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EPIDEMIOLOGICAL PATTERNS OF LYME BORRELIOSIS IN LITHUANIA IN 1995-2006

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LAIMO BORELIOZĖS EPIDEMIOLOGINIAI DĖSNINGUMAI LIETUVOJE 1995-2006 M.

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1. INTRODUCTION

Tick-borne zoonosis is a relevant and emerging threat to the public health across various regions of northern hemisphere, including majority of the European Union (EU) countries. Lyme borreliosis (LB) is the most common vector-borne infection in the EU countries, with estimated 85,000 cases occurring annually. The change in prevalence of LB is observed during the last two decades, the morbidity of LB increased in many central and eastern EU countries. Till now factors causing the spread of the disease have not been thoroughly investigated.

The cases of LB are registered in Lithuania since 1991. During this period the number of cases ranged from 60 cases (morbidity rate 2/100 000 population) registered in 1991, the first year the registration started, up to 3688 cases (morbidity rate 106/100 000 population) registered in 2003. There are approximately 1500 cases of LB registered annually in Lithuania, the average morbidity rate was 34/100 000 population in the years 1991-2006.

Spread of LB is determined by abundance of ticks, their infectivity rate (with LB agent) and freaquency of human contacts with ticks. *Ixodes ricinus* ticks, which usually carry LB agents in Europe, as well as in Lithuania, are prevalent in all districts of country. 3-58% of adult ticks in Europe are infected with *Borrelia* (an average of 17.4%, nymph 2-43% (on average 10.8%), larvae of 0-8% (on average 1.9%). According to the previous studies the ticks collected in various areas from all regions of Lithuania are found to be infected with *Borrelia*. Adult ticks are infected with *Borrelia* in average from 6.9 to 13.7%, but in separate areas it ranges from 0 to 38.7%. The data about nymph's infection with *Borrelia* in Lithuania is insufficient.

What genotypes of *Borrelia* are prevalent in a particular territory is important aspect both clinically and ecologically. In Lithuania in adult ticks most often are detected these genotypes of *Borrelia*: *B. afzelii* - from 9.3 to 10.1%, *B. garinii* - from 1.3 to 2.5%, *B. sensu stricto* - about 1%.

The increased spread of recently discovered novel tick-borne organisms with known or potential medical importance, such as various *Borrelia*, *Rickettsia*, *Anaplasma*, and *Babesia* species, raises concerns, but the identification of prevalence of these organisms in Lithuania is not sufficient.

There is a big attention is paid in Europe towards identification of factors, influencing tick abundance and also incidence of LB. Climate and its changes are the most important and most frequently examined factors influencing tick-borne diseases (TBD), however the morbidity of TBD is influenced not only by natural-climatic factors, but also by social factors. Various factors, such as landscape naturalization, the impact of human activity on natural ecosystems by fragmentation of the landscape, land use, global climate warming and the increasing mobility of the population, socio-economic changes and changes of behavior in nature, directly or indirectly causes to the changes in tick abundance and in spread of LB and other TBD.

The risk of LB and TBD in Lithuania is not described in details, it is not evaluated which natural-climatic or social factors are mainly related to the LB and the TBD.

The aim of the study is to assess epidemiological patterns of Lyme borreliosis and the main factors (natural-climatic and social) leading to them in Lithuania 1995-2006.

The objectives of the study

1. To describe the epidemiological patterns of LB incidence: dynamics, seasonality, territorial spread, the incidence in various age and social groups.
2. To characterize the relationship between natural-climatic, social factors and the incidence of LB in Lithuania.
3. To assess prevalence of tick bites, other risk factors of tick borne diseases and their changes over 5 years among the Lithuanian population; to assess what factors are associated with the tick bite most.
4. To evaluate the tick abundance and the prevalence of *Borrelia* in nymphs in new, previously not examined biotopes; to identify *Borrelia* genotypic structure.

1.1. Relevance of the study

The high incidence of TBD, including LB, determines the high attention to the epidemiological situation in Lithuania. The analysis of the diseases patterns and causative factors is needed for management of the situation, planning and development of disease preventive strategies, improving the diagnostics, providing recommendations to the public and others.

The studies of LB in Lithuania were mostly performed for determination of the abundance of ticks and rate of *Borrelia* infection, especially focusing on adult ticks' infections and genotyping of *Borrelia* detected. It is important that adult ticks and nymphs can transmit LB to humans but the role of nymphs in the epidemiology LB might be much more significant, because the nymphs are smaller than adult ticks and they need shorter feeding time so they are often undetected and that ensure opportunity for feeding and agent‘s transmission. Untill now the studies about infection level of tics in this stage performed in Lithuania were only occasional, small in scope, not allowing to make any conclusions about the *Borrelia* infection rate in nymphs and about the genotypes widespread *Borrelia*. The detection of prevalence of new *Borrelia* transmitted by ticks found in other countries may be important clinically, for example while searching the cause of fever with unknown etiology.

The morbidity of LB and other TBD in Lithuania may be determined not only by risk factors common in all Europe but also by some specific factors, unique only Lithuania or to the Baltic countries. During the last 15 years due to economic reforms the changing use of land in Lithuania reasonably have led to the transformation of the natural environment – arable land areas decreased significantly while the areas of revegetated, abandoned agricultural land and grassland increased. During this period an active deforestation causing revegetation of clear-cuttings of forests was observed. The areas of new clear-cuttings

increased from 162 448 ha in 1995 up to 224 026 ha in 2000 (an increase of 38%), revegetating clear cuttings composed 3% of all Lithuanian land cover.

The landscape of Lithuania also had an active decline in continuous forest areas – forest fragmentation due the development of communication infrastructure, such as new roads and telecommunications networks, development of new settlements in the suburb territories, development of forestry and recreational services and other.

Human activity and the changes of it are related to various social and economic factors that may lead to more frequent visits of people to the forests and consequently more frequent exposure to ticks, but until now no research has been carried out to establish the prevalence of various TBD risk factors, tick bites and spread of LB among the population of Lithuanian.

All these mentioned factors could have caused directly or indirectly the changes in the abundance of ticks, but the impact of changed or newly occurred biotopes to the TBD spread in Lithuania has not yet been examined. The correlation of various risk factors with the incidence of LB and the abundance of ticks has not been assessed too.

1.2. Novelity of the study

LB is the most common tick-borne disease in Lithuania, but despite the relevance of this disease no studies have been carried out until now to determine the epidemic patterns of the LB incidence and their most important natural-climatic and social determinants.

In this study the distribution of LB incidences is defined by gender, age, seasonality, geographical spread, the trends of perennial morbidity are assessed both for Lithuania in general and for separate administrative territories.

Analysis of LB risk factors is performed, the main natural, climatic and social risk factors influencing the spread of LB in Lithuania are determined, correlation of various risk factors with LB incidence and abundance of ticks are identified and assessed in this study in order to identify the key factors affecting the incidence of LB in Lithuania.

