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Clinical Syndromes and Epidemiological Factors of Lyme Disease in Lithuania

SUMMARY OF DOCTORAL DISSERTATION

Medicine and Health Sciences,
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This dissertation was written from 2016 to 2021 at the Clinic of Rheumatology, Orthopaedics Traumatology, and Reconstructive Surgery.

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VILNIAUS UNIVERSITETAS

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Laimo ligos klinikiniai sindromai ir epidemiologiniai rodikliai Lietuvoje

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THE LIST OF ABBREVIATIONS

AAN - American Academy of Neurology

ACR – American College of Rheumatology

ACA - Acrodermatitis chronica atrophicans

AV - atrioventricular block

B. burgdorferi - Borrelia burgdorferi

B. afzelii - Borellia afzelii

B. burgdorferi sl - Borrelia burgdorferi sensu lato

B. burgdorferi ss - Borrelia burgdorferi sensu strictu

B. garinii - Borrelia garinii

BL - borrelial lymphocytoma

CRP - C reactive protein

ECG - electrocardiogram

EM - erythema migrans

ESR - erythrocyte sedimentation rate

ESCMID - European Society of Clinical Microbiology and Infectious Diseases

I. hexagonus - Ixodes hexagonus

I. ricinus - Ixodes ricinus

I. uriae - Ixodes uriae

IDSA - Infectious Diseases Society of America

USA - United States of America

LA - Lyme arthritis

LB - Lyme borreliosis

LDe - Lyme dermatitis

LC - Lyme carditis

LD - Lyme disease

LP - lumbar puncture

MMPs - metalloproteinases

n. facialis - facial nerve

NB - Neuroborelliosis

NICE - National Institute for Health and Care Excellence

SD - standard deviation

CCDA - Center for Communicable Diseases and AIDS

BCVR - Bioethics Committee for Vilnius Region

HVU SC - Hospital of Vilnius University Santara Clinic

CID HVU SC - Center for Infectious Diseases of the Hospital of Vilnius University Santara Clinics

WB – Western blot

WBC - leukocytes

CONTENTS

1. INTRODUCTION

1.1. Research problem and relevance of the dissertation

Lyme disease (LD), also known as Lyme borreliosis (LB), is the most common tick-borne infectious disease in Europe. The pathogen is the gram-negative bacterium spirocheta *Borrelia burgdorferi (B. burgdorferi)*. More than 20 genotypes of this bacterium are isolated, four of which are pathogenic to humans and result in the development of LL: B. burgdorferi sensu strictu (*B. burgdorferi ss*), *B. afzelii*, *B. garinii*, *B. bavariensis*. (1) Each of these genotypes is associated with different clinical manifestations of Lyme disease. *B. burgdorferi ss* is associated with joint damage, *B. afzelii* is associated with neurological changes, *B. garinii* is associated with dermatological damage. The global prevalence of these *B. burgdorferi* genotypes varies, *B. burgdorferii ss* is found in North America, but can also be found in Europe, and *B. afzelii* and *B. garinii* are prevalent in Europe and Asia. (2)

Lyme disease has been described in historical sources in essence since the second half of the 20th century. In 1976, an epidemic of arthritis in children was described in Lyme, Connecticut, then it was observed that the onset of the disease was recorded in late summer to early autumn, and before that patients had a rash of erythema migrans. (3) Erythema migrans was mentioned in Europe as early as the early 20th century and in America in the 1970. (4) A significant break in the understanding of the disease occurred in 1981, when Burgdorfer reported the detection of spirochetes in the ticks *Ixodes scapularis* and *Ixodes ricinus*. (5) Although 40 years have passed since the isolation of the pathogen, the incidence of Lyme disease still remains high.

About 85,000 cases of LD are reported in Europe each year, but this is not an accurate enough reflection of the situation for a variety of reasons. Based on data from different sources, it is likely that there should be more than 200 000 cases each year, most of which affect people in the age groups of 5-15 and 45-65 years of age.

According to the Center for Communicable Diseases and AIDS official registration of LD in Lithuania started in 1991. 51 528 cases of LD were registered from 1991 to 2019. The highest incidence in the last ten years was recorded in 2003. (106 cases per 100 thousand of residents), 2009 (107.5 cases per 100 thousand of residents), 2016 (101.6 cases per 100 thousand of residents), and in 2019 (117.8 cases per 100 thousand of residents) It is believed that the incidence of LD recorded in 1991-1994 was relatively low (1.6–18.1 cases per 100 thousand of residents) due to insufficient diagnosing of the "new" disease at that time.

It is likely that the onset of an increase in the incidence of LD since 2000 is associated with improved diagnosis of the disease, climatic conditions conducive to an increase in tick abundance and the enlargement of the tick habitat. The high level of LD morbidity is also related to socio-economic conditions - a large number of unemployed, increasing areas of uncultivated land, felled and unmanaged forests.

The most common sign of the disease is a rash of erythema migrans (EM). The rash usually manifests as a small erythematous papule or macula at the site of tick bite, within 1-2 weeks after tick bite (time may vary from 3 to 32 days), the rash gradually increases, its center fading. Erythema migrans can be asymptomatic, itch may be irritating, sometimes painful when left untreated, may even grow to 61 cm in diameter, and remain for about 3-4 weeks before disappearance. (6)

About 80 percent of patients develop a local form of Lyme disease and only a rash of erythema migrans is observed, but the bacterium can spread hematogenically to both the skin and other systems. Early-spread infection can occur with multiple EM rashes. Widespread disease, regardless of whether an EM rash has been observed, may present with neurological damage such as cranial neuropathy (especially facial nerve) and meningitis, as well as cardiac damage such as carditis, mainly atrioventricular block (AV), and rheumatological damage - inflammation of the joint (usually the knee joint).

The main natural reservoirs of the pathogen *B. burgdorferi*, are mice, chipmunks, other small mammals and birds. Deer are not true reservoirs, but they are important in maintaining the life cycle of vectors - ticks. Ticks feed once during each of their life cycles (larvae, nymphs, and adults). During feeding, Borrelia are transferred from the infected animal and can be passed on to humans during bite. This transmission of the pathogen to humans is most likely when the tick is in the nymph stage, in spring and summer, due to the small size of a tick at that time, the bite of a tick goes unnoticed. Accordingly, an increased incidence of erythema migrans was observed during the same period. Risk factors include both leisure and work activities in the fields and forests in endemic areas, as well as outdoor activities in residential yards near forests.

Although Lyme disease is the most common infectious disease in Europe, the incidence continues to rise, with new outbreaks appearing in non-endemic areas. This is due to many factors, such as a warming climate, milder winters, which results in a longer period of tick activity. Therefore, it is expected that a large number of new cases will remain in the future, and even likely to increase. The increasing incidence of the disease can also be explained by an improved understanding of the disease both among physicians and among patients. As the number of cases increases, it is very important to be able to recognize this disease, which, due to the multifaceted clinical manifestations of the disease, is faced by doctors of several specialties. The diagnosis of borreliosis remains problematic, as only non-standardized molecular methods currently exist, making it difficult to grow the pathogen. Laboratory diagnostics is based on serological methods, their interpretation is complicated, in endemic areas 30 percent of subjects have positive results without clinical manifestations of the disease, so it is very important to know the clinical manifestation of the disease very well. (7–9) For these reasons, there remains the possibility of hyperdiagnosis, unnecessary use of antibiotics, which promotes the development of antibiotic resistance. With timely diagnosis and initiation of antibiotic treatment, the disease

is completely cured, if there is a delay, residual effects are possible. It is important to note that the duration of treatment varies depending on the clinical syndrome and stage of the disease.

1.2. The Aim of the Research

1. To identify the epidemiological factors of Lyme disease in Lithuania.
2. To identify the clinical syndromes of Lyme disease that manifested in Lithuania and to distinguish their main characteristics.

1.3. Objectives of the Research

1. To investigate the epidemiological data on diagnosed cases of Lyme disease of the Center for Communicable Diseases and AIDS for the period from 2014 to 2016.
2. To collect and analyze clinical data of patients, diagnosed with Lyme disease in the outpatient department at the Center for Infectious Diseases of the Hospital of Vilnius University Santara Clinics (HVU SC), of 2014-2016.
3. To collect and analyze clinical data of persons, hospitalized in Center for Infectious Diseases of HVU SC hospitalized for Lyme disease, of 2014-2019.

1.4. Novelty of the Research

The data analyzed in the study cover the period from 2014 to 2019. Data on Lyme disease in Lithuania for this period are not available in international databases. This doctoral dissertation provides a detailed overview of Lyme disease factors in Lithuania, includes data on epidemiological disease factors and clinical manifestations of the disease.

1.5. Practical Significance of the Research

Since the manifestations of the disease are diverse, this disease is faced by doctors of many specialties. Therefore, it is very important to know how the disease manifests itself in our country, to single out individuals of the risk group.

Knowing the main epidemiological factors of the disease and the predominant clinical syndromes of the disease, facilitates:

1. Diagnosis of Lyme disease, which leads to targeted and timely antibiotic therapy, which can be expected to reduce the number of cases of late disease or residual effects of the disease.
2. It is also easier to identify people of the risk group and educate them.

2. METHODOLOGY

The study was performed at Hospital of Vilnius University Santara Clinics (HVU SC). The permission of the Bioethics Committee for Vilnius Region (BCVR) (No. 158200-17-900-420) and the permission of the State Data Protection Inspectorate were obtained for the conduction of biomedical research.

A retrospective-cohort study was performed (data collection period 2014–2019) and consisted of three separate, independent study groups. Due to the retrospective analysis and the sending of personal information and consent forms to the patients by mail, the achieved responsiveness was low and all responding patients agreed to have their data analyzed during the study.

Patient data collected and included in the study were encrypted (each patient was assigned a unique identification code) and stored in a database created specifically for the study. Physical, clinical, laboratory, instrumental, and treatment data were collected.

Data were collected from the Center for Communicable Diseases and AIDS (CCDA) and from the Center for Infectious Diseases of the

Hospital of Vilnius University Santara Clinics (CID HVU SC). Center for Infectious Diseases of HVU SC is a reference center for infectious diseases in adult patients and serves for population of 809 000, which is 27% of the total population of Lithuania.

2.1. The First Group of Subjects

The study included all patients diagnosed with Lyme disease in 2014-2016 and reported to the Center for Communicable Diseases and AIDS (CCDA). Data obtained from CCDA. Based on the data of this research group, epidemiological indicators of the disease, which are important to know in order to identify persons of the risk group, have been analyzed and evaluated, and epidemiological factors often become a differential diagnostic factor of the disease.

