ANALYSIS OF THE INCIDENCE OF LYME DISEASE IN THE DISTRICT OF ČADCA IN THE YEARS FROM 2016 TO 2019
ANALÝZA VÝSKYTU LYMSKEJ BORELIÓZY V OKRESE ČADCA V ROKOCH 2016-2019

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ABSTRACT

Background: In our study, we focused on *Borrelia burgdorferi sensu lato*, the causative agent of Lyme disease, which is a multisystemic disease; and on its clinical manifestations, symptoms and post-borreliosis symptoms.

Objectives: The objectives of our research were to evaluate the trend of the time-dynamics of the Lyme disease occurrence and its incidence according to the age, sex, and seasonality in the catchment area of the district of Čadca in the years from 2016 to 2019.

Method: Detection of IgG and IgM antibodies against *Borrelia burgdorferi* was determined by ELISA and subsequent confirmation of positive results by confirmatory Western blot.

Results: Out of the total number of 6 452 blood samples analysed, 24.9 % (n = 1 609) were positive and 71.1 % (n = 4 590) were negative. Women (58.2 %) were infected more often with Lyme disease than men (41.8 %). The most common incidence of the disease was in the categories 49 to 58 years of age. When evaluating the incidence of the disease according to seasonality, we scored the most cases of the diseases in the summer period.

Conclusion: The results of our study regarding to epidemiological factors point to the need for vaccine development and the associated preventive measures that would reduce the incidence of Lyme disease.

Key words: Lyme disease. Laboratory diagnostics. Multisystemic disease.

ABSTRAKT

Východiská: V našej štúdii sme sa venovali problematike zameranej na *Borrelia burgdorferi sensu lato*, pôvodcovi ochorenia lymská borelióza, ktorá patrí medzi multisystémové ochorenie, jej klinickým prejavom, symptómom a post-boreliovým príznakom.

Ciele: Cieľom štúdie bolo vyhodnotenie trendu vývoja lymskej boreliózy a jej výskytu v závislosti od veku, pohlavia a sezonality v spádovej oblasti okresu Čadca v rokoch 2016 – 2019.

Metódy: Dôkaz protilátok IgG a IgM proti *Borrelia burgdorferi* boli stanovené metódou ELISA a následné potvrdenie pozitívnych výsledkov konfirmačnou metódou Western blot.

Výsledky: Z celkového počtu 6 452 analyzovaných vzoriek krvi bolo 24,9 % (n = 1 609) pozitívnych a 71,1 % (n = 4590) negatívnych. Častejšie boli infikované lymskou boreliózou ženy (58,2 %) ako muži (41,8 %). Najčastejší výskyt ochorenia bol zaznamenaný vo vekovej kategórii 49 – 58 rokov. Pri vyhodnotení výskytu ochorenia podľa sezonality sme najviac ochorení zaznamenali v letnom období.

Záver: Výsledky našej práce vzhľadom na epidemiologické faktory poukazujú na potrebu vývoja očkovacej látky a s tým spojené preventívne opatrenia, ktoré by znižovali výskyt lymskej boreliózy.

Kľúčové slová: Lymská borelióza. Laboratórna diagnostika. Multisystémové ochorenie.

INTRODUCTION

Lyme disease, also known as Lyme borreliosis, has become the most common infection transmitted by ticks in many parts of Europe and the USA. The US Centers for Disease Control and Prevention estimate around 300 000 new cases of Lyme borreliosis per year in the US based on the results of two studies. A WHO report stated that about 85 000 cases were reported annually in Europe with wide variability across European countries, but they noted that many Lyme disease infections remained unrecognised due to inconsistent and incomplete methods used in ascertainment of Lyme disease. As for Western Europe, more recent estimates were about 232 000 cases of Lyme borreliosis per year. However, some countries included exclusively centralised reporting [1].

In Slovakia, the morbidity of Lyme disease was 5.00/100 000 in 1989, 19.08/100 000 in 2010 and 21.08/100 000 in 2016, thus indicating a rising trend of this disease [2].

Lyme borreliosis is a compulsorily notifiable disease in some countries. In countries without mandatory notification, qualified estimates are calculated based on epidemiological studies or incidence estimates from neighbouring comparable countries. Under- and over-reporting, as well as differences in case definitions, diagnostic difficulties, and different laboratory methods, are recognised issues for Lyme borreliosis diagnostics and surveillance [3, 4].