After performing a population survey in this study the prevalence of tick bites and TBD risk factors among the Lithuanian population is determined and the change of these factors over five years is assessed.

For the first time ever this study has evaluated the possible ecological significance of newly formed biotope‘s to the LB spread – tick abundance and nymph’s infection rate was examined in different biotopes: old LB infection sites, i.e. in the forests with known incidences of LB infection, and newly formed previously not investigated biotopes – forests and revegetating clear-cuttings close to the areas of uncultivated lands.

Utill now only infection level of adult ticks was examined in more detail though the role of the nymphs in transmission of LB agents to humans is very important. In some of these previously performed studies the nymphs were investigated, but the results of small scope nymph‘s infection rate investigations are insufficient to describe the LB risk and. After molecular tests in our work the prevalence and genotypes of *Borrelia* in *Ixodes ricinus* nymphs collected from different biotopes was determined.

While performing molecular tests on infection level of ticks this study succeded to detect new, until now not detected in Lithuania tick born *Borrelia*, i.e. *Borrelia miyamotoi* belonging to relapsing fever group. These results allow us to confirm that *B. miyamotoi* habitat is greater than was currently set out and it is spreading not only in Scandinavia, but also in Lithuania, and possibly in other neighbouring countries.

1.3. Practical significance of the study

The determination of epidemiological patterns and their causing factors create good preconditions for epidemiological care and for the management of the infection. The data of the study supports LB epidemiological patterns and the strategy of preventive measures for LB and other TBD in Lithuania. Based on a detailed analysis of such data, it is possible to assess a real situation of LB, justify and prepare more accurate LB prevention programs, to predict the need and necessity for preventive measures and evaluate the effectiveness of the measures applied.

This study shows that the dynamics of LB incidence in Lithuania in 1995-2006 was determined by territorial, although focal, spread of the disease, which was particularly prominent in areas with low incidence rate observed in previous years. The study showed that tick abundance, which correlates with local climatic factors, is the main determinant of LB incidence.

The indicator of tick bites prevalence determined in the study after conducting the same or similar studies may be used in the future as an indicator for spread of tick bites and changes in risk assessment of TBD in Lithuania. The questionnaire used for analyzing population's behavior and its changes could be applied in the future for similar studies.

The study of tick abundance and prevalence of nymphs' infection rate in different biotopes showed that the natural cycle of LB is quickly restored in newly formed, high-risk biotopes and it can cause the spread of the disease. The studies of infection rate have confirmed the importance of nymphs in epidemiology of LB, whereas the nymph's infection rate is higher than the infection rate of adult ticks determined in the previous studies.

During the study of the ticks (nymphs) infection level there were the main types of *Borrelia* prevalent in Lithuania determined, compared between the different biotopes and detected new *Borrelia* species (*B. miyamotoi*) which have not been found until now in Lithuania. The identification of LB pathogen in different sites helped to define the specific risk areas/territories. While making the diagnosis of LB based on main clinical symptoms, it is useful from clinical point of view for infectious disease doctors and other health care specialists to know *Borrelia* species prevalent in Lithuania so the results of this study might be useful as well for mentioned professionals. In addition, while trying to determine the cause of fever of unknown origin, it is important to take in to account that the causative agent may be *B. miyamotoi*.

1.4. Defensive statements of the dissertation

- Natural – climatic and social factors influence the spread of tick habitat and LB incidence in Lithuania.
- The naturalization of landscape and newly formed habitats cause the occurrence of LB infection sites.
- The infection rate of nymphs in Lithuania does not differ from the infection rate of adult ticks.
- The habitat of *Borrelia miyamotoi* is not limited to the Scandinavian countries and is wider than previously determined.

2. MATERIAL FOR RESEARCH AND METHODOLOGY OF THE STUDY

The descriptive analysis of LB incidence and influencing risk factors, analysis of prevalence of risk factors of TBD among population, research on the tick abundance and prevalence infectivity rate in different biotopes was performed in order to reach the aim and the tasks of the study.

2.1. Data and Descriptive analysis of LB incidence

The official data from the State Registry for Communicable Diseases and from the Centre for Prevention and Control of Communicable Diseases were used to perform the analysis LB and TBD morbidity.

The data about the LB from 1991-1998 collected from the epidemiological investigation protocols (Form 357/A), which were stored as the filled in copies in the archive of the Centre for Prevention and Control of Communicable Diseases. TBD (LB and TBE) aggregated data collected from annual statistical report No. 4 „Sergamumas užkrečiamosiomis ligomis“ (sveikata, mėnesinė, metinė) (describing incidence of communicable diseases by month and year). This statistical report form since 1998 was modified by adding new data (incidence by age (children under 16 years), place of residence (urban/rural), incidence according to the administrative divisions). The data about the TBE incidence in the period 1968-1990 collected from statistical report forms No. 85.

Since 1999 the data about the incidence of LB and TBE was provided to the Centre for Prevention and Control of Communicable Diseases once a year by filling in the statistical report No. 44 „Parazitozių epidemiologinė būklė“ (describing epidemiological situation of parasitical diseases and Lyme borreliosis by epidemiological signs), No. 38-1 „Sergamumas erkių platinamomis ligomis“ (sveikata metinė) (discribing incidence of tick-borne diseases by epidemiological signs), No. 45 „Entomologinė higieninė būklė“ (sveikata metinė) (discribing entomological situation). Since 1999 the copies of the epidemiological investigation protocols (Form 357/a) were not sent any more to the Centre for Prevention and Control of Communicable Diseases. In 1999-2006 the data about LB and TBE were

collected from the mentioned statistical report forms. Since 2004 all statistical report forms were specified and complemented.

The dynamics of data collected were analyzed identifying and evaluating statistically the trends of incidence, morbidity by age, gender, seasonality and geographic distribution.

2.2. Data of LB risk factors and descriptive analysis

The data of tick abundance and surveillance methods

Ixodes ricinus abundance in Lithuania is observed in nine stationary sites for ticks surveillance: the cities of Vilnius (Bukčių forest) and Klaipėda (Girulių forest), the districts of Biržai (Biržų Forest), Kaunas (Vaišvydavos forest), Kelmė (Patytaukio forest), Kedainiai (Babėnų forest), Marijampolė (Varnabūdės forest), Panevėžys (Žalioji Forest) and Šilutės (Usėnų forest), which were established and operates since 1986. The sites for ticks surveillance (a 1 km section of the forest) are selected in the areas least-affected by human activity and are constant.

The abundance of tick in surveillance stationaries is observed from April till October once in every 10 days and is expressed in the number of ticks in the section of 1 km.

The data of the observations during the period of tick activity are provided to the Centre for Prevention and Control of Communicable Diseases in the statistical report form No. 45

The average monthly tick abundance index, which was calculated from average of the three indexes of the "10 days" periods, or from average annual rate of tick abundance, which is calculated from average of all tick abundance indexes, were used for descriptive analysis.