Patient demographics (age, gender, place of residence, social status), epidemiological data (location of infection, time of tick bite, time of onset), clinical data of the disease (symptoms), laboratory parameters (serological markers of Lyme disease in the blood) were evaluated.

2.2. The Second Group of Subjects

The study included all adults for whom a diagnosis of Lyme disease had been confirmed during an outpatient visit at the Center for Infectious Diseases of the Hospital of Vilnius University Santara Clinics (CID HVU SC) from 2014 to 2016.

Cases of Lyme disease were selected on the basis of outpatient card data, clinical characteristics, laboratory parameters, electrocardiogram (ECG), and skin biopsy results were evaluated. All confirmed cases of Lyme disease are classified by clinical syndromes according to the following factors: Erythema migrans (EM), Neuroborelliosis (NB), Lyme arthritis (LA), Lyme carditis (LC), and Lyme dermatitis (LDe).

The factors used to diagnose the clinical syndromes were as follows: In the EM group - rash of erythema migrans; In the NB group - neuropathological signs of the central nervous system (facial muscle paralysis, radiculitis) and tick bite and/or erythema migrans rash were observed; In the LA group - a swollen joint and tick bite and / or erythema migrans rash have been observed; In the LC group - changes in the ECG (atrioventricular block) and tick bite and/or erythema migrans rash were observed; LD confirmed through skin biopsy.

Laboratory enzyme-linked immunosorbent assay (ELISA) for Lyme immunoglobulins M and immunoglobulins G was evaluated.

2.3. The Third Group of Subjects

The study included all adults who had been confirmed with a diagnosis of Lyme disease during hospitalization at the Center for Infectious Diseases of the Hospital of Vilnius University Santara Clinics from 2014 to 2019.

Data from patients consulted in the outpatient department do not reflect one of the clinical syndromes of Lyme disease, i.e. Neuroborelliosis. To confirm this LD syndrome, it is necessary to perform a spinal fluid puncture and test the fluid. Therefore, it was necessary to include in the study the data of individuals hospitalized due to LD. The data collection period of this group is longer, because the evaluation of the data from the period of 2014-2016 revealed that the cerebrospinal fluid test in the second quarter of 2016 became more informative, the calculation of the immunoglobulin index was commenced, therefore a larger sample was needed to obtain more accurate indicators.

Cases of Lyme disease were selected on the basis of medical history data, clinical characteristics, laboratory parameters, and ECG were evaluated. All confirmed cases of Lyme disease are classified by clinical syndromes according to the following factors: Erythema

migrans (EM), Neuroborelliosis (NB), Lyme arthritis (LA), and Lyme carditis (LK).

The factors used to diagnose the clinical syndromes were as follows: In the EM group - rash of erythema migrans; In the NB group - neuropathological signs of the central nervous system and indicators of spinal tap fluid; In the LA group - a swollen joint and tick bite and / or erythema migrans rash have been observed; In the LC group - changes in the ECG (atrioventricular block) and tick bite and/or erythema migrans rash were observed.

Laboratory enzyme-linked immunosorbent assay (ELISA) for Lyme immunoglobulins M and immunoglobulins G was evaluated. Examination of cerebrospinal fluid - cytosis, lymphocyte percentage, neutrophil percentage, protein content, albumin content, IgG/albumin ratio, glucose, Lyme IgM and their index, Lyme IgG and their index.

2.4. Statistical Analysis

A retrospective-cohort study was performed. In the descriptive part of the statistical analysis, the following numerical characteristics were used to describe the categorical variables: minimum and maximum values, mean with standard deviation or median with interquartile difference. Quantitative variables distributed according to the normal law are presented as the mean with the indication of the standard deviations, not distributed according to the normal law as the mean with the indication of the minimum and maximum values.

Student’s t and Chi-square tests were used to retrospectively compare and evaluate two independent sets of quantitative and qualitative data. A P value less than 0.05 was considered statistically significant. Statistical analysis was performed using R Commander (Rcmdr) software (version 4.0.0).

2.5. Limitations of the Research

According to the clinical symptoms among the patients diagnosed with Lyme disease in the outpatient department, some of them were classified as having Neuroborelliosis Clinical Syndrome, but none of them underwent lumbar puncture (LP) and the diagnosis was not confirmed, although these patients were classified in the results as having Neuroborelliosis, but in the final conclusions this could not be stated and this possibly results in a lower incidence of this syndrome among the subjects studied.

A retrospective study was performed, therefore, the practical-clinical records of physicians, which are not adapted for the collection of scientific information, were evaluated. An analysis of 3-year data was also chosen, which at first glance may appear to be too short a period of time, but the sample of data collected is sufficient to meet the set research objectives.

There was an intention to link LD clinical syndromes to the geographical area of infection and the infestation of ticks that are prevalent there with a particular genotype of Borrelia, but during the study it became apparent that a large portion of patients did not notice the tick bite or could not indicate the location of infection, and the tick studies published so far do not provide data on different Borrelia genotypes according to geographical area, only data on tick infestation with different Borrelia genotypes depending on the predominant vegetation.

3. RESULTS

3.1. The First Group of Subjects. Epidemiological Data of the LD

7424 cases of Lyme disease were recorded at the Center for Communicable Diseases and AIDS from 2014 to 2016. The larger portion of patients was women - 4633 cases (62.4%), the smaller

portion was men - 2791 (37.6%). . At the time of diagnosis more than half of the individuals were older than 51 years of age (56.2%). The age of women was statistically significantly older than that of men, the mean age of women was 52.0 (± 36.15), and that of men was 44.3 (± 36.98) (p <0.001). 5368 (72.3%) persons indicated their place of residence as urban and 2056 (27.7%) as rural. However, in 2016 (in the last year of the study) the number of people living in rural areas increased statistically significantly and reached 857 (29.4%), (p = 0.031) (Table 1).

Table 1. Demographics of people with Lyme disease in 2014-2016 in Lithuania.

Demographics	**Calendar year**			**Total**
	2014	**2015**	**2016**	
	n (%)	**n (%)**	**n (%)**	**n (%)**
Total	2257 (30.4)	2252 (30.3)	2915 (39.3)	7424 (100.0)
Age, years				
Average (±SD)	48.5 (19.61)	48.9 (19.57)	49.8 (19.23)	49.1 (19.45)
Interval	1-90	1-90	0-91	0-91
0-10	112 (5.0)	116 (5.2)	124 (4.3)	352 (4.7)
11-20	118 (5.2)	127 (5.6)	154 (5.3)	399 (5.4)
21-30	225 (10.0)	183 (8.1)	232 (8.0)	640 (8.6)
31-40	248 (11.0)	267 (11.9)	353 (12.1)	868 (11.7)
41-50	374 (16.6)	357 (15.9)	461 (15.8)	1192 (16.1)
51-60	479 (21.2)	502 (22.3)	657 (22.5)	1638 (22.1)
61-70	404 (17.9)	411 (18.3)	520 (17.8)	1335 (18.0)
71-80	249 (11.0)	250 (11.1)	352 (12.1)	851 (11.5)
≥81	48 (2.1)	39 (1.7)	62 (2.1)	149 (2.0)

Demographics	Calendar year			Total
	2014 n (%)	2015 n (%)	2016 n (%)	n (%)
Gender				
Female	1430 (63.4)	1427 (63.4)	1776 (60.9)	4633 (62.4)
Male	827 (36.6)	825 (36.6)	1139 (39.1)	2791 (37.6)
Place of residence				
Village	600 (26.6)	599 (26.6)	857 (29.4)	2056 (27.7)
City	1657 (73.4)	1653 (73.4)	2058 (70.6)	5368 (72.3)

SD: standard deviation.

Assessing the prevalence of the disease in social groups, it was observed that 41.77% of persons did not have any employment relationship at the time of diagnosis (unemployed, retired, disabled, on parental leave), and 38.81% had direct employment, including attending educational institutions and university studies. (Figure 1) In 19.42% of cases, the social status of individuals was unknown.

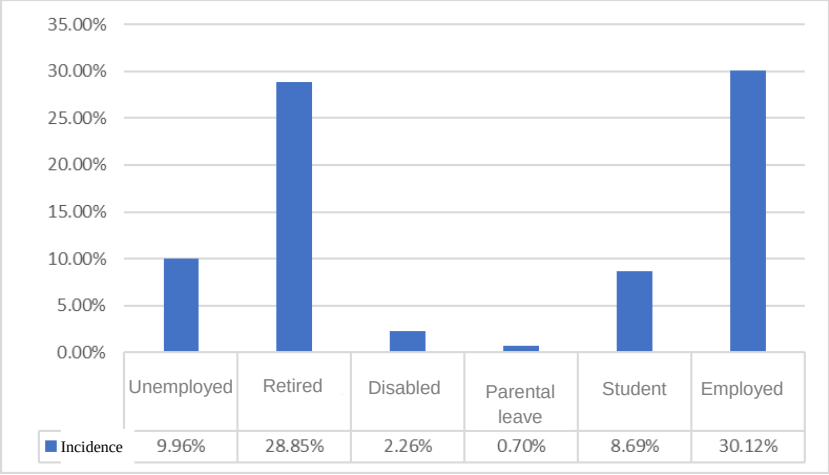

Figure 1. LD incidence by social groups in 2014 - 2016 in Lithuania.

In 2014 - 2016 a total of 7424 cases of LD were identified, in 2014 - 2257, in 2015 - 2252, and in 2016 - 2915 cases. The average estimated incidence of Lyme disease was 85.5 cases per 100 000 of residents in 2014 - 2016, the highest incidence was recorded in 2016 - 101.6 per 100 000 of residents. (Table 2.)

Table 2. Morbidity of Lyme disease in 2014-2016 in Lithuania.

	Number of cases (n)	Indicator per 100 000 of residents
2014	2257	77.6
2015	2252	77.5
2016	2915	101.6
Total	7424	85.5

Highest morbidity rates in 2014 - 2016 were registered in Kaunas, Vilnius, and Panevėžys counties, and the lowest in Alytus county (Table 3, Figure 2).

Table 3. Geographical distribution of Lyme disease incidence in Lithuania in 2014 - 2016.

