (13.1%)	17/362 (4.7%) [10]	p<0.00001
Serological response of rural inhabitants to antigen of *Borrelia burgdorferi*	31/94 (33.0%)	15/110 (13.6%) [11]	p<0.001
Incidence of clinical cases of Lyme borreliosis	15/7,500 (0.002%)	1,631/2,166,213 (0.00075%) [27]	p<0.001

infection rate of *Ixodes ricinus* ticks with *Borrelia burgdorferi* s.l. found in the present study (13.1%) was similar to the rates reported by these authors for Gdańsk-Gdynia-Sopot area and Elbląg region (12.4% and 13.2% respectively), and higher than the rates reported for Słupsk and Bydgoszcz regions (9.1%, and 7.4%, respectively).

Studies on LB natural foci have been conducted in various countries in Europe [2, 3, 6, 7, 9, 13, 15, 17, 18, 20, 21, 24, 26, 29, 30, 31, 32, 33, 34, 38, 44]. In the study conducted by Ferquel *et al.* [15] on the territory of Alsace in northeastern France (described as the focus of Lyme borreliosis) the percentage of adult ticks infected with *B. burgdorferi* fluctuated from 21.0%–36.4% and was comparable to the results obtained in this study, where the rates of infected females and males were 26.7% and 20.1%, respectively. The active natural foci of Lyme borreliosis were shown by Biletska *et al.* on the territories of 8 provinces in the Ukraine; the mean percentage of infected ticks was lower than in the present study and amounted approximately to 10% [3]. The seroprevalence among people living in LB foci in the Ukraine was comparable to the current study and amounted to 34.3%.

Potential foci of Lyme borreliosis were also identified in towns. Nadzamova *et al.* reported that on the territory of Košice (Slovakia), the infection rate of ticks was similar to the present study and amounted from 12.8%–15.0% [30]. Compared to own research, the higher infection rate (35.0%) of *B. burgdorferi* infected ticks was found by Danielova *et al.* [13] in *I. ricinus* from selected South Bohemian locations in the Czech Republic. The cited authors suggested the rapid formation and establishment of natural focus of Lyme disease on territory with a low incidence of Lyme borreliosis. The higher prevalence of *B. burgdorferi* in *I. ricinus* ticks (from 13.9%–24.0%) was also shown by Oehme *et al.* in a study upon the foci of tick-borne diseases in the State of Baden-Wuertemberg in southwestern Germany [31]. Similarly, a high infection rate (19.1%) of infected *Ixodes persulcatus* ticks in natural LB foci in Novosibirsk region in Russia was found by Fomenko *et al.* [17]. By contrast, the prevalence of *B. burgdorferi* s. l. in ticks obtained in the present study (13.1%) was higher than in the study of Makinen *et al.* (5.0%) conducted on the area of suburban and rural territories of the Turku archipelago in Finland, which is an endemic LB area [26].

In the present study 3 pathogenic genomic species of *Borrelia burgdorferi* s.l. were found on the examined area and *Borrelia afzelii* was a dominant genospecies in single infections in *I. ricinus*. This genospecies was indicated by Stańczak *et al.* [40] as the most prevalent genospecies in *Ixodes ricinus* collected from various localities in Poland. *Borrelia afzelii* was also the most frequent genomic species in *Ixodes ricinus* ticks from the majority of European regions [7, 18, 28, 31, 32, 44, 45]. The results of the present study also show that in co-infections with 2–3 *B. burgdorferi* pathogenic species, *Borrelia burgdorferi* sensu stricto (s.s.) was the most common species. *Borrelia burgdorferi* s.s. was found as a dominant *B. burgdorferi* genospecies in our previous study conducted on the territory of the Lublin region [12], and also in some localities in Europe [7, 13, 23, 43].

The seroepidemiologic studies conducted in many countries showed a high risk of *B. burgdorferi* infection among farmers and other outdoor working people, manifested by high prevalence of anti-*B. burgdorferi* antibodies [8, 14, 19, 24, 46]. This is in accord with the prevalence of anti-*B. burgdorferi* antibodies among rural inhabitants found in the present study which was relatively high (33.0%), and significantly greater compared to the control group of healthy urban dwellers.

The variety of clinical symptoms (rheumatic manifestations, skin changes, neurologic symptoms) found in LB cases presented in this work does not substantially differ from the cases hitherto described in Europe [3, 8, 19, 24].

In conclusion, the results of this study confirm the presence of areas of enhanced risk of contracting LB and indicate a need for searching such areas – the natural LB foci. The knowledge of such foci would be very helpful for planning prevention measures against LB which represents an emerging threat to public health in Poland and other countries.

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