Data of risk factors influencing Lyme borreliosis incidence

The data officially published by various institutions or collected from these institutions were used for factors influencing LB incidence analysis:

- The number of unemployed (share of unemployed in the total labor force and unemployment rate) in the years 1992-2005 – the data from Lithuanian Department of Statistics under the Republic of Government of Lithuania;
- The data of the Ministry of Environment about the hunted wild animals (hind, red deer, elk), records for the years 1992-2005;
- Lithuanian Hydrometeorological Service under the Ministry of Environment data about the average daily air temperature, relative humidity, rainfall, snow cover (Vilnius, Kaunas and Klaipėda cities) in the years 2000-2004. The metereological stations from different geographical areas with different LB incidence as well as those areas with permanently monitoring sites of ticks were choosen for assessment of influence of climatic factors to LB;
- National Land Service under the Ministry of Agriculture and State Registers office data - the farmland areas in the years 1991-2005.
- Environmental Protection Agency – the data about land cover (project "Lithuanian CORINE Land Cover 2000").

2.3. Material and methods of the study for prevalence of risk factors among Lithuanian population

Sample size

Sample size necessary for the study of prevalence of risk factors was compiled from the 18-74 years old Lithuanian population. In the beginning of 2006 there were 2.470.544 inhabitants of 18-74 years old residing in Lithuania according to the data of Department of Statistics under the Government of Republic of Lithuania,

The sample size was estimated using program EpiInfo (3.5.1 version (2008)). Taking in to account the risk factors with the least expected prevalence, when the least risk error of α is not greater than 0.05, and the power of the study is 80%, the sample size was estimated - 1670 adult Lithuanian.

A multistage sampling was used taking into account the distribution of 18-74 Lithuanian population by place of residence, age, gender and education. Taking in to account the data of Lithuanian Statistics Department the locations were selected maintaining the proportions of Lithuanian population in the rural and urban areas.

The study instrument

The survey of Lithuanian population for determination of prevalence of TBD risk factors was performed by mean of direct interview. The preparation of the questionnaire was based on epidemiological particularities of LB and other TBD after assessing similar studies carried out in other countries. The respondents were presented with original questionnaire out of 47 questions.

The questionnaire compiled in order to determine:

- The demographic data (age, gender, nationality, education, profession, area of residence, the number of children and other family members, average monthly incomes per family member);
- The risk factors of TBD (tick bites, visits to the forested areas, hobbies (fishing, hunting, berry picking, gathering of mushrooms), work and recreation in nature, gardening, homestead) and their frequency;
- The changes of TBD risk factors over the past five years;
- The preventive measures used (vaccinations, repellent measures, proper clothing, physical examination)
- The main TBD symptoms, previous incidence of TBE and LB.

Before the main study was carried out a pilot survey was performed questioning a group of 30 people. The wording of question and the rationale for their understanding were tested in this survey. Following the survey the remarks of the respondents were assessed and some questions were clarified, Because of the plain wording of the questions, reliability of the questionnaire was not counted.

Performance of the survey

The survey was conducted in the period from 30th November till –13th December 2006.

After the selection of the starting point in the site a person performing the survey was selecting every 3rd house and 5th apartment in it; if there were no apartments in the house, respondent was selected from every third house. According to the birthday rule (asking family members whose birthday will be the first) one respondent was selected and interviewed in the selected house or apartment. If the respondent refused to participate in the survey, the next apartment or house was selected. In case the respondent was not at home, two additional attempts were made to interview the unquestioned person.

1078 people agreeded to participate in the survey and were interwieved.

2.4. The study of tick abundance and infectivity with *Borrelia* in different biotopes

Tick collection sites selected for the study comprised closely situated biotopes: old forest with a record of LB cases, transitional young forest at previously clear-cut area, and interface between an old forest and retired farmland.

29 public health facilities agreed to participate in the study: (Alytus, Druskininkai, Lazdijai, Varėna, Kaunas, Raseiniai, Prienai, Kedainiai, Klaipėda, Klaipėda distr., Kretinga, Skuodas, Šilutė, Marijampolė, Panevėžys, Akmenė, Joniškis, Kelmė, Pakruojis, Radviliškis, Šiauliai, Utena, Ignalina, Zarasai, Ukmergė, Šalčininkai, Širvintos, Švenčionys, Vilnius). During specially arranged meeting the research methodology was introduced to the representatives of the Public Health Centres that expressed consent to participate in the study, the tick collection methodology was distributed in a written form too.

Tick collection

In coordination with the study's organizers the specialists of the Public Health Centers following a single methodology selected tick collection sites, which were composed out of three different biotopes: (1) old forest with a record of LB cases, (2) transitional young forest at previously clear-cut area, and (3) interface between an old forest and retired farmland. In one district 1-2 tick collection sites could be selected. Selected sites could not be changed throughout the year.

The ticks were collected during the year 2005 in 41 area of Lithuania. The collection of ticks was carried out 6 times during peak of tick activity (3 times between May and June and 3 times in August-September). Ticks were collected by flagging along 1 km path at each site. The ticks collected in separate sites were placed in separate tubes also separating females, males and nymphs from each other. Tubes were numbered and labeled indicating the number of tube, name of the area, biotype and collection date.

Totally 9195 Ixodes ricinus ticks were collected during all six attempts: 6652 ticks were collected in spring (May-June.), 2543 ticks in autumn (August-September).

Sorting and preparation of the ticks for tests

The ticks collected were delivered to the Centre for Prevention and Control of Communicable Diseases for check up, counting and resorting according to the type, gender and form of development (nymphs and imago), the information in cover letter was reviewed, the tick abundance index was calculated for 1 km section.

Laboratory investigation of the ticks

All nymphs selected in spring were analysed for *Borrelia* infection.

Quantitative PCR on ticks. NA was obtained from *I. ricinus* nymphs using alkaline extraction with amonium hydroxide procedure. The DNA extracts were assayed for LB and relapsing fever group spirochetes in a quantitative real time PCR (qPCR) assay with the probes and primers specific for the 16S rRNA gene of *Borrelia* species. For standards, control DNA was extracted from *Borrelia burgdorferi* B31 and *Borrelia hermsii* HS1.

Identification of *Borrelia* species and genetic variants. *Borrelia* species were identified by direct sequencing of the amplicons generated by nested PCR of partial *rrs* (16S)-*rrl* (23S) intergenic spacer (IGS) region. Sequences were initially aligned using the CLUSTAL X algorithm and then manually using MacClade 4.04 software. Positions with at least two different characters in at least two sequences each were considered polymorphic and included in the analysis. With few exceptions, singletons, i.e. variant nucleotides found in only one sequence, were ignored. Novel sequences detected were deposited in the gene bank (GeneBank).

2.5. Statistical analysis of the data

Statistical analysis of descriptive analysis of LB incidence and risk factors influencing it.

Taking in to account that the official registration of LB was started in Lithuania since 1991 it is considered due to inadequate diagnostics of this "new" disease the incidence rate of LB during the period 1991-1994 was relatively low - an average of 380 cases per year (from 60 to 676 cases), the average morbidity index of 10.2/100000 population (from 1.6 to 18.1/100000 population). Because of inadequate diagnostic in 1991-1994 this period is not included in the assessment of LB incidence trends. The period 1995-2006 is selected for assessment of LB incidence trend.

