

## EPIDEMIOLOGICAL SURVEY OF HUMAN BORRELIOSIS DIAGNOSED IN EASTERN SLOVAKIA

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**Abstract:** 439 sera of patients from eastern Slovakia suspected of Lyme borreliosis were examined for anti-*Borrelia* IgG and IgM antibodies by ELISA (Ezygnost, Dade Behring, Germany). Out of the total number of 64 sera, e.g. in 14.6% found anti-*Borrelia* antibodies were. Among seropositive patients, 54.7% were women and 45.3% men. The highest incidence of the disease was diagnosed in the group of women aged 55–64 and men aged 45–54. Out of 29 positive sera of men, 55.2% had IgG antibodies, 27.6% IgM antibodies and 17.2% both types of antibodies. Out of 35 positive sera of women, 48.6% had IgG antibodies, 40.0% IgM antibodies and 11.4% both types of antibodies. *Erythema Chronicum Migrans* - ECM (31.3%) and arthritis (25.0%) prevailed among clinical symptoms, in contrast with only 7.8% of neurological cases. In men, arthritis most frequently occurred (27.6%), while in women erythema migrans forms (37.1%). Other manifestations of the disease appeared in 13 patients (20.3%), and 10 patients (15.6%) had no record of clinical manifestations. As shown by patients' records, 32.8% reported attachment of tick, 20.3% insect bites and 29.7% were not aware of being bitten by vectors. Seasonal dynamics of diagnosed cases reached three peaks with the highest numbers in February, May and October.

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## INTRODUCTION

Lyme borreliosis is a polysystemic disease of humans and animals. The disease is caused by recently discovered spirochete *Borrelia burgdorferi* [9]. There are still many unknown issues concerning borreliosis, such as a resistance of *Borrelia burgdorferi* to the immune system of vertebrates, and changes of surface antigens in the course of the disease and cultivation [4, 39, 44]. The importance of host reservoirs in the circulation and survival of the causative agent in the environment is also being studied [15, 17, 30,]. The disease diagnostics has proven troublesome, especially in cases with an asymptomatic course of the disease or frequent non-specific symptoms

[10, 24]. In such cases serological tests comprising indirect fluorescent-antibody assay (IFA), indirect enzyme-linked immunosorbent assay (ELISA), and Western immunoblot appear very useful [12, 18, 23, 38]. Heterogeneity of the agent of Lyme borreliosis, as well as differences in methods applied in the preparation of antigens and in tests procedures, cause significant discrepancies between the results of serological examinations obtained in different laboratories [1, 2, 3, 32, 43].

In Slovakia, first observations of human borreliosis were reported by Doutlík *et al.* [13]. In the following years the number of clinical cases considerably increased, and now Lyme borreliosis is a subject of broad interest in Slovakia.

In this study, we provide an analysis of human cases of borreliosis diagnosed in eastern Slovakia throughout the years 1999-2000.

## MATERIALS AND METHODS

439 sera of patients suspected of Lyme borreliosis were repeatedly examined by health care centres during 1999–2000. Commercial test ELISA (Enzygnost, Dade Behring, Germany) was used to examine the sera. Detergent extract of clinical isolate of *Borrelia afzelii*, strain Pko, was used as antigen. In order to determine IgG and IgM antibodies, tested and reference sera were prediluted by extract of *Treponema phagedenis* to eliminate non-specific reactions. To prevent interference of rheumatoid diseases in assessing IgM antibodies, the sera were also prediluted by rheumatoid factor (RF - sheep antibodies against human IgG). Intra-assay variability was modified by correction factor obtained from dividing values of the absorbance of nominal positive control stated in an enclosed table by values of the absorbance of positive control measured in the test. Cut-off value was assessed as an average value of negative control plus respective test-specific value for IgG (0.130) and IgM (0.280).

## RESULTS

Out of a total 439 patient sera suspected of Lyme borreliosis in 64 cases (e. g. 14.6%) anti-*Borrelia* antibodies were found. Among the 64 positive patients there were 35 (54.7%) women and 29 (45.3%) men. The highest incidence of the disease was diagnosed in the group of 55–64 women aged (15 cases) and 45–54 men aged (9 cases). The incidence of Lyme disease was much lower in younger age groups (Tab. 1). Out of 29 positive sera of men, 16 sera (55.2%) were positive with IgG antibodies, 8 sera (27.6%) with IgM antibodies and 5 sera (17.2%) with both types of antibodies. Out of 35 positive sera of women 48.6% had IgG antibodies, 40.0% IgM antibodies and 11.4% both types of antibodies (Tab. 2). *Erythema Chronicum Migrans* - ECM (31.3%) and arthritis (25.0%) prevailed among clinical symptoms, in contrast with only 7.8% of neurological cases. In men, arthritis most frequently occurred (27.6%) while in women erythema migrans forms (37.1%). Other manifestations of the disease appeared in 13 patients (20.3%), and 10 patients (15.6%) had no record of clinical manifestations (Tab. 3).

