ORIGINAL ARTICLES AAEM

Ann Agric Environ Med 1998, 5, 183–186

SEROEPIDEMIOLOGIC STUDY ON LYME BORRELIOSIS IN THE LUBLIN REGION

Jolanta Chmielewska-Badora

Department of Occupational Biohazards, Institute of Agricultural Medicine, Lublin, Poland

Chmielewska-Badora J: Seroepidemiologic study on Lyme borreliosis in the Lublin region. *Ann Agric Environ Med* 1998, **5**, 183–186.

Abstract: The frequency of anti-*Borrelia burgdorferi* antibodies in human sera of various groups of people from the Lublin region was studied. In the indirect immunofluorescence test (IFT) sera from 836 forestry workers and 56 farmers occupationally exposed to ticks were examined. Fifty healthy blood donors from the city of Lublin were examined as a control group. Forestry workers showed positive response in 26%, whereas farmers in 11% and the control group in 6%. In ELISA test, sera from 44 forestry workers, 217 farmers, 458 patients from the dermatologic and neurologic clinics, and 50 blood donors (controls) were examined. A positive response was found in 38.6% of forestry workers, 28.1% of farmers, 12.2% of patients of the neurologic clinic, 27.1% of patients of the dermatologic clinic and in 6% of controls. During this study, in one of an forestry workers the diagnosis of Lyme borreliosis as the occupational disease was clinically confirmed. High percent of anti-*Borrelia burgdorferi* antibodies found in people exposed to ticks may suggest that *Borrelia burgdorferi* is widespread in the forest environment of eastern Poland and that infection often has an occupational character.

Address for correspondence: Jolanta Chmielewska-Badora, MS, Department of Occupational Biohazards, Institute of Agricultural Medicine, Jaczewskiego 2, P.O. Box 185, 20-950 Lublin, Poland.

Keywords: Lyme borreliosis, seroepidemiology, immunofluorescence test, ELISA, Lublin region, forestry workers, farmers.

INTRODUCTION

Lyme disease (L.D.) is a chronic multisymptomatic zoonosis with three clinical phases and dermatologic, osteoarticular, neurologic and organ symptoms. Etiologic factor of this zoonosis is a spirochete, *Borrelia burgdorferi*, isolated in 1982 from the digestive tract of ticks and described by Willy Burgdorfer [3, 5, 6, 31]. The spirochetes were also isolated from skin, cerebrospinal fluid and blood of human patients in whom specific antibodies were also found [4, 25]. Epidemiologic studies identified as the pathogen reservoirs small rodents living in forests, and as vectors ticks *Ixodes ricinus* and *Ixodes persulcatus* in Europe and *Ixodes dammini (scapularis)* in Northern America [1, 18, 19, 31].

L.D. is common worldwide and is associated with areas inhabited by ticks [14, 27]. Since the first isolation of *B. burgdorferi* from *Ixodes dammini*, numerous bacteriologic examinations of ticks have been done to evaluate the frequency of infected ticks in various areas of the world [16, 22, 28, 29, 30]. The results from Poland show that infection of ticks with *B. burgdorferi* vary from 0.77% to 58.6% [16, 29]. According to a WHO report [13], all of Europe should be regarded as an endemic area of L.D., although the number of infections in particular countries varies from several to over 100 per 100,000 inhabitants per year. The frequency of specific antibodies in blood of European populations, with special regard to Poland, is shown in Fig. 1 [23]. The range of positive results in chosen regions of Poland was from 12.2% to 16.7% [18]. The serologic studies performed in Europe showed that populations exposed to contact with ticks (forestry workers and farmers) more often have specific antibodies against *B. burgdorferi* than the rest of population [23].

Routine diagnostics of borreliosis in man is based on the determination of the level of specific antibodies of

Received: 9 November 1998
Accepted: 20 November 1998

A	Szczecin district	12.2%
B	Słupsk district	20.0%
C	Olsztyn district	14.3%
D	Suwałki district	23.4%
E	Białystok district	18.8%, 49.7%
F	Krosno district	16.7%
G	Jelenia Góra district	71.7%
H	Lublin region examined in this study	

Figure 1. Incidence of anti-*Borrelia burgdorferi* antibodies in human sera in Poland and other European countries. Numbers concerning occupationally exposed groups in **bold face**. According to Prokopowicz [14], modified.

IgM and IgG classes, mainly in blood, cerebrospinal fluid and synovial fluid. The methods commonly used are indirect immunofluorescence test (IFT) and ELISA.

The aim of the present study was an evaluation of the frequency of antibodies anti-*Borrelia burgdorferi* in human sera collected from forestry workers, farmers and patients of the neurologic and dermatologic clinic from the Lublin region.