The incidence trend line (segmental regression) was analyzed by broken line regression model. This is one of the non-linear regression models. Joinpoint regression program, v. 2.7 (2003, U.S. National Cancer Institute) was used for this analysis. This program establishes linear trend. The analysis starts with the minimum (0) number of break points (one line segment) and determine whether the 1 (2 line segments), 2 (3 line segments), 3 (4 line segments) break points are statistically significant. These points are used to build the model. The final regression model defines statistically significant changes of trend and the annual changes of trends for each segment for the period are calculated in percentages. The significance level 0.05 was applied for hypothesis testing.

For assessment of correlational links of LB incidence, the abundance of ticks and other risk factors Kendell correlation coefficient was used, which was calculated using the program SPSS 11.0. The significance level 0.05 was applied for the statistical correlation assessment.

Comparison of incidence rates was done by WinPepi (1.45, 2003) program, using the Chi square (χ^2) method (Yate).

The seasonality was assessed by calculating the share (in percentages) of the diseases caused by seasonal factors.

Statistical analysis of the TBE risk factors study.

Statistical analysis was performed by using SPSS 11.0 program.

For categorical data analysis chi-square (χ^2) and Fisher's exact methods were used, for correlation strength evaluation Cramer V coefficient was used, for rank data Kruskal - Wallis method was applied.

For assessment of correlational relationship Spearman correlation coefficient (r_s) was used. For risk factors assessment logistic regression was used. Bivariate tests were used for variable screening procedure.

The variables were selected into the model when the obtained p value was <0.25. Hierarchical backward elimination procedure was used for model bilding. However, epidemiologically important variables were included in to the model despite their statistical significance. The purpose for this was to ensure control of confounding factors. χ^2, Hosmer-Lemshov test, classification table were used in the model for the assessment of the compatibility of the model. The significance of the coefficient β was assessed by Wald test. The statistical significance level was chosen $\alpha=0.05$, the results were regarded as statistically significant when $p\leq0.05$.

Statistical analysis of prevalence of ticks and infectivity

The t-test and χ^2 test was used for comparing the average means and the proportions for independent samples.

3. RESULTS OF THE STUDY

3.1. Analysis of Lyme borreliosis incidence

Trends of Lyme borreliosis and their changes in Lithuania

The average incidence of LB was 42.3/100000 population (average annual number of registered LB cases 1502.4) in the period 1995-2006. LB incidence varies every year – the highest was in 2003, when the incidence rate was 106/100000 population (3688 cases), the lowest incidence was in 1999 – the incidence rate 20.7/100000 population (766 cases) and in 1996 – the incidence rate 21.2/100000 population (785 cases).

After comparison of the incidence of LB in different periods, i.e. in the years 1995-2000 and 2001-2006, there were no statistically significant differences detected (Table 1).

Table 1. Comparison of the incidence of Lyme borreliosis in different periods in 1995-2006.

Year	Average number of cases	Average incidence rate per 100000 population.	*p*
1995-2000	1227.3	33.14	0.178
2001-2006	1777.5	51.4	
1995-2006	1502.4	42.3	

The LB incidence trend observed in the period 1995-2006 was not statistically significant (linear regression coefficient (b) equal to 0.068, *p = 0.083*). The average annual change of LB incidence in the period 1995-2006 was 7.08% (-1.06, 15.88, 95% CI). The broken line regression model did not detect any statistically significant change of LB incidence dynamics in any segment. The p-value of 1-4 line segment conversion test (permutation test) was equal to 0.850, 0.964, 0.955 while checking the zero hypothesis with alternative 1, 2 and 3 points of the joint point hypothesis.

Taking in to account the fact that vectors of *Borrelia* and TBE virus are the same (Ixodes ticks), besides there is a strong relationship (Kendall correlation coefficient 0.97, *p<0.01*) in the dynamics of LB and TBE incidence, the changes of TBE dynamics were analyzed too. The observed trend of TBE incidence during this period was declining, but not statistically significant (b=-0.195, *p=0.081*). The average annual change in incidence of TBE in period 1995-2006 was -17.71 (-34.21, 2.92, 95% CI). As in the case of LB's no statistically significant change in the dynamic of incidence was observed. Checking the zero hypothesis (joint point score of zero) with the alternative 1, 2 and 3 the permutation test p-values 0.979, 0.993 and 0.996 received respectively.

The period 1991-1994 is not included in the assessment of LB incidence trend, because of inadequate diagnosis, but this period was assessed after evaluating the incidence trend of the other tick-borne diseases – TBE. According the perennial TBE dynamics (1968-2006) it can be assumed that in 1992-1994 a real increase of TBE and LB incidence could occur (Figure. 1).

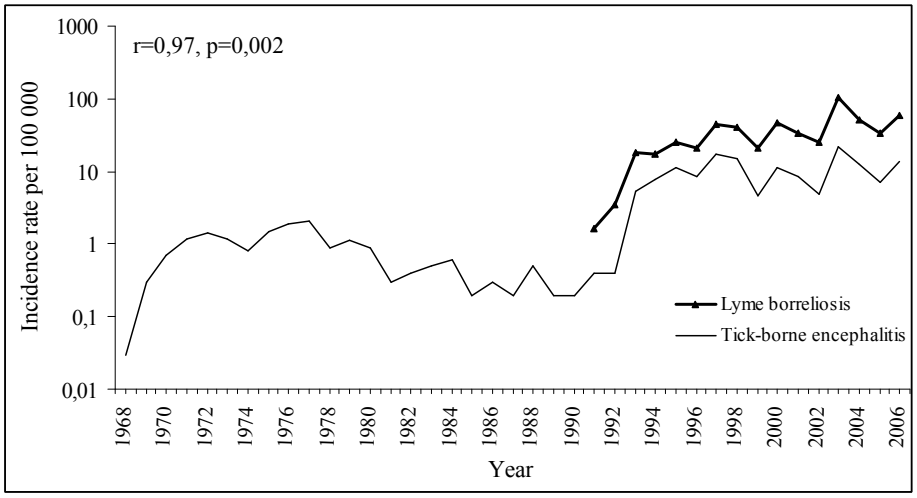


Figure 1. LB and TBE incidence in Lithuania, 1968-2006.

LB incidence by age, gender and employment

The LB incidence correlates with age - this means that incidence is increasing with age (b=9.911, *p=0.011*), i.e. LB is more frequent in adults. Children composed 9.4% during the period 1999-2006 among all cases. There were no cases of children younger than 1 year age recorded in 1999-2004.

Increased incidence of LB observed among older people: the highest in age group of 50-59 years and the averaged in 1999-2006 reaches 85.6/100000 population, the lowest incidence of the youngest 0-9 years age group (Table 2).

Table 2. Lyme borreliosis incidence and it's structure by age groups, 1999-2006.