County	**Number of cases (n)**	**Indicator per 100 000 of residents**	**Percentage (%)**
Alytus	143	32.7	1.9
Kaunas	1864	107.3	24.9
Klaipėda	353	36.1	4.8

County	Number of cases (n)	Indicator per 100 000 of residents	Percentage (%)
Marijampolė	187	41.6	2.4
Panevėžys	733	105.3	9.8
Šiauliai	620	74.2	8.4
Tauragė	244	80.0	3.2
Telšiai	345	80.9	4.6
Utena	386	93.0	5.2
Vilnius	2549	105.4	34.6
Total:	7424	85.5	100

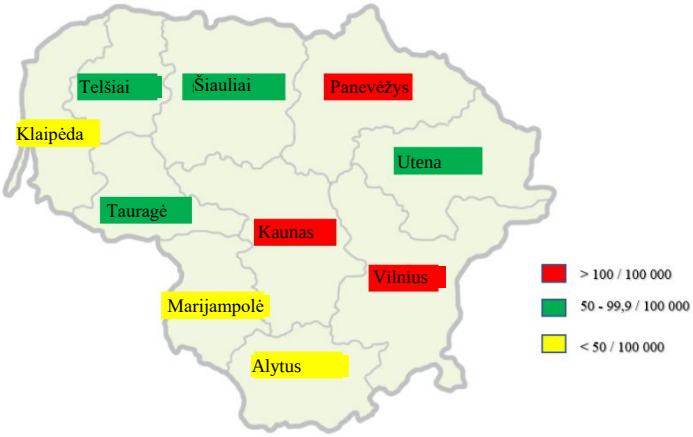

Figure 2. Geographical distribution of Lyme disease incidence in Lithuania in 2014 - 2016.

6120 persons indicated the area where the tick bit them. The highest number of LD infections occurred in the forest (n = 2039), residential area (n = 1784), and nature (n = 1177). (Figure 3.).

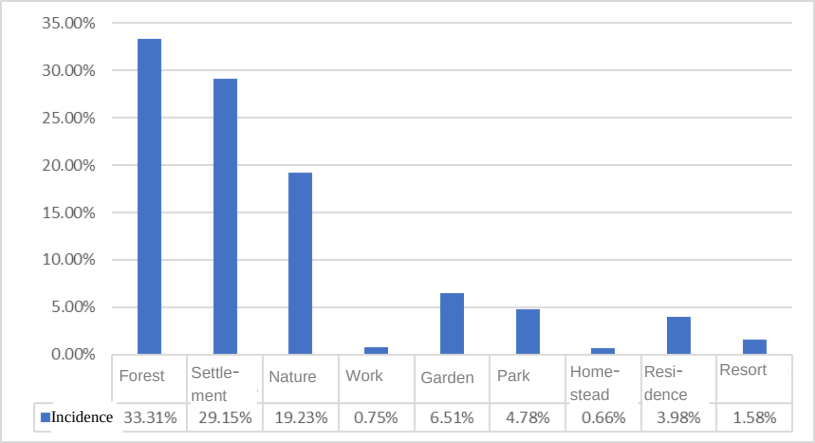

Figure 3. The observed tick bites in Lithuania from 2014 to 2016 according to location.

Ticks that bit in were observed in more than half of the patients with Lyme borreliosis n = 4576 (61.6%). No significant statistical difference was observed between men and women in the retrospective assessment of the noticed tick bite (p = 0.107). In general, tick bite was observed in the warmer months of the year, from May to September (90%), with the highest frequency recorded in July (Figure 4). The tick bite was more often observed in urban than in rural population (p = 0.003).

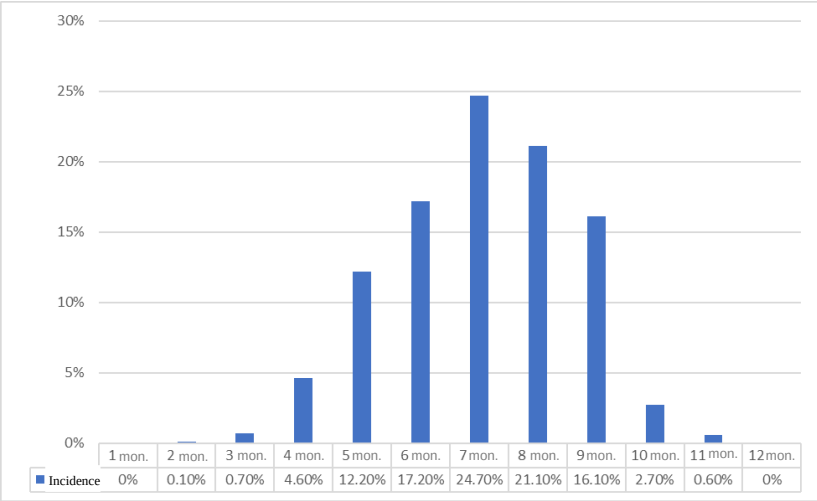

Figure 4. The incidence of tick bites was observed in Lithuania from 2014 to 2016 according to months.

Growth in confirmed cases of LD has been observed since April, but has increased more significantly since May, peaked in September, and then began to decline. Less than one-sixth (15.32%) of all cases of LD were detected from December to May. (Figure 5.)

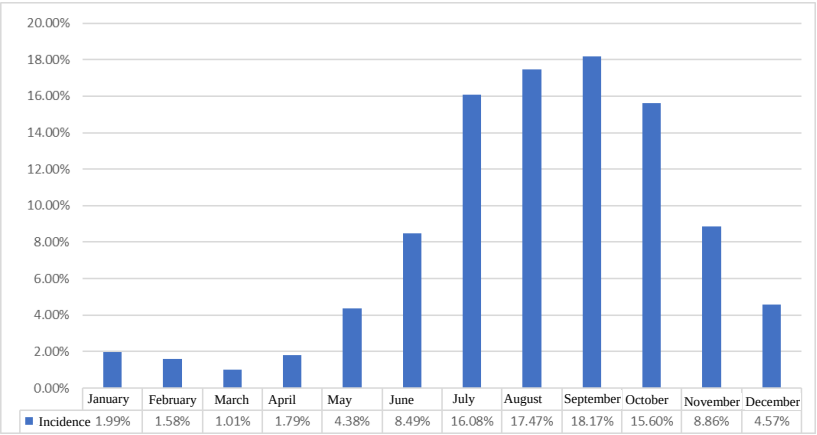

Figure 5. Incidence of diagnosis of Lyme disease in Lithuania from 2014 to 2016 according to months.

996 patients were aware of their clinical symptoms, and no data were provided to the Center for Communicable Diseases and AIDS on the clinical manifestations of the remaining 6428 patients. Erythema migrans occurred in 753 (75.6%) subjects and was the most common fixed symptom, followed by joint pain (n = 220, 22.1%), headache (n = 151, 15.2%), and general weakness (n = 124, 12.4%), fever (n = 101, 10.1%), muscle pain (n = 78, 7.8%), and dizziness (n = 64, 6.4%) (Figure 6). Clinical symptoms were independently distributed between men and women (p = 0.651), but patients with recorded clinical symptoms were statistically significantly older with a mean age of 52.1 (± 18.30) *vs* 48.7 (± 19.59) years (p <0.001). Erythema migrans was observed more frequently in urban residents than in rural residents. (p < 0.001). The incidence of clinical symptoms did not differ between subjects with noticed tick bites and subjects with unnoticed tick bites (p <0.05 for all symptoms).

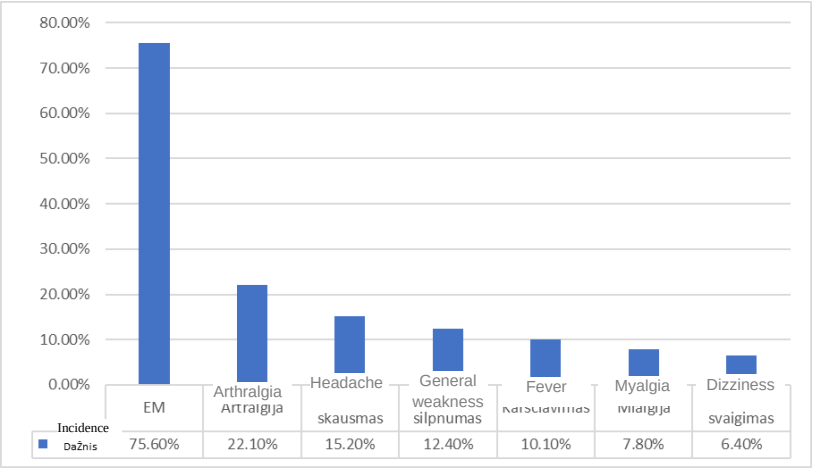

Figure 6. Clinical symptoms of Lyme disease in 2014-2016 in Lithuania.

The disease was mainly diagnosed on the basis of clinical symptoms, 5357 (72.2%) cases. For other 1849 (24.9%) individuals the disease was confirmed on the basis of clinical symptoms and

laboratory diagnosing of the disease, finally, for 189 (2.5%) patients the disease was diagnosed only on the basis of laboratory tests. Thus, the majority of cases, i.e. 7353 (99.6%), was diagnosed on the basis of clinical symptoms or a combination of clinical symptoms and performed LD diagnostic laboratory tests. 1720 (23.2%) patients were tested for Lyme disease immunoglobulins, of them positive immunoglobulins M were found: in 1283 (17.3%) patients, positive immunoglobulins G in 952 (12.8%) patients, and in 536 (7.2%) patients both positive IgM and IgG were found. It is interesting to note that Lyme disease immunoglobulins were more frequently tested in rural areas 26.0% *vs* 22.1% ($p < 0.001$).

Among those patients with identified erythema migrans, 277 (36.8%) were tested for immunoglobulins of Lyme disease, positive IgM were found in 198 (71.5%) patients, while positive and IgG in 142 (51.3%) patients, both positive immunoglobulins were found in 67 (24.2%) patients, and both negative immunoglobulins were found in 3 patients (1.1%).

3.2. The Second Group of Subjects. Data From Patients Diagnosed with LD in the Outpatient Department

1005 persons are confirmed with Lyme disease during an outpatient visit to the Center for Infectious Diseases of HVU SC in 2014 - 2016. The disease was more common in women (n = 663, 66%) than in men (n = 342, 34%). The majority of patients were in the 35-55 age group (38.2%), with a median age of 53 years. More than half of the patients noticed tick bite (59.6%), mainly on the lower extremities (60.5%). As many as 96% of people experienced a typical Lyme disease rash - erythema migrans, which was most commonly observed on the lower extremities (67.2%) as a single element of the rash (95.8%). All cases of multiple erythema were recorded in the EM clinical syndrome group. In most cases, the site of tick bite and erythema migrans coincided (94.7%), Spearman rho = 0.911, p

<0.001. Lyme immunoglobulins in serum were tested in 52.5% of subjects, IgM-positive in 63.6% and IgG-positive in 46.6% of subjects. Clinical symptoms that manifested: joint pain (33.2%), general weakness (19%), headache (13.1%), muscle pain (11.2%), fever (6.2%), joint swelling (5.9%), dizziness (5.1%), neuropathy of n. facialis (2.4%), and radiculitis (2.0%). (Table 4.)