Current global climate changes have expanded the range of tick vectors, suggesting that Lyme borreliosis will remain an important epidemiologic issue in the forthcoming decades. It is possible to only

approximate estimates of the Lyme borreliosis incidence in Europe because only few European countries record Lyme borreliosis as a compulsorily reported disease [5].

Further, the incidence rates of Lyme borreliosis across Europe are influenced by geographical, environmental and climatic factors. Additionally, human behaviour, including free-time and hobby activities, can play a role in Lyme borreliosis seasonality. Geographical expansion of the distribution of Lyme borreliosis cases has been observed across the whole European continent [6].

Lyme disease is caused by spirochaetes of the *Borrelia burgdorferi* sensu lato species complex, which are transmitted by *Ixodes ricinus* ticks. Various pathogenic species are responsible for the Lyme disease at different regions in the world. For example, in North America, the species of Lyme borrelia known to cause human disease is *Borrelia burgdorferi* sensu stricto while in Europe, at least five species of Lyme borrelia (*Borrelia afzelii*, *Borrelia garinii*, *Borrelia burgdorferi*, *Borrelia spielmanii*, and *Borrelia bavariensis*) can cause the disease, leading to a wider variety of possible clinical manifestations in Europe than in North America [7].

Various *Ixodes* subspecies can serve as vectors for the Lyme disease transmission: The main vector of Lyme disease in Europe is *Ixodes ricinus*, whereas *Ixodes persulcatus* is the main vector in Asia. *Ixodes scapularis* is the main vector in northeastern and upper midwestern USA and *Ixodes pacificus* serves as the main vector in western USA [8].

Erythema migrans, an expanding skin rash that occurs around the site of the tick bite, is the most common symptom of early Lyme borreliosis. It manifests several days up to weeks after the tick bite and can be accompanied by influenza-like symptoms such as fever, headache, mild stiff neck, arthralgia, and myalgia. When untreated, dissemination of the bacteria to other tissues can occur and lead to more severe manifestations that include several skin, neurologic, cardiac, musculoskeletal and ocular manifestations [9].

Most patients with Lyme disease are cured by a 3–4 weeks' course of antibiotics. However, a smalller fraction of them (approximately 10 %) have prolonged somatic and neurocognitive symptoms, such as fatigue, difficulty in sleeping, arthralgia, myalgia, memory impairment, and headache. This condition is called post-Lyme disease syndrome (PLDS) or post-treatment Lyme disease syndrome (PTLDS) [10].

Interestingly, the appearance of post-Lyme disease symptoms seems to correlate with disseminated disease, greater severity of illness and delayed antibiotic treatment. They do not correlate with the duration of the initial antibiotic treatment [11].

Lengthy courses of antibiotics are not recommended in patients with PLDS because of the lack of benefit, and they are fraught with hazards. Most patients with PLDS recover from persistent symptoms with time. However, it can take months before they feel completely well. Based on the above facts, it is clear, that developing preventive strategies against Lyme borreliosis is important for reducing its negative impact on people's health and countries' economies [10].

OBJECTIVES

In our study in the population of the catchment area of Čadca in the years 2016-2019, we had the following objectives:

- monitor the incidence of Lyme disease by sex and age categories,
- analyse the incidence of Lyme disease according to seasonality,
- evaluate the development trend of Lyme disease.

MATERIAL AND METHODS

All samples for confirmation of IgG and IgM antibodies against *Borrelia burgdorferi* were examined by ELISA method. A positive IgM antibody result indicated evidence of ongoing infection and a positive result for IgG antibodies suggests for past infection. In the case of a negative ELISA result, no further testing of the patient was required to assess that *Borrelia burgdorferi* presence.

In the case of a positive result in a patient, positivity was subsequently confirmed by Western blot, if this test was negative, the infection was considered not presented in the patient.

False positive ELISA results may be due to cross-reactivity with antibodies to viral infections (e.g. infectious mononucleosis) or to *Treponema pallidum*. Laboratory diagnostics can also be influenced by ongoing immunopathological diseases such as rheumatoid arthritis or systemic lupus erythematosus. False-negative test results may be present in the early stages of the disease, when anti-

bodies have not yet formed, or when antibiotics are used early.

For IgG-antibodies detection was used antigen combination test kit *Borrelia burgdorferi sensu lato*: VlsE, p83, internal flagellin – p41i, p39, OspC and p17; detection of IgM antibodies was performed with the use of the antigen combination of OspC, internal flagellin -p41i and p39. The results were evaluated by means of an instrument Vidas [13, 14].