Age group	Average incidence rate per 100 000 population	Percent
0-9 years	17.6	4.0
10-17 years	21.0	5.4
18-29 years	33.4	11.8
30-39 years	51.2	15.9
40-49 years	62.6	19.1
50-59 years	86.5	20.1
60 and over	56.7	23.6
Total	47.5	100.0

The incidence rates in adult age groups (30 years and older) are 2-3 and more folds higher than in children's age group of 0-9 years and 10-17 years. The incidence in the age groups of 0-9 and 10-17 years was statistically significantly different compared to the incidence in 18-29, 30-39, 40-49, 50-59, 60 years old and older age groups ($p<0.05$). There were no difference of the incidence among older age groups (18-29, 30-39, 40-49, 50-59, 60 years of age and older persons).

Analysis of the LB incidence by gender showed that although the annual incidence of women were higher than in men during the period 1999-2006, but statistically significant difference between these groups has not been established ($\chi^2=3.17$, $p=0.08$).

LB incidence analysis by occupation is inadequate and very limited, because only few risk groups have official records of incidence (foresters, agricultural workers, field workers, the unemployed, pensioners). The major part of official data at national level is not detailed by occupation or profession.

According to data analysed the unemployed people (retired and unemployed) compose a big part of LB incidences. The retired people in 1999-2006 composed in average 24.3% (in average 391 case/year) of cases, the unemployed people in 2004-2006 - 12.4% (in average 197.3 cases/year).

By occupations, which can be attributed to LB risk groups, 109 cases were recorded in 1999-2006 among foresters (an average 13.6 cases/year, 0.84%), 52 cases were recorded in 2004-2006 among agricultural workers (an average 17.3 cases/year, 1.04%), 46 cases among field workers (an average 15.3 cases/year, 0.9%).

LB incidence by place of residence

A comparison of incidence by place of residence shows that urban residents more frequently suffer from LB than rural residents. The LB incidence between the urban population is nearly two fold higher than among rural ($p<0.05$).

The incidence rates in urban and rural population were 40.7/100000 population and 60.83/100000 population among urban and 19.52/100000 population and 32.83/100000 population among rural population in 1995-2000 and 2001-2006 respectively, but the

difference between separate periods was not statistically significant neither among rural nor among urban populations. Though both urban and rural population increased in morbidity during 2001-2006 period, but the ratio remained unchanged - the city population of almost 2 folds more often was suffering from LB than rural residents (χ^2=5.676, *p=0.017*).

In 41-50% of LB cases from the period 2004-2006 it was indicated that the patient could have been infected in the forest, 10-17% in home environment.

Seasonality of Lyme borreliosis

Lyme borreliosis is recorded throughout the year in Lithuania, but the disease is characterized by clearly expressed seasonality. During 1999-2006, compared to seasonal morbidity each year separately, it was noted that the LB is characterized by incidence peaks almost at the same time every year.

The seasonal peak of LB incidence was observed from August to September in 1999-2006. During 3 month period of seasonal increase from 52.9 to 62.5% cases were recorded with LB out of all cases. (Table 3).

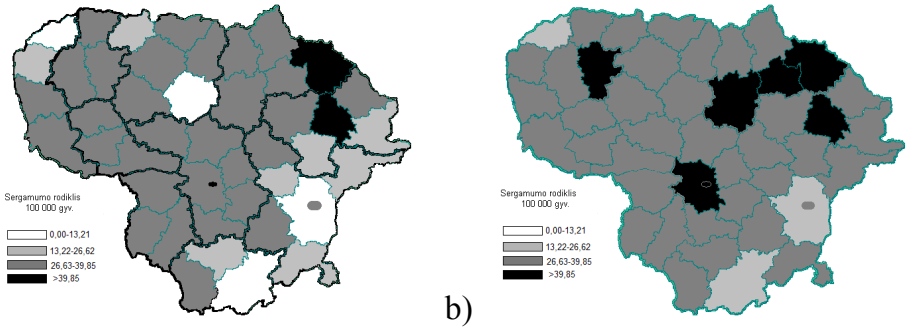
Table 3. The main indicators of Lyme borreliosis seasonality in Lithuania, 1999-2006.

Year	Peak date (χ^2) *	Sezoninio pakilimo laikotarpis The period of seasonal peak				
		2 month (cases %*)	3 month (cases %*)	4 month**	5 month**	6 month**
1999	August 25 (472.39)	August-September (37.5)	July - September (55.4)	June - September	June - October	June - November
2000	August 26 (1446.15)	August - September (35.7)	July - September (62.5)	July - October	June - October	June - November
2001	August 27 (682.8)	August - September (19.8)	July - September (54.6)	July - October	June - October	June - November
2002	August 1 (558.78)	July - August (22.6)	June - August (59.6)	June - September	May - September	May - October
2003	September 12 (3400.34)	September - October (49.6)	August - October (59.6)	July - October	July - November	June - November
2004	August 29 (664.91)	August - September (22.5)	July - September (52.9)	July - October	June - October	June - November
2005	August 11 (823.64)	August - September (24.2)	July - September (61.2)	June - September	June - October	May - October
2006	September 12 (1710.51)	September - October (38.51)	August - October (62.2)	July - October	July - November	June - November

**p<0,01*
*** p<0,05*

Geographical distribution of Lyme borreliosis incidence and the analysis of trends in the counties

Ixodes ricinus ticks are prevalent throughout the territory of all Lithuania, as well as in all areas of the Lithuania ticks infected with *Borrelia* are found, but the analysis of incidence shows that the geographical distribution of LB is not uniform (Figure 2).



a) b)

Figure 2. Geographical distribution of Lyme borreliosis in 1995-2000 (a) and in 2001-2006 (b).

In 4 districts (Skuodas, Radviliskis, Vilnius and Varėna) an extremely low incidence was recorded and only in two districts (Rokiskis and Utena) it was particularly high in 1995-2000. In subsequent years the highest incidence rates of LB were determined in 6 areas situated in Panevėžys, Kaunas, Šiauliai and Utena counties. During this period the number of districts with low LB incidence rate decreased.

The highest LB incidence rate registered in Panevėžs, Kaunas and Utena counties in 1995-2006.

After comparison of the incidence in the counties in the periods 1995-2000 and 2001-2006 it is observed that it increased in all counties. More than four-fold increase observed in Telšiai county and more than two fold in Alytus county.

The analysis of LB incidence trend across counties in Lithuania indicated that there were no statistically significant breaks of trends observed in the period 1995-2005. However, certain pecularities of the overall trends in separate counties were discovered (Table 4).

Table 4. Trends and changes of LB incidence rate in the counties, 1995-2006.