Table 4. Descriptive statistics of data on persons diagnosed with LD in the outpatient department in 2014 - 2016 at Center for Infectious Diseases of HVU SC

		(n = 1005) n (%)
Age	Average (SD)	51.5 (15.86)
	Median (min-max)	53 (18 - 91)
Age groups	18-35	187 (18.6)
	36-55	383 (38.2)
	56-70	311 (30.9)
	71-99	124 (12.3)
Gender	Female	663 (66.0)
	Male	342 (34.0)
Tick bite was observed		600 (59.6)
Tick bite site	Head	6 (1.3)
	Neck	3 (0.6)
	Belly	56 (12.9)
	Lower limbs	261 (60.5)
	Chest	18 (4.1)
	Genitals	3 (0.6)
	Back	17 (3.9)
	Upper limbs	67 (15.5)
Erythema migrans (EM)		968 (96.2)
EM site	Head	6 (0.7)
	Neck	6 (0.7)
	Belly	84 (10)
	Lower limbs	563 (67.2)
	Chest	34 (4.0)
	Genitals	3 (0.3)

	Back	33 (3.9)
	Upper limbs	108 (12.9)
Location of a tick bite/EM	Coincided	341 (94.7)
	Did not coincide	19 (5.2)
One EM		791 (95.8)
Multiple EM		34 (4.1)
Tested Lyme Ig		529 (52.5)
Lyme IgM	Positive	317 (63.6)
	Negatives	139 (27.9)
	Marginal	42 (8.4)
Lyme IgG	Positive	168 (46.6)
	Negatives	170 (47.2)
	Marginal	22 (6.11)
Clinical symptoms	Joint pain	180 (33.2)
	General weakness	103 (19.0)
	Headache	71 (13.1)
	Muscle pain	61 (11.2)
	Fever	34 (6.2)
	Swelling of the joints	32 (5.9)
	Head dizziness	28 (5.1)
	N. facialis neuropathy	13 (2.4)
	Radiculitis	11 (2.03)
	Nausea	6 (1.1)
	Chills	2 (0.3)

The following clinical syndromes of LD have been identified: Erythema migrans n = 945 (94%), Lyme arthritis n = 32 (3.1%), Neuroborelliosis n = 23 (2.28%), Lyme carditis n = 4 (0.3%), and Lyme dermatitis n = 1 (0.1%). (Figure 7.)

Table 5. Clinical symptoms of LD according to LD clinical syndromes among persons diagnosed with LD in the outpatient department of CID HVU SC in 2014-2016

	EM (n=945)	LA (n=32)	NB (n=23)	LC (n=4)	LD (n=1)	Total, n
Joint pain	140	32	8	-	-	180
General weakness	92	5	6	-	-	103
Headache	57	7	7	-	-	71
Muscle pain	47	3	9	2	-	61
Fever	31	2	1	-	-	34
Swelling of the joint(s)	0	32	-	-	-	32
Head dizziness	21	3	3	1	-	28
N. facialis neuropathy		-	13	-	-	13
Radiculitis	6	-	5	-	-	11
Nausea	4	-	2	-	-	6
Chills	2	-	-	-	-	2

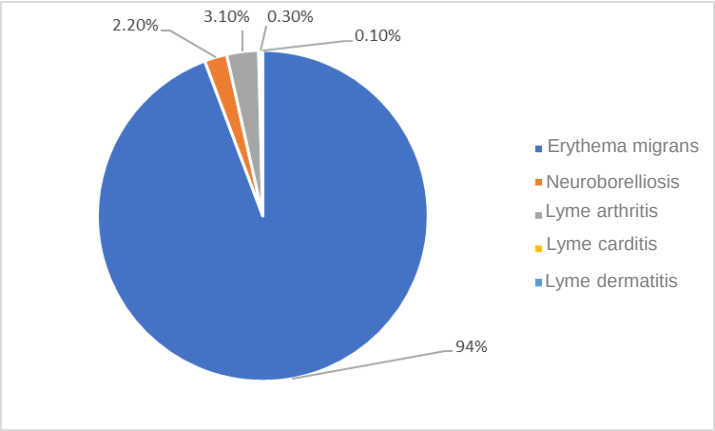

Figure 7. Clinical syndromes of LD among individuals diagnosed with LL in the outpatient department in 2014 - 2016 at Center for Infectious Diseases of HVU SC

No statistically significant difference was observed between Lyme disease syndromes in different age groups. (Table 6.)

Table 6. Clinical syndromes of Lyme disease in different age groups in 2014 - 2016

	EM	NB	LA	LC	LD	Total
18-35	94.1	2.7	3.2	0.0	0.0	187
36-55	93.5	2.3	3.4	0.8	0.0	383
56-70	95.2	1.9	2.6	0.0	0.3	311
71-99	92.7	2.4	4.0	0.8	0.0	124

Pearson's Chi-squared test X-squared = 7.2419, df = 12, p-value = 0.8412

Most of the tick bites observed during the warm season, from May to September, the highest number of observed tick bites occurred in July. In most cases, a rash of erythema migrans appeared between one week and one month after tick bite.

Erythema migrans. Among patients diagnosed with EM clinical syndrome of Lyme disease, female prevalence was observed - 65.7%, the syndrome manifested between the ages of 18 and 91, and the mean age was 53 years. 51% of individuals with a rash of erythema migrans had their Lyme immunoglobulins in serum tested.

Lyme arthritis. Lyme arthritis was diagnosed in 4 men (12.5%) and 28 women (87.5%), with patients ranging from 19 to 80 years of age with a median age of 55 years. The affected joints were: knee 13 (40%), ankle 9 (28%), hand 8 (25%), and elbow 3 (9%). Monoarthritis of one joint was observed in 11 (34%) persons and oligoarthritis, damage to multiple joints, in 19 (59%) persons, in 2 cases it is not known which joint was affected.

Neuroborreliosis. In this group of clinical syndromes, the following gender distribution was observed: 16 women (69.6%) and 7 men (30, 4%), with patients age ranging from 18 to 77 years, with a mean age of 51 years. The main symptoms observed are: n. facial neuropathy (47, 8%), muscle pain (39%), headache (26%), and radiculitis (21%).

Lyme carditis. Lyme carditis manifested as atrioventricular block in 3 men and 1 woman, with patients age ranging from 37 to 72 years, with a median age of 51 years.

Lyme dermatitis. Acrodermatitis was diagnosed in a 61-year-old woman, who developed a red, painful rash on the arm, and the diagnosis was confirmed by histological skin biopsy.

In search of a possible association between the time to onset of erythema migrans after tick bite and the onset of Lyme disease clinical syndrome, a statistically significant association was observed in the Lyme arthritis group. The longer it takes for erythema migrans to appear after tick bite, the more likely it is that Lyme arthritis will occur (p value 0.013). Such a link could not be established in the case of Neuroborelliosis, and due to the small amount of data in cases of Lyme carditis and Lyme dermatitis, it was not possible to calculate possible links

The main treatment chosen was doxycycline, with a median duration of treatment of 18 days. Amoxicillin treatment was chosen for treatment during pregnancy or in elderly patients. (Table 7) In some cases, one drug was switched to another due to allergic reactions. When evaluating treatment options for different clinical syndromes of LD, doxycycline is the most commonly selected in all groups. (Table 8.).

Table 7. Treatment of persons diagnosed with LD in the outpatient department, duration of treatment in 2014 - 2016 at Center for Infectious Diseases of HVU SC.

Antibiotic	**Frequency**	**Percentage (%)**	**Duration of treatment in days Average (SD)**
Amoxicillin	68	6.91	17.8 (10.73)
Amoxicillin, Azithromycin	3	0.30	29.0 (22.63)
Amoxicillin, Doxycycline	10	1.02	32.1 (13.50)

Antibiotic	Frequency	Percentage (%)	Duration of treatment in days Average (SD)
Azithromycin	18	1.83	7.4 (3.14)
Azithromycin, Cefuroxime	1	0.10	16.0 (-)
Azithromycin, Doxycycline	8	0.81	22.3 (6.22)
Azithromycin Doxycycline, Ceftriaxone	1	0.10	63.0 (-)
Ceftriaxone	2	0.20	21.0 (-)
Ceftriaxone, Doxycycline	2	0.20	23.5 (6.36)
Cefuroxime	8	0.81	14.3 (2.71)
Doxycycline	858	86.99	18.1 (4.69)
Doxycycline, Cefuroxime	2	0.20	29.5 (2.12)
Clarithromycin	3	0.30	8.0 (5.57)

Table 8. Treatment choice between different LD clinical syndromes in 2014 - 2016 at Center for Infectious Diseases of HVU SC

	EM	LA	NB	LC	LD
Doxycycline	833	28	19	2	1
Amoxicillin	78	2	1	-	-
Azithromycin	29	1	-	-	-
Cefuroxime	9	2	-	1	-
Ceftriaxone	-	-	4	1	-
Clarithromycin	3	-	-	-	-

3.3. The third group of subjects. Data from hospitalized LD patients

Data of 120 patients with Lyme disease, confirmed during the hospitalization at Center for Infectious Diseases of HVU SC in 2014-2019, in total were analysed. Among the subjects, 76 (63.3%) were women and 44 (36.6%) were men, with age ranging from 18 to 99 years, with a median age of 57 years. About half of the patients (51.6%) observed a tick bite, the localization of which was mainly in the lower extremities (54%). Erythema migrans rash occurred in ¾ of persons, mainly in the lower extremities (64%). In most cases, the site of tick bite and the rash of erythema migrans coincided. There were 4 cases of multiple erythema migrans rash, 3 of them in the EM group and 1 in the NB group. The duration of hospitalization of patients ranged from 1 day to 63 days, with an average duration of 11 days. Lyme disease immunoglobulins were tested in 95 (79.1%) subjects. The clinical symptoms that manifested were as follows: general weakness (18.2%), headache (15.5%), fever (12.2%), muscle pain (9.7%), radiculitis (9.5%), facial muscle paralysis (7.7%), joint pain (6.7%), nausea (5.5%), chills (4%), diarrhea (1%), and impaired consciousness (1%). (Table 9.)

The identified clinical syndromes of the disease: Migratory erythema (60%), Neuroborellosis (35.8%), Lyme arthritis (2.5%), Lyme carditis (1.6%). (Figure 8.)

Table 9. Descriptive data statistics. Persons hospitalized due to LD at Center for Infectious Diseases of HVU SC in 2014-2019.