MATERIALS AND METHODS

Altogether, samples of 1,711 human sera were examined in this study. Sera were collected from 1,153 persons occupationally exposed to ticks (880 forestry workers and 273 farmers), from 458 patients suspected of L.D. (362 from neurologic clinic and 96 from dermatologic clinic), and from 100 blood donors (healthy inhabitants of the city of Lublin) who were considered as the control group. Forestry workers came from five districts and farmers from four districts of the Lublin region. Before serologic examination, the epidemiologic anamnesis was performed. This included questions about profession, place and time of work, contact with animals, tick bites and symptoms concerning skin, nervous system and osteoarticular system.

Sera were examined with quantitative indirect immunofluorescence test (IFT) "Lyme Spot IF" (*bioMerieux*, France), or ELISA IgM and ELISA IgG "Enzygnost Borreliosis" (*Behring*, Germany). In IFT, whole cells of the reference strain *Borrelia burgdorferi* B31, isolated in USA, were used as an antigen and fixed on a slide. After incubation with sample sera, antiglobulin antibodies labelled with fluorescein were added and bright complexes were searched for in fluorescence microscope.

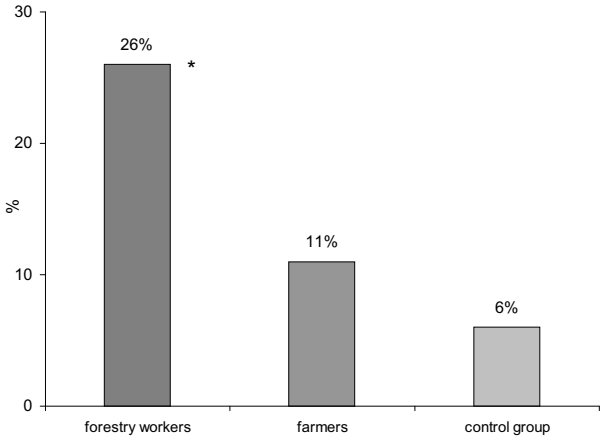

Figure 2. Results of seroepidemiologic survey by immunofluorescence test (IFT) in occupationally exposed people and control group. * Significantly greater compared to the control group ($p < 0.01$).

In immunoenzymatic assays, detergent extract from a mix of membrane proteins from PKo strain *Borrelia afzelii*, isolated in Europe from skin, was used as an antigen. The mixture of antigens includes at least the proteins: 100 kD, 41 kD, 39 kD, Osp A, Osp B, Osp C and 17 kD. This ensures very high sensitivity of the assay in subsequent phases of the disease. Specificity was increased by the addition of *Treponema phagediens* ultrasonificate in sample buffer which decreases frequency of cross reactions.

In IFT, 942 sera were examined: 836 sera from forestry workers, 56 from farmers and 50 from the control group. Immunofluorescence test was read in fluorescence microscope JENAMED 2. A result was considered as positive if in titre 1/160 or higher bright complexes were found.

In ELISA, 769 sera were examined: 44 sera from forestry workers, 217 from farmers, 96 from patients of dermatologic clinic, 362 from patients of the neurologic clinic, and 50 sera from the control group. Assay was read in ELISA reader with filter 450 nm. A result was considered as positive if absorbance was greater than cut-off evaluated according to instruction. The frequency of results in particular groups was compared with the control group using chi-square test.

RESULTS

Results of indirect immunofluorescence test (IFT). In epidemiologic anamnesis, 90% of forestry workers examined by IFT stated to have been bitten by ticks. As much as 26% of forestry workers showed the positive

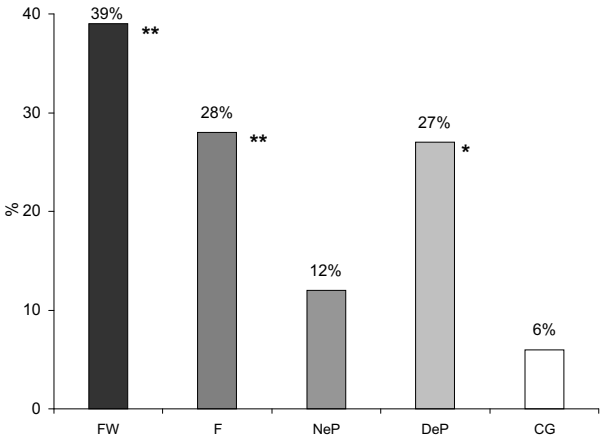

Figure 3. Results of seroepidemiologic survey by ELISA in occupationally exposed people, patients and control group. FW: forestry workers; F: farmers; NeP: neurologic patients; DeP: dermatologic patients; CG: control group. *-** Significantly greater compared to the control group: *p < 0.01, ** p < 0.001.

reactions with the *Borrelia burgdorferi* antigen in the IFT. Farmers showed 11% of positive reactions and control group 6% (Fig. 2). The incidence of positive results in forestry workers was significantly greater compared to the control group (p<0.01).