Western blot was performed using a commercial kit on test strips on which antigens are coated. These were subsequently detected using antibodies against human IgG or IgM, which were labelled with the enzyme and elicited a colour reaction, which was evaluated by the programme Immunoblot Software [15].

RESULTS

During the monitored period of 2016 to 2019, a total of 6,452 blood samples were examined from patients from the catchment area of the Čadca district. The samples were examined at the Department of Microbiology at the Hospital and healthcare centre in Čadca for the presence of *Borrelia* antibodies. Out of the total of 6 452 blood samples, 4 590 samples were tested by ELISA and 1 862 by Western blot (table 1).

The number of samples examined by Western blot for the years 2016 – 2019 was a total of 1 862 (table 1). The largest number of examined samples was in 2018, namely 557, and the least examined samples were identical in 2017 and 2019 (n = 434).

The following graphs 1 – 8 provide a general evaluation of the incidence of Lyme disease in relation to the interpretation of the results of confirmed antibodies IgG and IgM by Western blot confirmation.

In Graph 1, we evaluated the incidence of Lyme disease in women for the period under review. The highest incidence of Lyme disease up to 31.7 % (n = 297 cases) was in 2017 and, conversely, the lowest 19.3 % in 2016 (n = 181).

Graph 2 shows the incidence of Lyme disease in men, where, as in women, we recorded the highest incidence in 2017 at 33.3 %, which represented 224 cases and the least diseases in 2016 (n = 133, 19.8 %).

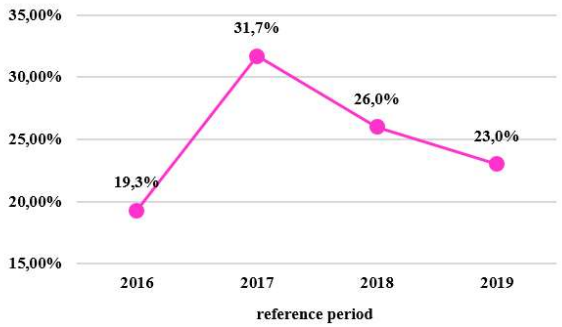
In the overall comparison, we noted that women (58.2 %) were more often infected with Lyme disease than men (41.8 %).

In graphs 3 – 6, we evaluated the incidence of Lyme disease in patients according to age categories for the observed years 2016 – 2019. The most positive samples for Lyme disease in 2016 (Graph 3) were in patients aged 49-58 years (23.9 %). The fewest positive samples were between 79 – 88 years of age (1.6 %).

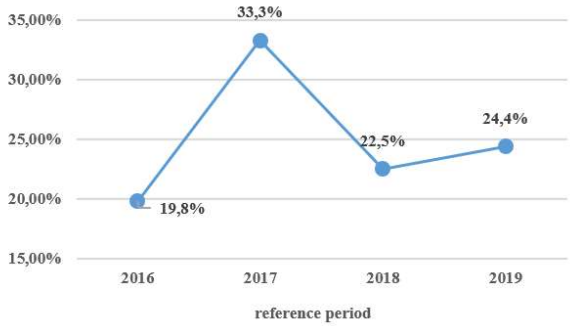
Graph 4 shows the year 2017, where we recorded a relatively equal incidence of the disease in age categories 49 to 58 years and 59 to 68 years (23.1 % and 22.6 %). The lowest incidence of the disease was 3.8 % in patients in the highest age category, but compared to the previous year, the increase in the disease in this age group was more than 2-times.

Table 1 The total number of samples tested for antibodies against *Borrelia burgdorferi* by the methods ELISA and Western blot

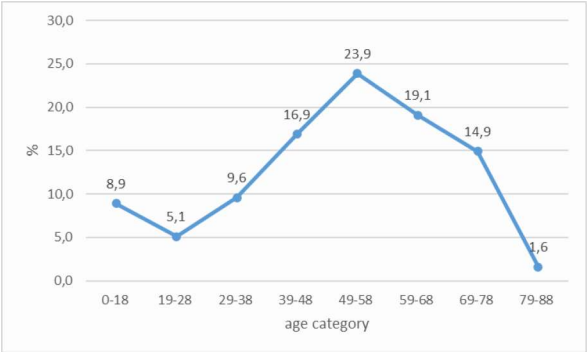
Period	Number of examined samples	
	ELISA	Western blot
2016	1077	437
2017	1079	434
2018	1253	557
2019	1181	434
Total	**4590**	**1862**