		(n = 120) n (%)
Age	Average age (SD)	54.3 (16.28)
	Median (min-max)	57 (18 - 90)
Age groups	18-35	18 (15.0)
	36-55	37 (30.8)
	56-70	55 (41.6)

	71-99	10 (8.3)
Gender	Female	76 (63.3)
	Male	44 (36.7)
Bed-days		11 (1-63)
Tick bite was observed		62 (51.6)
Tick bite site	Head	3 (8.1)
	Neck	1 (2.7)
	Belly	4 (10.8)
	Lower limbs	20 (54.0)
	Chest	4 (10.8)
	Upper limbs	5 (13.5)
Erythema migrans (EM).		91 (75.8)
EM site	Head	4 (4.4)
	Belly	7 (7.8)
	Lower limbs	57 (64.0)
	Chest	9 (10.1)
	Genitals	1 (1.1)
	Upper limbs	11 (12.3)
Tick / EM location	Coincide	32 (94.0)
	Does not coincide	2 (5.8)
One EM		87 (95.6)
Multiple EM		4 (4.4)
Tested Lyme Ig		95 (79.1)
Lyme IgM	Positive	71 (75.5)
	Negatives	15 (15.9)
	Marginal	8 (8.5)
Lyme IgG	Positive	64 (68.8)
	Negatives	21 (22.5)
	Marginal	8 (8.6)
Symptoms of the disease	Joint pain	27 (6.7)
	General weakness	73 (18.2)
	Headache	62 (15.5)
	Muscle pain	39 (9.7)
	Fever	49 (12.2)
	Head dizziness	34 (8.5)

	Facial muscle paralysis	31 (7.7)
	Radiculitis	38 (9.5)
	Nausea	22 (5.5)
	Chills	16 (4.0)
	Diarrhea	4 (1.0)
	Impaired consciousness	4 (1.0)
Clinical disease syndrome	Erythema migrans (EM).	72 (60.0)
	Neuroborelliosis (NB)	43 (35.8)
	Lyme arthritis (LA)	3 (2.5)
	Lyme carditis (LC)	2 (1.6)

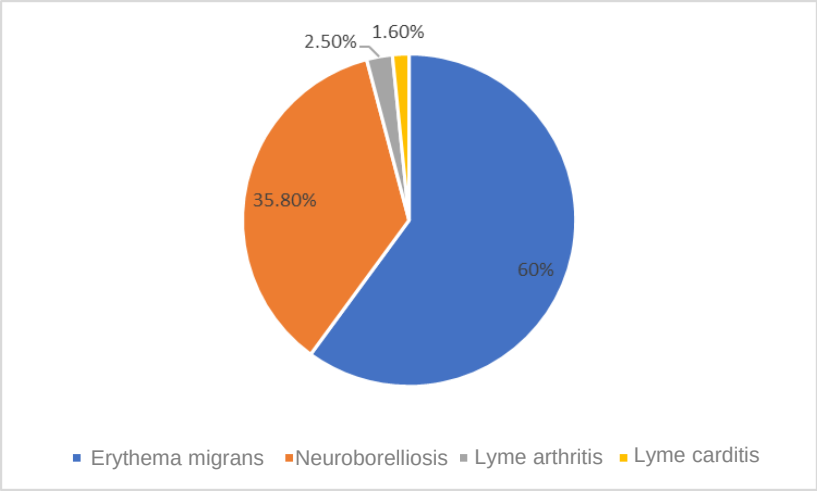

Figure 8. Distribution of LD clinical syndromes among individuals hospitalised at Center for Infectious Diseases of HVU SC in 2014 - 2019

During the assessment of the distribution of indicators between different clinical syndromes of LD, a comparison could only be made between the EM and NB groups because the number of subjects in the LA and LC groups was too small. A statistically significant older age (60 years) (p-0.02) was observed in patients with NB compared to patients in the EM group, and the gender distribution was similar

between these groups. Tick bite was noticed statistically significantly more often in the EM group, and the time from onset of symptoms to hospitalization was statistically significantly longer in the NB group, averaging 28 days, and longer duration of hospitalization was observed, also a higher leukocyte count was observed in the NB group. No statistically significant differences were observed during the assessment of other indicators.

In all patients, it took an average of 16 days from tick bite to the manifestation of erythema and 34 days from tick bite to other symptoms. Inflammatory indicators of the blood usually were not expressed. (Table 10.)

Table 10. Statistical analysis of identified LD clinical syndromes in 2014 - 2019 at Center for Infectious Diseases of HVU SC

	EM (n = 72)	**LA (n = 3)**	**LC (n = 2)**	**NB (n = 43)**	**P***
Age	51 (16)	54 (24)	44 (18)	60 (15)	0.02
Gender (male)	27 (37.5%)	-	1 (50%)	16 (37.2%)	0.59
Tick bite (yes)	45 (62.5%)	-	1 50.0%)	16 (37.2%)	0.01
Time from the tick bite to the onset of erythema, days	16 (3 - 60)	-	-	18 (14-21)	0.36
Duration of symptoms before	21 (0 - 120)	62 (2 - 180)	43 (30 - 55)	28 (0 - 120)	0.03

	EM (n = 72)	**LA (n = 3)**	**LC (n = 2)**	**NB (n = 43)**	**P***
hospitalizatio n, days					
Time from tick bite before symptoms, days	31 (4 - 150)	-	-	42 (10-120)	0.09
Hospitalizatio n duration, days	7 (1 - 22)	7 (2 - 11)	10 (5 - 16)	17 (3 - 63)	<0.00 1
WBC (leukocytes)	7.76 (2.90-26.82)	6.79 (4.21-11.46)	7.39 (6.85-7.94)	9.60 (4.95-23.60)	0,002
CRP (C reactive protein)	20.10 (0.00-147.00)	58.62 (0.93-124.81)	2.55 (0.86-4.25)	13.46 (0.42-128.62)	0.52
ESR (erythrocyte sedimentation rate)	14 (1-73)	47 (3-116)	7 (5-8)	18 (2-88)	0.42

* reliability is presented by comparing EM clinical syndrome with NB. It has not been compared with other syndromes due to the small number of cases.

In the 5-year period from 2014 to 2019, EM syndrome prevailed among hospitalized individuals with LD, except in 2018, when number of persons treated for NB was by one case higher than number of persons with EM, also the lowest number of hospitalized persons with LD was observed in that year. The highest number of hospitalizations for LD was in 2016. (Figure 10) Distribution of LD clinical syndromes by year:

In 2014 EM (11), NB (8), LA (2);

In 2015 EM (13), NB (6), LC (1);

In 2016 EM (19), NB (9);

In 2017 EM (13), NB (6), LA (1), LC (1);
In 2018 EM (6), NB (7);
In 2019 EM (10), NB (7). (Figure 9.)

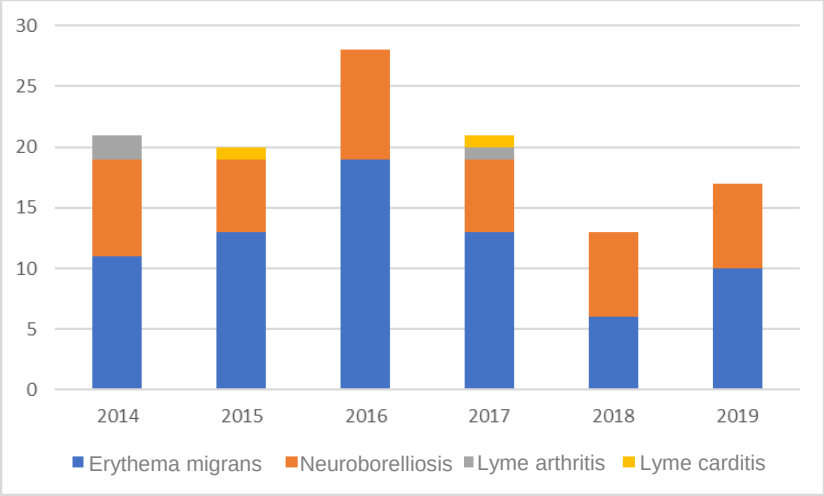

Figure 9. Distribution of clinical syndromes by year of hospitalization in 2014 - 2019 at Center for Infectious Diseases of HVU SC

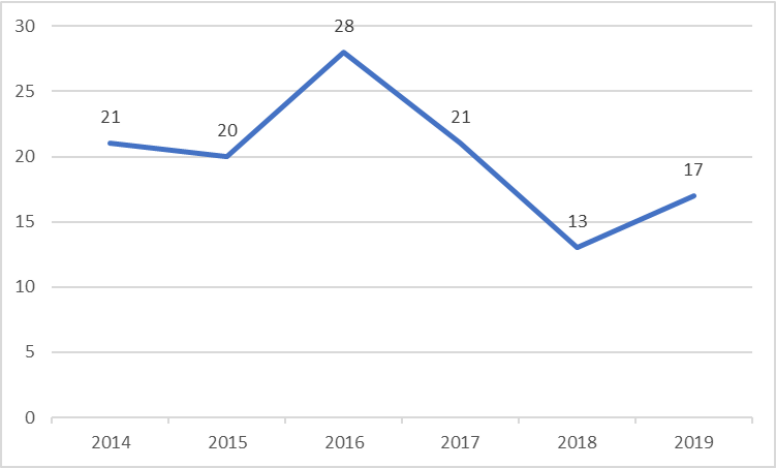

Figure 10. Distribution of LD cases by year 2014 - 2019 at Center for Infectious Diseases HVU SC.

During the assessment of the monthly distribution of LD clinical syndromes, the majority of EM cases were detected in May-September

(72%) and those of NB in June-September (72%). (Figure 11) More than half of all cases of LD were detected in June-September (55.8%). (Figure 12.)