Results of ELISA. In ELISA, positive results were found in 38.6% of forestry workers and in 28.1% of farmers. Patients from the neurologic clinic showed positive response in 12.2%, patients from dermatologic clinic in 27.1%, and control group in 6% (Fig. 3).

The frequency of positive ELISA reactions was significantly higher in forestry workers (p<0.001), farmers (p<0.001) and dermatologic clinic patients (p<0.01) compared to the control group. Results of ELISA in particular classes of antibodies are shown in Table 1. Positive results in IgG class were higher than in IgM class. This may suggest the presence of long-standing phase of borreliosis or signs of past infection.

Clinical case of occupational Lyme borreliosis. During this study, the diagnosis of Lyme borreliosis as an occupational disease was clinically confirmed in one forestry worker from Roztocze National Park in Zwierzyniec. In the years 1994-1996, anti-*Borrelia burgdorferi* antibodies had been detected in patient's serum by ELISA both in IgM and IgG classes. After treatment, a significant decrease of antibody level in both classes of immunoglobulins was found. The results are shown in Table 2.

DISCUSSION

The highest number of cases of L.B. is noted in USA, where the disease is found in all states. In Far East (Japan and China), Australia, South America and Western Africa the disease is diagnosed less frequently. In Europe, the disease was found in almost every country [11].

Table 1. Results by ELISA test in particular classes of antibodies.

Group	N	IgM positive	IgG positive	IgM and IgG positive
Forestry workers	44	0,2%	2,5%	1,1%
Farmers	217	0,4%	2,1%	0,3%
Neurology patients	362	0,5%	0,6%	0,1%
Dermatology patients	96	0,7%	1,7%	0,3%
Control group (blood donors)	50	0,2%	0,4%	0,0%

The first cases of L.B. in Poland were described by Januszkiewicz and Kieda in 1987 [15]. Next, Szechiński *et al.* described an endemic region of tick spirochetosis in the forest area of Piła district (northern Poland) [26]. The studies conducted in subsequent years evidenced that those most exposed in Poland are people living in the following endemic regions: Białowieża Forest, Białystok area, Mazury Lake District, Suwałki area (north-eastern Poland) and Karkonosze Mountains (south-western Poland) [11, 12]. Seroepidemiologic studies in the Białowieża Forest showed 49.7% positive results in inhabitants and 60-70% in forestry workers [12, 21, 24]. In the Karkonosze Mountains, 71% positive responses were found in forestry workers [9]. Our earlier studies in the years 1994-1996, performed in the Lublin region, showed 23.7% positive results in occupationally exposed people. This result does not seem to indicate the Lublin region as being an endemic region [7, 8].

Data from literature shows high numbers of seropositive results in groups at risk (people occupationally exposed to ticks, mainly forestry workers and farmers) [10]. In various European countries the frequency of antibodies anti-*Borrelia burgdorferi* in risk groups ranged from 19% in the Netherlands, 22% in Switzerland, 26% in Sweden, 34% in Bavaria, to 43% in Croatia. In general populations the values of positive results were lower and ranged from 2% in Sweden to 9.7% in Croatia [13]. In serological

Table 2. Antibodies to *Borrelia burgdorferi* in serum of a forestry worker from National Park in Zwierzyniec with clinically diagnosed borreliosis.

Initials	Year of birth	Date of examination	Results	
Z. M.	1968	94.10.10[1]	(-)	
		95.12.04[2]	IgM(+)	IgG (-)
		96.01.05[3]	IgM (+++)	IgG (-)
		96.01.26[3]	IgM (++++)	IgG (+)
		96.08.29[3]	IgM (+)	IgG (-)
		97.10.10[4]	IgM (±)	IgG (-)

[1] Biomerieux IFT; [2] Biomedica ELISA qualitative; [3] Biomedica ELISA semiquantitative (from 1 to 4 pluses); [4] Behring ELISA (IgG: quantitative - units per ml, IgM: qualitative)

study performed in Poland by Anusz *et al.* [2], among veterinary staff, forest workers, farm workers from state farms and hunters, antibodies anti-*B. burgdorferi* were found in 17.7% of sera in Suwałki district, 16.7% in Krosno district and 14.4% in Olsztyn district.

Results of our study suggest that L.D. in Poland may have an occupational character. The frequency of positive results found by us in an non-endemic region was higher than that reported by Anusz *et al.* [2] and close to that from Bavaria [20], thus confirming the view that Lyme borreliosis is often a work-related disease.

CONCLUSION

High percentage of anti-*Borrelia burgdorferi* antibodies found in people occupationally exposed to ticks may suggest that *Borrelia burgdorferi* is widespread in the forest environment of eastern Poland, and that infection often has a work-related character.

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