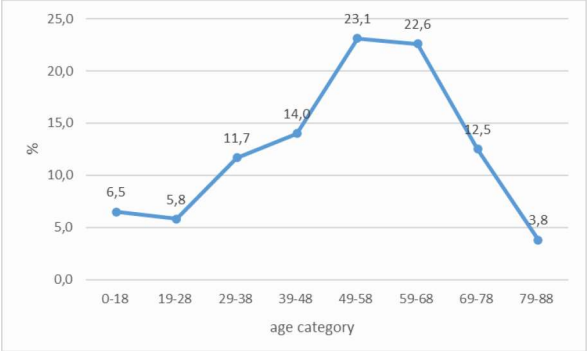
Graph 1 Evaluation of Lyme disease in women in the years 2016-2019



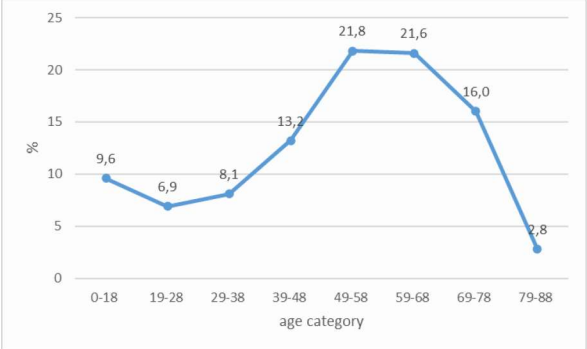
Graph 2 Evaluation of Lyme disease in men in the years 2016-2019



Graph 3 Evaluation of Lyme disease according to age categories in 2016



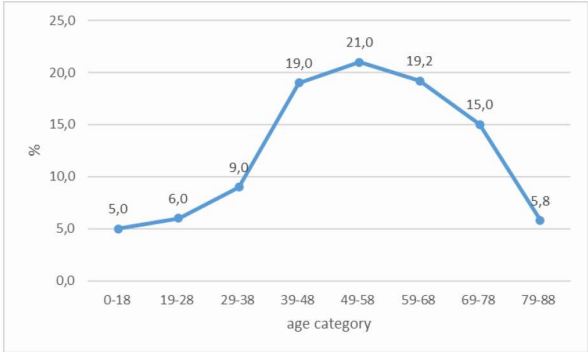
Graph 4 Evaluation of Lyme disease by age categories in 2017



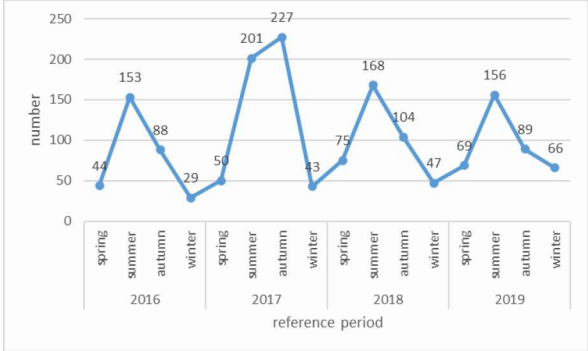
Graph 5 Evaluation of Lyme borreliosis by age categories in 2018

The highest incidence of borreliosis in 2018 (Graph 5) was in the same age categories as the previous observed year 2017, namely 49 – 58 years and 59-68 years (21.8 % and 21.6 %).

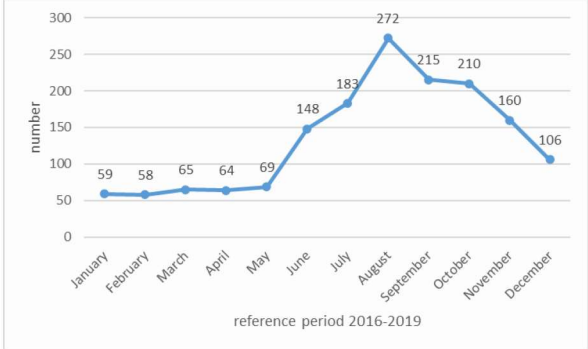
In 2019, in addition to the age categories 49 –58 years (21.0 %) and 59-68 years (19.2 %), we recorded a higher incidence of borreliosis also in the category 39 – 48 years (19 %). Compared to 2016 – 2018, the incidence of diseases in the highest age category increased again to 5.8 % (Graph 6).



Graph 6 Evaluation of Lyme borreliosis by age categories in 2019



Graph 7 Occurrence of Lyme disease by seasonality 2016-2019



Graph 8 Trend of Lyme borreliosis development during the observed period 2016-2019

In our study, we focused on evaluating the incidence of positive samples for Lyme disease according to seasonality in 2016 –2019 based on the evaluation of positive IgG and IgM antibodies.