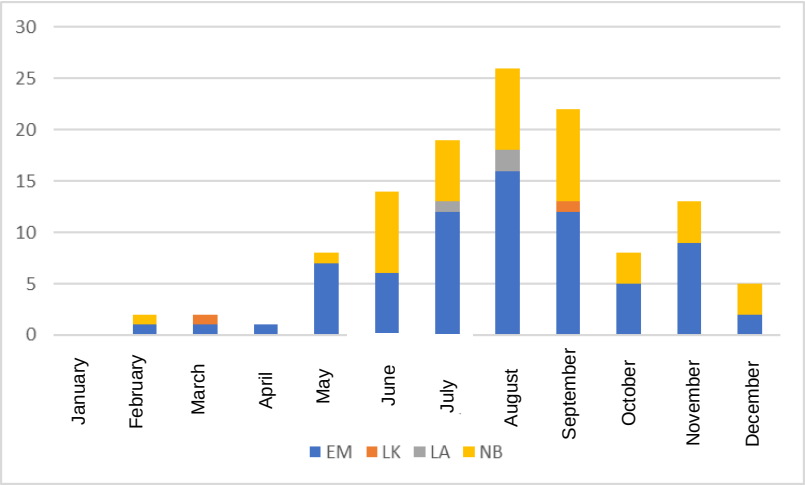

Figure 11. Distribution of clinical LD syndromes by months in 2014 - 2019 at Center for Infectious Diseases of HVU SC

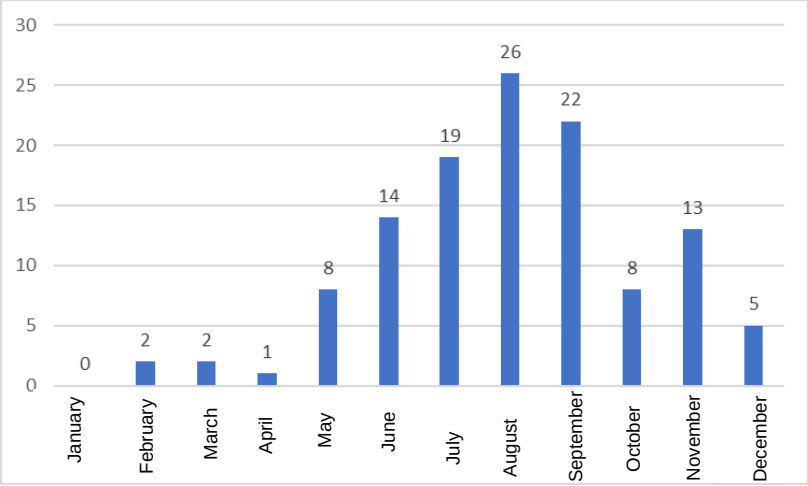

Figure 12. Distribution of LD cases by months in 2014 - 2016 at Center for Infectious Diseases of HVU SC

Erythema migrans. Clinical syndrome of erythema migrans was identified in 72 persons, mainly women (62.5%), with a mean age of 51 years. The rash most commonly occurred on the lower extremities as a single rash element and, in many cases, coincided with the site of the tick bite. The time to onset of the rash of erythema migrans after the observed tick bite ranged from 3 to 60 days. Most patients were treated with doxycycline, 3 subjects treated with amoxicillin, intravenous ceftriaxone in 4 cases due to observed febrile fever, and in 12 cases the suspected NB during the early hospitalization, diagnosis of which was discarded by lumbar puncture and spinal fluid testing, as well as in 1 case of multiple EM were treated with azithromycin.

Neuroborreliosis. Neuroborelliosis was diagnosed in 43 individuals, mostly women (62.8%), with a mean age of 60 years. Among all NB patients, erythema migrans rash was observed in only 19.8 percent. The main symptoms noted were: radiculopathy (62.8%), facial muscle paralysis (46.5%), and headache (27.9%). The diagnosis was confirmed in all patients by examination of the cerebrospinal fluid. (Table 11) Intravenous Ceftriaxone was prescribed for treatment, 3 subjects treated with Doxycycline, 1 patient was treated with Ampicillin, Penicillin.

Table 11. Clinical and cerebrospinal fluid indices of the neuborelliosis group in 2014 - 2019 at the Center for Infectious Diseases of HVU SC

	Neuroborelliosis (n = 43) **N (%)** **Average (min-max)**
Erythema migrans	18 (19.8%)
Radiculopathy	27 (62.8%)
N. facialis neuropathy: One-sided Two-sided	20 (46.5%) 16 (37.2 %) 4 (9.3 %)
Fever: Body temperature	8 (18.6 %) 37.9 (37-40)

	Neuroborelliosis (n = 43) N (%) Average (min-max)
Headache	12 (27.9 %)
Head dizziness	8 (18.6 %)
Nausea	3 (7.0 %)
Cerebrospinal fluid	
Cytosis	147 (5-586)
Lymphocyte percentage	90 (10-100)
Protein	1.41 (0.36-6.09)
Albumin	807.8 (39.9 - 5110)
Total IgM	46.7 (0.42-555)
Total IgG	180.5 (5.5-934)
Intrathecal IgM (%)	26 (60.5%)
Intrathecal IgG (%)	31 (72.1%)
IgM index	6.86 (0.82-25.96)
IgG index	5.20 (0.41-17.92)

Lyme arthritis was diagnosed in 3 women, age ranging from 30 to 78 years, with a mean age of 54 years. In 2 cases monoarthritis was observed, the knee and ankle joints were affected; in case 1, oligoarthritis was observed, affecting the joints of the knees, ankles, hands, and wrists. Fever occurred in two cases (max t - 39.5 ºC). Doxycycline treatment has been prescribed for monoarthritis and intravenous Ceftriaxone has been selected for oligoarthritis.

Lyme carditis manifested as atrioventricular block in 1 woman (31 years of age) and 1 man (56 years of age). In both cases, tests of Lyme IgM and IgG in the serum were positive. Intravenous Ceftriaxone was prescribed for treatment.

Among all hospitalized individuals, LD was not the primary diagnosis for hospitalization in 14 persons. The following diagnoses were observed in these cases: tick-borne encephalitis (6), erysipelas (3), fever of unknown origin (1), shingles (1), salmonellosis (1),

gastroenteritis and colitis (2). 79 (65.8%) individuals were suspected of having neuroborelliosis at the beginning of hospitalization and underwent lumbar puncture and fluid testing to clarify the diagnosis.

4. DISCUSSION OF THE RESULTS

4.1. Discussion of the Results of Epidemiological LD Data of the CCDA

Lyme disease is the most common tick-borne infection in Europe, with the exception of the northernmost regions (Iceland has a prevalence of 2 cases per 100 000 of residents) (10) and the hottest places in the south of the continent (in north-western Spain, the incidence is 2.5-11.6 per 100 000 residents) (11), so it is not surprising that Lithuania is among the endemic countries of Lyme disease. The incidence of the disease in Lithuania in 2014-2016 was 85 cases per 100 000 residents (12), and during the period from 2014 to 2019 - 95.95 cases per 100 000 residents. This incidence is similar to other countries with the highest number of cases in Europe - Slovenia, Germany, Austria, and a southern part of Sweden. The estimated incidence rate in these countries is more than 100 cases per 100 000 of residents. (13) However, the interpretation and comparison of epidemiological indicators between countries is difficult: in many European countries, registration of borreliosis is optional, the disease can often be undiagnosed, and different European laboratories use different serological tests to detect antibodies.

As a result of global warming and milder winters, the period of activity of ticks is getting longer, which means that the high number of newly diagnosed cases of the disease can be expected to continue in the future and is likely to increase even more. The higher incidence of Lyme disease may also be due to the improved knowledge of the disease both among doctors and among patients.

The disease was found more often in women (62.4%) than in men (37.6%). The gender distribution is the same as described in other European countries: Finland (14), Norway (15), Sweden (16), Germany (17), with the exception of parts of the United Kingdom (England and Wales), where men prevail (18). This may be because women are more likely to notice changes in their skin and are more likely to see doctors as a result.

The study found that the disease is found mainly in the urban population of the 51-60 year age group, one decade earlier than in the publications of other authors (18, 14). There was also no age-related bimodal distribution, with the first peak observed in early childhood (6-9 years of age) and the second in the late middle age (60-69 years of age) (14, 18, 17, 19). The reason for this is not clear, it is possible due to the fact that pediatricians less often report cases of the disease in Lithuania.

The disease was more common in urban residents (72.3%) than in rural areas (27.7%), which may be due to the fact that urban residents have easier access to treatment services and urban residents are more likely to turn to health care professionals. A similar distribution of patients in Lithuania between urban and rural areas was observed in 1995-2006, with 79.9% in urban areas and 20.0% in rural areas. (20)In the assessment of social groups of patients, the disease affects both employed and unemployed persons equally, therefore social status is not relevant for the diagnosis of the disease and should not be classified as a risk factor.

During the assessment of the geographical distribution of the disease by counties the highest indicators were observed in Kaunas, Vilnius, and Panevėžys counties. These counties could be dominant in terms of the number of cases identified for the following reasons: Vilnius and Kaunas have the largest medical centers in the country, which provide third-level services and serve a large number of the country's patients. And the reason why such high disease prevalence rates are observed in Panevėžys County remains unclear, possibly due to the natural characteristics of the Green Forest that is located there.

Alytus county has the highest forest cover in Lithuania, but the number of cases of LD is the lowest in the population, it is difficult to say what determines such indicators, perhaps the fact that people in this county tend to treat themselves with folk medicine or that doctors are less likely to report identified cases. This may also be possible due to the predominance of pine forests (40.7%) in the forests of this county, and according to the research conducted on Borrelia-infected ticks in Lithuania, it has been established that they are most common in deciduous and mixed forests. (21)

The lowest incidence of LD is observed in Marijampolė and Klaipėda counties. These counties also have the smallest forest cover in Lithuania.

Assessment of the infection frequency according to location, the highest rates of infection were observed in the forest (33.31%) and residential areas (29.15%), while infections related to the working environment accounted for only 0.75%. Forests are associated with the habitat of the ticks' hosts and the place where the ticks themselves spread, and the habitats are already associated with insufficient maintenance of the greenery by humans.

The main marked symptom of the disease is a rash of erythema migrans, which is also a pathognomonic diagnostic marker of the disease. In more than a third of cases of erythema migrans rash, patients were also tested for Lyme immunoglobulins, mainly in rural areas. The higher frequency of serological markings of Lyme disease performed in rural areas, when the EM is observed, may be explained by a worse understanding of the disease among physicians. According to the latest European and American diagnostic guidelines for the disease, no additional laboratory tests are required to confirm a diagnosis of Lyme disease when EM is observed. (22, 23) (24, 25). When there is no evidence for EM or an atypical rash, clinical signs are assessed and a laboratory test of the disease is performed.

The highest number of tick bites was observed in May-September, their peak became apparent in July. This coincides with a known period of tick activity, which also occurs during the warmer months of

the year, from May to September. In Lithuania, ticks *Ixodes ricinus* are responsible for the spread of the disease, the development, activity and survival of which require certain natural conditions (ambient temperature and relative humidity). (26) It usually takes 2 to 30 days after the tick bite for the predominant symptom of Lyme disease, the manifestation of erythema migrans. If symptoms of the disease occur, some time passes before the patient visits the doctor. If it is decided to perform a serological blood test to confirm the disease, the patient finds out the final results during a secondary visit to the doctor, at which time a diagnosis of LD is formed. Therefore, it may take 1-2 months after the tick bite before the final diagnosis is made. In 2014-2016, the diagnosis of the disease was mainly made in June-November, most diagnoses were made in September. Compared to the frequency of tick bites by months, a shift of 1-2 months is observed. In Finland, most cases are diagnosed in August. (14)

In summary, Lithuania is an endemic country of LD. LD is characterized by seasonality, the dominance of middle-aged women living in the urban environment, with the highest probability of contraction in Kaunas, Vilnius, and Panevėžys counties. The main symptom of the disease is a rash of erythema migrans. Social status is not a risk factor for LD, and the probability of contracting LD is similar in both the forest and the residential area.