At all clinical diagnoses the IgG antibodies were more developed, except for ECM, where IgM antibodies prevailed (Tab. 4). Out of seven patients in whom the disease manifested as a combination of clinical signs, four patients contracted erythema migrans accompanied with arthritis, one patient suffered from erythema migrans and neurologic manifestations, one patient from arthritis and neurologic manifestations and one patient contracted arthritis and cardiac manifestations (Tab. 5). As noted in patients' records, 32.8% of them reported attachment of tick, 20.3% insect bite, and 29.7% were not aware of being bitten by vectors (Tab. 6).

**Table 1.** Seropositive patients with Lyme disease by age and gender.

Age/Gender	Men	Women	Total
1–4	1	0	1
5–9	0	1	1
10–14	4	0	4
15–24	1	2	3
25–34	3	2	5
35–44	5	6	11
45–54	9	8	17
55–64	2	15	17
> 64	4	1	5
Total	29	35	64

**Table 2.** IgG and/or IgM antibodies in patients with Lyme borreliosis.

Antibodies	Men		Women		Total	
	No	%	No	%	No	%
IgG	16	55.2	17	48.6	33	54.6
IgM	8	27.6	14	40.0	22	34.3
IgG/IgM	5	17.2	4	11.4	9	14.1

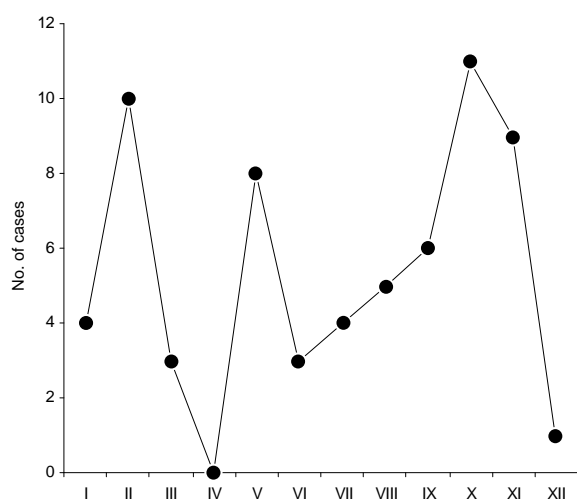
**Table 3.** Clinical symptoms in seropositive patients.

Symptoms	Men		Women		Total	
	No	%	No	%	No	%
ECM	7	24.1	13	37.1	20	31.3
Arthritis	8	27.6	8	22.9	16	25.0
Neuroborreliosis	2	6.9	3	8.6	5	7.8
Other symptoms	7	24.1	6	17.1	13	20.3
Not given	5	17.3	5	14.3	10	15.6

The seasonal dynamics of diagnosed cases reached three peaks with the highest numbers of cases in February, May and October (Fig. 1).

## DISCUSSION

*Borrelia burgdorferi* mostly occurs and retains in the areas with appropriate living conditions for ticks contrary to the geographically defined areas with foci of tick encephalitis virus [45]. The first surveys on ticks conducted in Slovakia presented the borreliae tick infestation on the entire territory [20, 21]. Furthermore, a high infestation of *I. ricinus* ticks was confirmed by studies of other authors [14, 34, 36, 37]. From the epidemiological reports of the Specialized State Health Institute in Banská Bystrica it appears that the number of patients with Lyme borreliosis has increased from 59 cases recorded in 1986 to 998 cases in 1998. When



**Figure 1.** Incidence of human borreliosis in eastern Slovakia by seasons.

analysing our set consisting of 64 cases with Lyme borreliosis we found that eastern Slovakia lies at the focus of this zoonosis. This has been proved also by 5–20% infestation of ticks with borreliae [20, 34]. The positive serological findings were also observed in forest workers (16.8%) and blood donors (5.4%) of this region. The incidence of infection among forest workers proved to be statistically greater than among blood donors [33]. The substantial seropositivity of domestic and farm animals confirmed the occurrence of borreliosis [40, 42].