County	Changes of incidence		Trend
	Percent	95 % PI	*p*
Alytus	14.51	3.96 – 26.14	0.011
Kaunas	4.80	-3.75 – 14.19	0.246
Klaipėda	1.08	-8.02 – 11.08	0.804
Marijampolė	4.34	-4.80 – 14.37	0.326
Panevėžys	-3.16	-16.14 – 11.84	0.630
Šiauliai	10.56	-1.39 – 23.97	0.079
Tauragė	0.03	-9.69 – 10.80	0.994
Telšiai	24.78	11.51 – 39.63	0.001
Utena	3.49	-3.75 – 11.28	0.317
Vilnius	10.71	3.14 – 24.78	0.009
In Lithuania	7.08	-1.06 – 15.88	0.083

The average annual change of LB incidence in the counties ranged from -3.16 to 24.78% during the period 1995-2006.

In the Alytus, Telšiai and Vilnius counties a statistically significant non-linear trend of incidence increase was observed during the period 1995-2006. However, statistically

significant changes of trends were not determined in different periods (Alytus county *p=0.644, p=0.748, p=0.438*; Telšių county *p=0.715, p=0.486, p=0.747*; Vilnius county *p=0.936, p=0.852, p=0.746*).

The trends of incidence increase in Kaunas, Klaipeda, Šiauliai and Utena counties were not statistically significant in 1995-2006. General trend observed in Tauragė county was also statistically insignificant. Incidence trend observed in Panevėžys county was slightly decreasing, but statistically insignificant in 1995-2006. There were no statistically siginificant changes observed too in the trends of incidence in these counties during different periods.

3.2. Descriptive analysis of risk factors

Analysis of tick abundance data

The mean tick abundance in 1995-2006 was 15.4 ticks in 1 km route. In different comparable periods the abundance of ticks in 2001-2006 was 16.2 ticks in 1 km route, 14.6 ticks in 1 km route in 1995-2000 (*p>0.05*) (Table 5).

Table 5. Comparison of tick abundance and Lyme borreliosis in 1995-2006.

Year	Average index of tick abundance in 1 km route	*p*	Average LB incidence rate per 100000 population	*p*
1995-2000	14.6	*0.178*	33.2	*0.540*
2001-2006	16.2		51.4	
1995-2006	15.4		42.3	

The morbidity of TBE, including LB, is associated with the activity of ticks. Statistical analysis showed a medium strength link between the incidence of LB and tick abundance (Kendall correlation coefficient 0.49, *p=0.028*). This confirms the assumption that the LB incidence directly depends on the ticks' activity.

Analysing the seasonal data of separate areas (Vilnius city and Vilnius district, Kaunas city and Kaunas district, Klaipėda city and Klaipėda district), i.e. monthly LB incidence and tick abundance in 2000-2004, a statistically significant correlation between the monthly incidence rate and the average monthly tick abundance was not found: Vilnius city and Vilnius district LB incidence rate and tick abundance index in Vilnius Bukčių ticks observation site, respectively r=0.049, *p=0.762* and r=-0.220, *p=0.227*, Kaunas city and Kaunas district incidence and abundance of ticks in Kaunas district Vaišvydava ticks observation site respectively r=-0.102, *p=0.405* and r=-0.139, *p=0.263*, Klaipėda city and Klaipėda district and the incidence of tick abundance in Klaipėda Girulių ticks observation site respectively r=-0.199, *p=0.110* and r=-0.045, *p=0.74*.

LB incidence rate and tick abundance correlation with climatic factors

The activity of ticks and the LB incidence dependence on climatic factors estimated analyzing the average air temperature, rainfall, relative humidity, snow cover.

Analysis of these factors in Vilnius, Kaunas and Klaipėda cities and districts showed a strong statistically significant correlation between the LB incidence and the average air temperature, negative, statistically significant correlation between the incidence and the snow cover and a weak but statistically significant relationship between incidence and rainfall. Statistically significant correlations were detected between tick abundance and air temperature in Vilnius city, between the tick abundance and rainfall in Klaipeda city. Statistically significant correlations were detected in Vilnius city and Kaunas district between tick abundance and relative humidity, a weak negative correlation between tick abundance and snow cover in Kaunas district (Table 6).

Table 6. Correlation of LB incidence, tick abundance and climatic factors, 2000-2004.

	Average monthly air temperature, °C		Average rainfall, mm		Average monthly relative humidity, %		Average monthly snow cover, cm	
	r	*p*	*r*	*p*	*r*	*p*	*r*	*p*
Monthly number of Lyme borreliosis cases								
Vilnius city	0.57	*<0.01*	0.20	*0.03*	-0.19	*0.03*	-0.57	*<0.01*
Vilnius district	0.16	*0.12*	0.10	*0.32*	0.03	*0.75*	-0.15	*0.16*
Kaunas city	0.44	*<0.01*	0.26	*0.01*	-0.03	*0.74*	-0.41	*<0.01*
Kaunas district	0.42	*<0.01*	0.26	*0.01*	0.01	*0.98*	-0.41	*<0.01*
Klaipėda city	0.44	*<0.01*	0.36	*0.01*	-0.09	*0.34*	-0.41	*<0.01*
Klaipėda district	0.49	*<0.01*	0.15	*0.14*	-0.26	*0.01*	-0.42	*<0.01*
Average monthly tick abundance index in observation sites								
Vilnius Bukčių forest	0.44	*0.01*	-0.03	*0.86*	-0.37	*0.02*	-0.13	*0.51*
Kaunas district Vaišvydavos forest	0.10	*0.39*	-0.22	*0.08*	-0.34	*0.01*	-0.33	*0.02*
Klaipėda city Girulių forest	0.10	*0.42*	-0.31	*0.01*	-0.14	*0.23*	-0.20	*0.17*

LB incidence and tick abundance correlation with the abundance of tick hosts

The accounting data from the period 1995-2006 of large wild animals (elk, red deer and hind) was used for the analysis of correlations between the fluctuation of wild animal abundance and the LB incidence and the abundance of ticks. A weak and not statistically significant correlation was detected between the abundance of large cattle (elk, red deer and roe deer) and LB incidence (respectively r=0.127, *p=0.586*, r=-0.200, *p=0.392* and r=0.273, *p=0.243*) and tick abundance (respectively r=0.164, *p=0.484*, r=0.127, *p=0.586* and r=0.091, *p=0.697*) (Table 7).

Table 7. Correlation between Lyme borreliosis incidence, tick abundance and large wild animals, 1995-2005

Year	LB incidence rate per100000 population*	Average annual index of tick abundance (No of ticks in 1 km)**	No of elk	No of red deer	No of hind
1995	25.3	8.1	2847	13787	40859
1996	21.2	15	2995	14124	39779

Year	LB incidence rate per100000 population*	Average annual index of tick abundance (No of ticks in 1 km)**	No of elk	No of red deer	No of hind
1997	44.9	16	3827	14988	36226
1998	40.5	17.8	4604	16071	44233
1999	20.7	14.7	4852	15429	54209
2000	46.26	16.2	5409	15181	68571
2001	33.03	16.8	2818	12660	68680
2002	25.69	11	4458	11098	69276
2003	106	16.3	4092	10584	72945
2004	50.65	17.6	3897	12599	81241
2005	33.79	10.1	3860	11199	75886
r (*p*)*			0.127 *(0.59)*	-0.200 *(0.39)*	0.273 *(0.24)*
r (*p*)**			0.164 *(0.48)*	0.127 *(0.59)*	0.091 *(0.70)*

Correlation of LB incidence with population employment data

The number of unemployed, i.e. unemployed share in the labor force and unemployment rate, was analyzed as one of the social factors that may influence the LB incidence, but statistically significant correlation between the incidence of LB and these factors were not found (Table 8).