4.2. Discussion of the Results of Persons Diagnosed With LD During Outpatient Consultation at Center for Infectious Diseases of HVU SC

The data of persons diagnosed with LD in the outpatient department, same as the data of persons diagnosed with LD during hospitalization described below, not only supplement the previously described epidemiological indicators of the disease, but also provide additional information reflecting the clinical progression, diagnosis, and treatment of the disease.

People of all age groups and of both genders can be ill with Lyme disease. However, women (66%) were more often diagnosed with the disease than men (34%), and people of working age (36–55 years) were affected the most. The same trend was observed when evaluating epidemiological data on the disease.

Specific symptoms of the disease include the rash of erythema migrans, facial nerve (n. facialis) neuropathy, radiculoneuritis, carditis, and migratory arthritis. (27). Other accompanying symptoms such as joint pain, muscle aches, headache, and general weakness; are general and non-disease specific. (28) .

Among all cases of Lyme disease diagnosed in the outpatient department, the following clinical syndromes have been identified: Erythema migrans (EM) 94.02%, Lyme arthritis (LA) 3.18%, Lyme carditis (LC) 0.39%, and Lyme dermatitis (LDe) 0.09%, although according to the predominant symptoms 2.28% cases could have been classified as Neuroborelliosis (NB), however the lumbar puncture and cerebrospinal fluid testing for Lyme disease were not performed. This distribution of clinical manifestations of the disease is similar to other endemic European countries. In France, the average annual incidence is 53/100 000 (2011-2016) and the distribution of the syndromes is as follows: EM (94%), LA (2.5%), LDe (acrodermatitis 0.89% and Borrelia lymphocytoma 0.59%) (29); In Germany, the incidence of Lyme borreliosis ranges from 26/100 000 (2015) to 41/100 000 (2013), and only EM manifests in 95% of cases, NB in 2.7% of cases, and LA in 2.1% of cases (30). According to health records, 9.3% cases of Neuroborellosis and 4.3% cases of Lyme arthritis were identified in Finland from 1995 to 2014. (14)

Although Lyme disease is a tick-borne infectious disease, only 59.6% of patients observed tick bites. Tick bite may go unnoticed due to the small size of the tick, especially when the tick is in the nymph stage. Nymph stage tick size is 1.2-1.5 mm, adult tick size is 2 - 4 mm, and they are easier to spot. Ticks are active during the warm season, from May to September, with a peak in July. Analysis of the data

showed that 92.5% of patients noticed tick bite between May and September, and almost a third of them observed tick bite in July.

Erythema migrans. Clinical syndrome of erythema migrans was detected in 94% (n = 945) of cases. Usually, ticks “lurk” for a host in the tall grass and their bites in humans is observed in the lower extremities, in many cases the location of tick bite and the location of rash of erythema migrans coincide. According to the analyzed data, the most common rash of erythema migrans was observed on the lower extremities (67.2%), and the location of tick bite and the location of the rash coincided (94.7%). It is known that after tick bite EM develops after 2-30 days, usually after 7 days. The study observed that 79.8% of cases occurred in the first month after tick bite. When the rash occurs within a few hours or days, then it is not erythema migrans but a skin reaction to tick saliva (31). It is important to note that sometimes the site of tick bite and erythema migrans do not coincide. Multiple erythema migrans (more than 1 element of the rash) has been observed in 34 persons, which already indicates the spread of the disease and such cases are classified as stage II of the LD.

Half (51%) of patients who developed a rash of erythema migrans had serum Lyme immunoglobulin tests to confirm the diagnosis, which is not required by the European and American diagnostic guidelines. (24, 25)

Lyme arthritis was observed in 3% (N = 32) of cases, more often as oligoarthritis, the most commonly affected joint was the knee joint (40%). According to the literature, Lyme arthritis manifests as mono or oligoarthritis, usually affecting the knee joint. (32) The analysis of the data showed a statistically significant relationship between the time over which the rash of erythema migrans manifests after tick bite and the onset of the clinical syndrome of LD - the longer the onset of the rash, the higher the likelihood of Lyme arthritis. (33)

Neuroborreliosis is a neurological manifestation of LD, occurring in 3 - 15% of all cases of LD. This clinical syndrome presents as polyradiculitis, meningitis, or encephalomyelitis (rare). The most commonly observed clinical symptoms are neuropathy of n. facialis

and radiculitis. (34). According to the analysed data, 2.2% (n = 23) of the cases could be classified as NB, but they were not confirmed by the testing of the cerebrospinal fluid. Symptoms observed were facial nerve neuropathy (47.8%), muscle pain (39%), headache (26%), and radiculitis (21%).

Lyme carditis. AV block of varying degree is the most common manifestation of Lyme carditis. LC can also manifest as acute myocarditis, pericarditis, myopericarditis, endocarditis, and pancarditis. The estimated incidence of this clinical syndrome is between 0.3% and 4% (35); According to the assessed data, Lyme Carditis occurred in 0.3% (n = 4) of cases. During Lyme carditis, male prevalence was observed, the male / female ratio was 3:1, and the same gender distribution was observed among persons consulted in the outpatient department. The course of the disease is often moderate, people fully recover after treatment with antibiotics. The following symptoms may be observed: weakness, dizziness, palpitations, chest pain. (36, 37). Atrioventricular block was identified in all patients.

Lyme dermatitis. Dermatological manifestations of LD may include Borrelia lymphocytoma or Acrodermatitis chronica atrophicans (ACA). (38). The incidence of Lyme's Acrodermatitis chronica atrophicans in Europe ranges from 1 to 10%, depending on the region. According to the analysed data, Acrodermatitis chronica atrophicans occurred in one person, which accounted for 0.1% of all LD cases analyzed. These data do not fully reflect the incidence of the syndrome in Lithuania, as patients go to dermatovenerologists for atypical manifestations of LD in the skin and such cases were not included in this study. ACA is a late-spreading LD that can last for years. ACA is described as a red or bluish-red skin lesion that gradually leads to skin atrophy. (39) The most common localization of skin damage is the limbs, although it can also affect other areas of the body, such as the face. In women, this clinical syndrome of LD is detected 2-3 times more often than in men. (40). During the analysis of the data, 1 case of ACA was observed, which occurred in a 61-year-old woman as a painful red rash on the hand. It is important to note

that ACA can manifest from 6 months to 8 years after a previous tick bite. (41).

According to the analysed data, stage I LD was detected in 911 persons (90.64%), stage II LL was detected in 93 persons (9.25%), stage III LD was detected in 1 person (0.09%).

LD is treated with antibiotics. The first-line treatment is doxycycline, the duration of treatment depends on the identified syndrome of LD: early local infection is treated for 10-14 days, early advanced infection is treated for 21-28 days. (42). For persons consulted in the outpatient department, doxycycline was the most commonly used to treat all clinical syndromes of the LD, and Amoxicillin was the second-line treatment.

4.3. Discussion of the Results of Persons Diagnosed with LD During Hospitalization at Center for Infectious Diseases of HVU SC

In 2014–2019, during hospitalization the diagnosis of LD was confirmed in 120 patients. For 14 persons among these patients, this was not the leading cause of hospitalization. The following diagnoses were made in these cases: Tick-borne encephalitis (6), Erysipelas (3), gastroenteritis and colitis (2), salmonellosis (1), shingles (1), fever of unknown origin (1). Thus, 106 individuals, specifically for LD, required inpatient examination and treatment over a five-year period. Among these 106 subjects, 82 patients (77%) were suspected of having neuroborelliosis at the beginning of the hospitalization.

The highest number of identified cases of LD was observed in 2016 (n = 28), and in the same year a high incidence of LD was observed in the population (101.6 cases per 100 000 of residents), the lowest number of cases was in 2018 (n = 13), the decrease in the incidence of LD in the population was observed in same year as well (81.9 cases per 100 000 of residents) Prevalence of EM syndrome was observed throughout the study year, followed by NB, except in 2018, when the number of detected NB cases was 1 case higher than that of EM. More

than half of all cases of LD were detected during the warm period of the year, from June to September (55.8%), with the majority of cases recorded in August. This coincides with the period of activity of ticks. In terms of clinical syndromes, the majority of EM cases occurred between May and November (95.8%), with the majority occurring in August (22%) and the NB clinical syndrome, mainly detected between June and September (72%), peaking in June. (18.6%), August (18.6%), and September (20.9%). It is interesting to note that during the five-year study period no case of LD was detected in January.

Among all the studied persons, women were predominant (63.3%), the median age was 57 years (4 years higher than among the persons diagnosed with LD in the outpatient department). Tick bite was observed by about half of the persons (51.6%), and rash of erythema migrans occurred in ¾ of the patients (75.8%). A rash of Erythema migrans was observed in 20 percent less persons when compared to persons diagnosed with LD in the outpatient department. An unnoticed rash of erythema migrans complicates the diagnosis of LD. 4 patients developed multiple EM, indicating the spread of the disease to the skin. The following clinical syndromes of LD have been identified: Migratory erythema (60%), Neuroborreliosis (35.8%), Lyme arthritis (2.5%), Lyme carditis (1.6%).

During the assessment of all identified clinical syndromes of LD, 57.5% of cases were detected at the stage of local early infection, 42.5% of cases were detected at the stage of widespread early LD, and these cases also included cases of multiple EM. No cases of widespread late-stage LD were observed.

It took an average of 16 days from tick bite to the onset of erythema migrans, and no statistically significant difference was observed between the EM and NB groups, it was tried to look for this difference in order to find possible link between the time from tick bite and the onset of erythema migrans rash and LD clinical syndrome, such link was observed among persons from the Lyme arthritis group consulted in the outpatient department. Of all subjects tested serologically for LD, only 5 subjects tested negative for both Lyme IgM and Lyme IgG,

all of these cases were observed in the EM group. Blood inflammatory indicators have no diagnostic value. The average duration of hospitalization was 10 days, with the longest hospitalization of patients with neuroborelliosis.

The group of hospitalized individuals for LD provides the most information on one of the clinical manifestations of LD, i.e. Neuroborelliosis, which is considered to be the second most common clinical manifestation of LD in Europe.