In Graph 7 we can see that the highest number of patients diagnosed with Lyme disease was observed in the summer of 2016 (n = 153), 2018 (n = 168) and 2019 (n = 156), except for 2017, when the highest incidence of the disease was in autumn (n = 227).

In connection with the evaluation of IgG and IgM antibodies, we focused on the development

trend of Lyme disease. We monitored its development according to individual months in the years 2016 – 2019. In Graph 8 we can see that the numbers of positive samples from January to May were linear, which began to change to ascending from May. The rising trend stopped in August with 272 diseases. From September to December, we observed a declining trend in the disease.

DISCUSSION

In the Slovak Republic, ticks are among the most important species of blood-sucking parasites that can transmit serious pathogens. Ticks transmit several diseases, including Lyme disease, which we have been working on. Recently, a slight increase in this disease has been observed in Slovakia, and this trend is also supported by our results. The increasing incidence of Lyme disease points to the importance and need for the population to realize that prevention of it is very important [15].

For each positive ELISA test, it is recommended to perform IgG and IgM confirmation by Western blot just to exclude cross-reactivity. It is also recommended to perform a negative ELISA test but present clinical signs in the patient. This implies the necessary communication between the laboratory and the clinical workplace. Our study confirmed that advantage of Western blotting is the ability to detect and analyse the antibody response against individual Borrelia burgdorferi antigenic proteins. Trajevo et al. they state in its study that laboratories using a combination of ELISA and Western blot methods have a combined sensitivity and specificity of an average of 85 % to 95 %. The antigens that are responsible for the onset and course of the disease are among the proteins found on the membrane. These are antigens responsible to produce early (IgM) or later (IgG) antibodies diagnosed by laboratory methods [14].

Manufacturers of test kits state that due to the reactivity of the organism to Borrelia burgdorferi it is necessary to consider the slow production of antibodies in the early stage of the disease, the possibility of influencing antibody production after previous application of antibiotics, atypical dynamics of antibody response, possibility of cross-reactions. In individuals with other spirochetal bacteria and non-pathogenic Borrelia. False positive findings may also be reported in pregnant women. It is also appropriate to allow for a possible laboratory error [14]. In one of our goals, we focused on the incidence of Lyme disease according to age categories. From the obtained results, we found that the number of diseases is increasing in the age category from 39 to 48 years. The most positive patients were in the age group 49 – 58 years. 361 people in this age group contracted Lyme disease. Ružek et al. states that this increased condition may be due to an aging population as well as a change in the health status of this age group [16]. Also, in his study carried out in the Czech Republic and Austria, Daneš noted that the increase in Lyme disease was in older age groups. He also justifies this by the fact that older people do not have time to find and remove ticks in time for their motor and sensory possibilities. This increase may also be related to the activity of this age group, as a large proportion of people of this age spend more time in nature [17].

In terms of monitoring the disease of Lyme disease depending on the season, the highest incidence was recorded in the seasons of summer and autumn. In addition to these seasons of Lyme disease, cases of the disease in the winter were also rarely registered. Bartunek et al. state that the incidence of Lyme disease also depends on the season, which affects tick activity and human behaviour in nature (cottages, hiking, summer sports, picking mushrooms and berries) and thus leads to more frequent contact with ticks [18].

In our results, the general statistical analyses of the trend of Lyme borreliosis presented by the author were partially confirmed. The high incidence, severity of the disease, non-existent vaccine, different clinical picture, complicated diagnostics, and treatment make Lyme disease a disease that is still the subject of intensive research.

CONCLUSION

In our study, we analysed 6,452 samples taken from both men and women, in the age category from 0 to 88 years. We confirmed 1,609 positive results for Lyme disease by ELISA, which represents 35.1 % of the total number of 4,590 samples analysed by the method. We confirmed 561 positive results for Lyme borreliosis by the confirmatory Western blot method, which represents 30.1 % of the total number of 1,862 samples examined by this method. When evaluating the total 4-year period, we recorded the fluctuating character of positive samples for Lyme disease with the lowest detection of the disease in 2016 (n = 314, 29.2 %), and conversely the highest detection in 2017 (n = 521, 48 %).

We found that women (58.2 %) were more often infected with Lyme disease than men (41.8 %). According to seasonality, the most positive results were in the summer. The most affected group were the age categories 49 to 58 years.

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