Table 8. Correlation of LB incidence with unemployed and the rate of unemployment, 1995-2006.

Year	LB incidence rate per100000 population *	No of unemployed persons (average annual No)	Share of unemployed persons in the labor force (%)	Unem-ployment rate
1995	25.3	109014	6.1	n.a
1996	21.2	124534	7.1	n.a
1997	44.9	104452	5.9	n.a
1998	40.5	113688	6.4	13.2
1999	20.7	148655	8.4	14.6
2000	46.26	204908	11.5	16.4
2001	33.03	223480	12.5	17.4
2002	25.69	198350	11.3	13.8
2003	106	167033	8.1	12.4
2004	50.65	142485	6.8	11.4
2005	33.79	132900	4.8	8.3
2006	59.4	89300	3.4	5.6
r (*p*)*		-0.091 *(0.68)*	-0.152 *(0.49)*	-0.389 *(0.14)*

Correlation of LB incidence and tick abundance with land cover data

Analizing the official land use areas a fluctuation in land use was observed from the year 1991 till 1995. The land areas designated for agricultural use decreased by 15% during the period 1991-2004. Weak negative correlation between agricultural land and LB incidence in 1995-2005 (r= -0.18, *p=0.411*) was identified. The changes of landscape and

transformation of land use undoubtedly are related with abundance of ticks and small rodents and also with the formation of new Lyme disease sites (Table 9).

Table 9. Correlation of LB incidence and tick abundance with agricultural land data, 1995-2006.

Year	LB incidence rate per100000 population *	Average annual index of tick abundance (No of ticks in 1 km)**	Agricultural lands, ha
1995	25.3	8.1	3196600
1996	21.2	15	3511600
1997	44.9	16	3524100
1998	40.5	17.8	3253197
1999	20.7	14.7	3513000
2000	46.26	16.2	3506000
2001	33.03	16.8	3504004
2002	25.69	11	3502104
2003	106	16.3	3496760
2004	50.65	17.6	3495534
2005	33.79	10.1	3488730
2006	59.4	25.4	3487121
r (*p*)*			-0.182 *(0.41)*
r (*p*)**			-0.152 *(0.49)*

The modernization of agriculture is one the directions of the EU market and environmental development policies. The modernization led to the reduction of cultivated farmlands and spreading of naturally revegetating areas in Central and Eastern European countries, especially in the Baltic states. The land use in Lithuania revealed about 15% increase in retired farmland areas during the last 14 years. In addition to this pattern of landscape change, in the 1990's forests in Lithuania were subject to over-exploitation and mismanagement. The latter factors manifested in clear-cutting, followed by formation of patches of young, transitional forests with brushes, weeds, and berry stalks within the tracts of old woodlands. The transitional forests in the country expanded from about 162450 ha to 224030 ha or by 38% during 1995-2000 and comprised about 3% of the country's total land cover.

3.3. The prevalence of risk factors of tick borne diseases among Lithuanian population

Description of respondents

1078 respondents were included in to the study of TBD risk factors prevalence in Lithuanian population. 45.6% (n = 492) men and 54.4% (n = 586) women participated in the survey. The average age of respondent was 46.7 years (men 46.75 years, women 46.65 years). 8.1% of respondents indicated having primary school education, basic - 11.0%, secondary - 34.3%, further - 25.2%, higher - 21.3%. Men and women groups were similar by level of education. 8.7% of respondents work is permanently or temporarily associated with the being in nature, 45.4% stated that tey do not work. The average monthly incomes

were 642 Lt per member of the family. 32.1% respondents lived in towns with less than 2,000 inhabitants, 18.2% - with a population of 2000-30000 people, 16.6% - with a population of 30000-190000 inhabitants and 33.1% - in the cities with a population more than 190,000 inhabitants.

Prevalence of tick bite in diferent groups of respondents

Assessing the prevalence of tick bites 46.1% (95% CI 45.51, 46.69) (n = 497) of respondents indicated that they had a tick bite once in their lifetime. 16.6% (95% CI 15.18, 18.02) (n = 179) stated that they had tick bite in survey year, 2.6% (95% CI 1.18, 4.02) (n = 28) had the first tick bite in survey year. 58.3% of individuals (n = 627) believes that ticks bites more often in recent year than five years ago.

The prevalence of tick bites in the lifetime and in the survey year was the same in men and women groups. Younger people less frequently identicated tick bites in the lifetime ($p<0.05$) – there were 37.7 and 38.2% of such individuals in the age groups 18-24 years and 25-35 ages respectively while in the groups of older age this number ranged from 46.0 to 54.4%. Assessing the tick bites in the survey years there were no differences between age groups ($p> 0.05$) - it is more likely that the tick bites with the same frequency both young and older persons, but over a longer period of life a more frequent exposure to ticks is observed. Persons with lower education (primary, secondary, further) more seldom indicated former tick bites in the life (41.4-48,7%) whereas among those with higher education there were more people - 55.2% ($p<0.05$). Assesing the observed tick bites during the year of survey that difference was not detected ($p>0.05$).

Persons whose work temporarily or permanently associated with the being in the forest/nature more often indicated a tick bites in the lifetime (58.1%) ($p<0.05$) and during the survey's year (31.2%) ($p<0.05$) than those who's work is not related to the being in the forest/nature - 43.6% in the lifetime and 14.5% in survey year. Unemployed people more often refer to the tick bites in the lifetime (46.0%) than employed respondents ($p<0.05$)

There was no dependence of tick bites observed related to gender, residential area and to amount of incomes per family member.

According to the place of residence the inhabitants of Utena, Alytus, Marijampole, Kaunas and Panevezys counties more frequently (from 51.6 to 64.3%) were indicating the tick bites, the inhabitants of Klaipėda, Telšiai, Šiauliai, Tauragė, Vilnius counties less frequently (30.3 to 45.7%) observed the tick bites.

Analyzing the frequencies of observed tick bites in areas with different LB incidence statistically significant differences were identified neither in the lifetime nor during the year of survey (Table 10).

Table 10. Distribution of respondents by observed tick bite in districts with different incidence level (average incidence rate per 100000 of population, 1995-2006).