Neuroborreliosis is a disease of the nervous system that occurs weeks or months after the bite of the infected tick. (43). The disease is called acute, when the symptoms of the disease last up to 6 months. (44) In subjects, symptoms occurred on average 42 days after previous tick bite. The most common symptoms of NB are facial muscle paralysis (n. facialis neuropathy) and radiculitis. (45). Other causes that can lead to facial paralysis include autoimmune diseases, trauma, tumours, metabolic diseases, and many other infectious diseases such as herpes virus infection, and shingles infection. (46). However, the cause of facial paralysis in about 80% of adults, to the exclusion of other possible diagnoses, remains unknown. (47)

The main symptoms observed in the NB group were: radiculitis (62.8%), facial muscle paralysis (46.5%), and headache (27.9%). The incidence of N. facialis damage is similar to that in a study in Hanover, Germany, where 68 persons were diagnosed with NB between 1999 and 2014, with the main symptoms observed being facial muscle paralysis (42.6%) and radiculitis (25%). (48)

Although facial nerve neuropathy and radiculitis manifest in about 80% of patients in Europe, NB may present with other non-specific signs of the disease and sometimes mimic other neurological diseases.

Seasonality of the disease may also be an important factor in differential diagnosis. Most of the observed cases of NB were distributed between June and September. Similar seasonality of NB is observed in Sweden, Finland, and Germany (49, 50, 48). During the analysis of the data, in 5 years no case was observed only during January, March, and April. Although the disease is mainly detected in

summer and September, it should be borne in mind that NB can be diagnosed at any time of the year. Thus, lower risk months are not a sufficient indicator for NB rejection.

69.7% of patients with Neuroborreliosis observed either tick bite or erythema migrans rash, thus ~ in 30% of cases where neither erythema migrans nor tick bite was observed, when assessing the anamnesis data, NB diagnosis becomes more complicated. This proves the need for serological testing and lumbar puncture to confirm the diagnosis.

In the serological blood tests performed by ELISA method for all subjects with NB found positive or borderline IgG, and positive or borderline IgM was observed in all but 5 cases where the tests were negative. It is important to mention that negative blood results do not rule out a diagnosis of NB. (51)

In patients with NB, inflammatory changes in the cerebrospinal fluid are observed, such as increased white blood cell count. (44) In patients with symptoms lasting 1-2 months, Lyme antibodies are found in the blood and cerebrospinal fluid. Lyme antibodies are sometimes detected in the cerebrospinal fluid of healthy individuals as well, in which case no inflammatory changes are observed in the fluid, this may be the case in individuals already treated for NB or in individuals who have recovered spontaneously. (52)

The observed indicators of cerebrospinal fluid correlate with those observed in other countries. (53)(51) (54, 48) In all patients with neuroborelliosis, increased cerebrospinal fluid cytosis was found with predominance of lymphocytes, intrathecal IgM antibodies were observed in 60.5% of cases, and intrathecal IgG antibodies in 72.1% of cases. These results indicate that NB is characterized by obvious changes in the lumbar fluid.

When differentiating NB-induced facial muscle paralysis with idiopathic facial muscle paralysis, it should be noted that in NB an increased cerebrospinal fluid cytosis and intrathecal Lyme antibodies, or inflammatory changes in cerebrospinal fluid, as well as positive Lyme IgG serology are observed, while during the idiopathic facial

paralysis serological tests are negative. Also, it has been observed that observation of facial muscle paralysis caused by NB is often accompanied by headache. (55, 56) In the analysis of the data, 29.6% of subjects also experienced n. facialis neuropathy and headache.

Lyme arthritis was detected in 3 women, in two cases as monoarthritis and in one as oligoarthritis, in two cases febrile fever was observed. By observing this clinical image of the disease, the disease can be differentiated with infectious septic arthritis. (57)

Lyme carditis was diagnosed in two individuals, with changes in AV block observed on the ECG, which is the most common cardiac manifestation of Lyme disease.

In all cases, treatment was selected based on the form of the disease and the assessment of the symptoms. Erythema migrans was treated with doxycycline in cases of febrile fever or if NB was suspected at the beginning of hospitalization treatment with intravenous ceftriaxone was prescribed; NB was treated with intravenous ceftriaxone, 3 subjects were treated with doxycycline, in case of LA doxycycline was prescribed, and in case of polyarthritis and monoarthritis, when febrile fever was observed, intravenous ceftriaxone was prescribed; and for LC ceftriaxone was prescribed. The choice of treatment is in line with LD treatment recommendations.

In terms of disease prevention, vaccines against the disease are not currently available. Ticks not only transmit LD, but their bite can also cause another disease commonly found in Lithuania - Tick-borne encephalitis. Based on the analysed data, both patients were diagnosed with both Lyme disease and Tick-borne encephalitis. People have the opportunity to get vaccinated against tick-borne encephalitis. (Radzišauskienė et al. 2018). Personal precautions should be taken to prevent Lyme disease: wear long-sleeved T-shirts, push the ends of long trousers into the worn socks when engaging in outdoors activity in endemic areas (e.g. when gathering mushrooms and berries, hunting, or hiking), use tick repellents, and thoroughly examine the entire body for possible tick bites. If a bit in tick is found, remove it as

soon as possible. The duration of tick bite is important for the transmission of infection.

4.4. Relationship between clinical syndromes of LD and the biological characteristics of the vector and pathogen

In Lithuania, the disease is mainly spread by ticks of the genus *Ixodes*, their infection with Borrelia reaches 13.4%. The infection of nymph-stage ticks is 10% and that of adults is 13.8%. The higher percentage of tick infection in adults is due to the fact that they have more time to get infected than nymph-stage ticks. Ticks that are infected with Borrelia are the most common in deciduous and mixed forest areas. (21) During the study of infected ticks, the prevalence of different Borrelia genotypes was as follows: *B. afzelii* 76%, *B. garinii* 10%, *B. burgdorferi sensu stricto* 7%. (58)

It is known that *B. afzelii* causes the clinical manifestations of LD on the skin, *B. garinii* is responsible for the neurological course of the disease, and *B. burdorferi ss* is responsible for joint damage. Given the frequency of ticks *Ixodes ricinus* infestation with different Borrelia genotypes, it is possible to determine clinical manifestations of the disease that can be expected most often.

The distribution of LD clinical syndromes observed in Lithuania during the entire study period was as follows: most often was observed EM 90.4% (n = 945 + 72), followed by NB 5.86% (n = 23 + 43), LA 3.11% (n = 32 + 3), LC 0.53% (n = 4+ 2), and LDe was the least observed one 0.08% (n = 1).

LD most often manifests in Lithuania as EM (90.4%), and B.afzelii is the most prevalent in ticks, NB is less common, and B. garinii infects only 10% of ticks, B.burgdorferi ss is least common in ticks, and LA is the least frequent. (Possible link between tick infestation with different Borrelia genotypes and the frequency of LD clinical syndromes is shown graphically - Figure 13)

5. CONCLUSIONS

1. Lithuania is an endemic country of Lyme disease, the registered incidence is one of the highest in Europe. In 2014-2016, the incidence of Lyme disease in Lithuania was 85.5 cases per 100 000 of residents.

2. The highest incidence was recorded in Vilnius, Kaunas, and Tauragė counties, the lowest in Alytus county.

3. LD was more common in women than in men, more than half of the individuals were older than 51 years of age at the time of diagnosis, and more often in urban than rural residents.

4. Seasonal increase in the morbidity was recorded in May-September, and correlated with observed tick bites, with the highest risk of contracting the disease in July.

5. Clinical syndromes of Lyme disease that were observed: Erythema migrans, Neuroborelliosis, Lyme arthritis, Lyme carditis, and Lyme dermatitis.

6. Erythema migrans mainly occurred in middle-aged women in the lower extremities as a single element of the rash, and in many cases coincided with the site of the tick bite, as well as tick bite is noticed more frequently.

7. Neuroborelliosis occurred predominantly in women, the observed mean age of 60 years is statistically significantly higher than in hospitalized persons of the EM group (p-0.02), with the main symptoms observed being facial muscle paralysis and radiculitis. The tick bite remains largely unnoticed. There was a statistically significant longer time from onset of symptoms to hospitalization, averaging 28 days, and a longer duration of hospitalization was observed compared to hospitalized persons of the EM group.

8. Lyme arthritis has mainly occurred in middle-aged women as oligoarthritis, mostly affecting knee and ankle joints. It was observed that the longer the time interval between tick bite and the onset of the rash of erythema migrans, the greater the likelihood of developing Lyme arthritis (p-0.013).

9. Lyme carditis manifested with atrioventricular block, a 5:2 ratio between men and women, with a mean age of 47 years.

10. Lyme dermatitis manifested as Acrodermatitis in a 61-year-old woman with a red, painful rash on the arm

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LIST OF PUBLICATIONS ON THE TOPIC OF THE DISSERTATION

1. Petrulionienė A, Radzišauskienė D, Ambrozaitis A, Čaplinskas S, Paulauskas A, Venalis A. Epidemiology of Lyme disease in a highly endemic European zone. Medicina (Kaunas). 5 Mar 2020;56(3):115. DOI: 10.3390/MEDICINA56030115. Pmid: 32151097; pmcid: pmc7143858.

2. Petrulionienė A, Radzišauskienė D, Paulauskas A, Venalis A. Lyme Disease among Patients at an Ambulatory Unit in a Highly Endemic Country: Lithuania. Medicina (Kaunas). 2021;57(2):184. Published 21 Feb 2021. doi:10.3390/medicina57020184

LIST OF PRESENTATIONS ON THE TOPIC OF THE DISSERTATION

1. Central European Congress of Rheumatology, CECR, Zagreb. 2018 Lyme disease. Most freguent observed symptoms. Is arthralgia among them?.

2. European Congress of Clinical Microbiology and Infectious Diseases, ECCMID, Amsterdam 2019 – Clinical presentation of Lyme disease in Lyme endemic European country Lithuania

3. Annual European Congress of Rheumatology, EULAR, Madrid 2019 – Rheumatic Lyme disease symptoms based on epidemiological data in high endemic Europe area

4. Annual European Congress of Rheumatology, EULAR, Madrid 2019 – Lyme arthritis in high Lyme disease endemic Europe zone;

5. School of Young Rheumatologists, Kėdainiai, 2019 - “Lyme Disease in Lithuania: Epidemiology, Clinical Overview, Rheumatic Syndromes”;

6. Pan-American Congress of Rheumatology – PANLAR, Miami, 2020 – Lyme arthritis in America‘s only? Eastern Europe‘s findings.

7. European Academy of Dermatology and Venerology, EADV, Spring Symposium 2021, Virtual Congress – Erythema migrans - a pathognomic marker of Lyme disease.

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