The occurrence of the disease in relation to gender and age found in our set does not differ from data already published [5, 22]. The highest disease rate was recorded in humans ranging from productive age up to elderly (34–65 years old) which seems to be associated with activities of people in this age group in the environment inhabited by ticks infested with borreliae.

Genotype heterogeneity of the causative agent of Lyme borreliosis is connected with different manifestations of the disease and uneven distribution of the different genospecies associated with particular symptoms. It has been identified that *Borrelia burgdorferi* sensu stricto mainly causes arthritis while *B. garinii* seems to be associated with neurologic symptoms and *B. afzelii* with skin manifestations. In our study, prevailing affections were those of skin - ECM (31.3%) and arthritis (25.0%),

**Table 5.** Occurrence of combined forms of disease.

Symptoms	Arthritis % <sup>a</sup>	Neuroborreliosis % <sup>a</sup>	Cardiological % <sup>a</sup>
ECM	4 6.2	1 1.6	– –
Arthritis	– –	1 1.6	1 1.6
Total	4 6.2	2 3.2	1 1.6

<sup>a</sup> Percent of the total group of 64 patients.

**Table 6.** Distribution of patients by the found vector.

Vector	Men		Women		Total	
	No	%	No	%	No	%
Tick	10	15.6	11	17.2	21	32.8
Insect	6	9.4	7	10.9	13	20.3
None	8	12.5	11	17.2	19	29.7
Not given	5	7.8	6	9.4	11	17.2

while neurological symptoms occurred only in up to 8% patients. This manifestation of clinical symptoms is not fully in accordance with the incidence of genospecies occurring in particular foci in the Euro-Carpathian region and eastern Slovakia, where *B. garinii* and *B. afzelii* are dominant while *B. burgdorferi* sensu stricto occurs rarely [16, 19, 28]. Nevertheless, genospecies associated with skin manifestations might also be identified in patients with neurologic and eye manifestations [29]. According to the study of Italian authors the number of Italian patients affected with rheumatological symptoms of Lyme borreliosis is almost as high as in the USA [11].

Data on the type of vector reported by patients from our set correspond with those reported by the State Health Institute and presented by Bartůnek *et al.* [5] and Kontrošová [22]. As indicated by the data, a tick appeared to be the most common vector (33%), insect bite (mosquito, horsefly) being reported by 20% of patients and epidemiological history proved to be negative in 30% of patients. The patients who were not aware of contact with a tick vector might have been infected with larval stages of *Ixodes ricinus*, which are likely to be overlooked.

**Table 4.** Mean values of absorbance in seropositive patients by type of antibodies and diagnoses.

	ECM		Arthritis		Neuroborreliosis		Others		Not given	
	No	Mean of absorbance ± S.D.	No	Mean of absorbance ± S.D.	No	Mean of absorbance ± S.D.	No	Mean of absorbance ± S.D.	No	Mean of absorbance ± S.D.
IgG	6	0.490 ± 0.29	10	1.945 ± 0.77	2	0.983 ± 0.18	9	0.944 ± 0.92	6	0.898 ± 0.29
IgM	11	0.754 ± 0.20	2	0.439 ± 0.0056	2	0.741 ± 11	4	0.471 ± 0.11	3	0.480 ± 0.11
IgG/IgM	3	0.330 ± 10/701 ± 0.30	4	1.948 ± 0.56/0.589 ± 0.17	1	0.694 / 0.515	0	0	1	0.529 / 0.256

Some authors indicate a possible way of transmission by contact infection, and through milk of infected cows [7, 8, 35].

In terms of the seasonal dynamics, an increased incidence was recorded in February, which might relate to late manifestations of the disease. Subsequently, the incidence increased in May, followed by a decrease, and repeatedly and gradually increased from August culminating in October. The seasonal dynamics of the disease in humans is influenced by the activity of the ticks which culminates and reaches its maximum twice a year, e.g. in spring and less so in autumn [34], and is also associated with various clinical manifestations and an incubation period [5].

As mentioned previously, serological diagnostics plays an important role in diagnosing Lyme borreliosis although the diagnostic value of serological tests is still under discussion. The use of an appropriate antigen in testing, as well as the relatively variable response of the organism during different stages of the disease, are the most serious problems. In Slovakia, there is a need for using antigens prepared from borreliae that most frequently occur in the Euro-Carpathian region [26]. Some other comparative studies on humans [6, 24, 31] and on animals [25, 42] demonstrate better detection of antibodies when using geographically related strains, compared to geographically distant strains of *Borrelia burgdorferi* sensu lato.

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