Tick bite in lifetime					
Districts by incident rate	Incident rate	No. of respondents	Yes	No	r_s *(p)*
			No. (%.)	No. (%.)	
Very high	>39.85	503	231 (45.9)	264 (52.5)	0.01 *(0.64)*
High	26.63-39.85	184	90 (48.9)	91 (49.5)	

Tick bite in lifetime					
Districts by incident rate	Incident rate	No. of respondents	Yes	No	r_s *(p)*
			No. (%.)	No. (%.)	
Moderate	13.22-26.62	205	99 (48.3)	99 (48.3)	
Low	0-13.21	186	77 (41.4)	105 (56.5)	
Total		1078	497 (46.1)	559 (51.9)	
Tick bite in the year of the study (2006)					
Very high	>73.15	314	48 (15.3)	263 (83.8)	-0.02 *(0.50)*
High	37.18-73.15	377	72 (19.1)	299 (79.3)	
Moderate	13.81-37.17	282	41 (14.5)	235 (83.3)	
Low	0-13.80	105	18 (17.1)	83 (79.0)	
Total		1078	179 (16.6)	880 (81.6)	

Prevalence of risk factors influencing tick bite

Hobbies, recreation or work with being in nature are the main risk factors influencing more frequent exposure of humans to ticks. 25.5% of the respondents were fishing, 4.4% hunting, 83.3% picking mushrooms/berries, 78.4% gardening, 91.3% hiking, 54.8% camping overnight, 31.5% taking dog for a walk.

It was found that fishing (χ^2=11.45, $p<0.05$), picking mushrooms/berries (χ^2=35.32, $p<0.05$), gardening (χ^2=7.05, $p<0.05$), taking dog for a walk (χ^2=3.94, $p<0.05$) leads to more frequent exposure to ticks in the lifetime (Table 11).

Table 11. Prevalence of risk factors of tick borne diseases and their relation with tick bite in lifetime.

Risk factor	No. of respondents		Prevalence of risk factor	Tick bite in lifetime		OR (95% CI)	χ^2(*p*)
				Yes	No		
			No. (%)	No. (%)	No. (%)		
Fishing	1048	Yes	267 (25.5)	147 (55.1)	116 (43.4)	1.61 (1.22-2.13)	11.45 (*0.001*)
		No	781 (74.5)	337 (43.1)	427 (54.7)		
Hunting	1041	Yes	46 (4.4)	25 (54.3)	20 (43.5)	1.41 (0.78-2.56)	1.31 (*0.25*)
		No	995 (95.6)	455 (45.7)	520 (52.3)		
Picking mushrooms/ berries	1071	Yes	892 (83.3)	448 (50.2)	428 (48.0)	2.82 (1.99-4.01)	35.32 (*0.00*)
		No	179 (16.7)	43 (24.0)	130 (78.6)		
Gardening	1067	Yes	837 (78.4)	404 (48.3)	419 (50.1)	1.49 (1.11-1.99)	7.05 (*0.01*)
		No	230 (21.6)	87 (37.8)	135 (58.7)		
Taking dog for a walk	1060	Yes	334 (31.5)	169 (50.6)	156 (46.7)	1.30 (1.00-1.68)	3.94 (*0.05*)
		No	726 (68.5)	320 (44.1)	393 (54.1)		
Hiking	1056	Yes	964 (91.3)	448 (46.5)	496 (51.5)	1.15 (0.78-1.70)	0.50 (*0.48*)
		No	92 (8.7)	37 (40.2)	53 (57.6)		
Camping overnight	1067	Yes	585 (54.8)	278 (47.5)	296 (50.6)	1.13 (0.89-1.44)	1.03 (*0.31*)
		No	482 (45.2)	212 (44.0)	259 (53.7)		
Total	1078			497 (46.1)	559 (51.9)		

Fishing (χ^2=12.34, $p<0.05$), picking mushrooms/berries (χ^2=45.60, $p<0.05$), gardening (χ^2=10.51, $p<0.05$) and camping overnight (χ^2=5.3, $p<0.05$) were the main risk factors which led to more frequent tick bites during year of the study (Table 12).

Table 12. Prevalence of risk factors of tick borne diseases and their relation with tick bite in the year of the study (2006).

Risk factor	No. of respondents		Prevalence of risk factor	Tick bite in lifetime		OR (95% CI)	$\chi^2(p)$
				Yes	No		
			No (%)	No (%)	No (%)		
Fishing	1038	Yes	226 (21.8)	55 (24.3)	165 (73.0)	1.89 (1.32-2.70)	12.34 (*0.00*)
		No	812 (78.2)	116 (14.3)	686 (84.5)		
Hunting	1037	Yes	40 (3.9)	9 (22.5)	29 (72.5)	1.48 (0.69-3.17)	1.04 (*0.31*)
		No	997 (96.1)	163 (16.3)	821 (82.3)		
Picking mushrooms/ berries	1066	Yes	769 (72.1)	165 (21.5)	590 (76.7)	5.76 (3.28-10.11)	45.60 (*0.00*)
		No	297 (27.9)	9 (3.0)	285 (96.0)		
Gardening	1064	Yes	792 (74.4)	149 (18.8)	628 (79.3)	1.98 (1.30-3.00)	10.51 (*0.001*)
		No	272 (25.6)	28 (10.3)	241 (88.6)		
Taking dog for a walk	1056	Yes	240 (22.7)	41 (17.1)	194 (80.8)	1.05 (0.71-1.53)	0.05 (*0.8*)
		No	816 (77.3)	129 (15.8)	673 (82.5)		
Hiking	1053	Yes	940 (89.3)	164 (17.4)	761 (81.0)	1.73 (0.99-3.04)	3.76 (*0.05*)
		No	113 (10.7)	10 (8.8)	100 (88.5)		
Camping overnight	1055	Yes	429 (40.7)	85 (19.8)	336 (78.3)	1.46 (1.06-2.02)	5.30 (*0.02*)
		No	626 (59.3)	89 (14.2)	527 (84.2)		
Total				179 (16.6)	880 (81.6)		

Assessing the prevalence and the changes of risk factors one can see that the majority of the activities remained unchanged, but five years ago people were more often camping overnight, picking mushrooms/berries. Also more people had to take a dog for a walk (Table 13).

Table 13. The prevalence of TBE riks factors and the changes of these factors in the year of survey (2006).

Risk factor	In study year		5 years before		Change	Wilcoxon test	
	No.	%	No.	%		z	*p*
Fishing	226	21.0	240	22.3	0.94	-2.24[a]	*0.03*
Hunting	40	3.7	42	3.9	0.95	-0.79[a]	*0.43*
Picking mushrooms/ berries	669	71.3	854	79.2	0.78	-4.21[a]	*0.00*
Gardening	792	73.5	797	73.9	0.99	-0.21[b]	*0.84*
Camping overnight	329	39.8	527	48.9	0.62	-1.07[a]	*0.29*
Hiking	940	87.2	917	85.1	1.03	-2.35[b]	*0.02*
Taking dog for a walk	240	22.3	284	26.3	0.85	-3.08[a]	*0.00*

a. 5 years before>study year
b. 5 years before< study year

After summarizing the results, risk factors were grouped into separate groups to identify the most significant tick-bite related risk factors.

A statistically significant association was found between the tick bites in lifetime and the group of risk factors, which included fishing (OR_p=2.43, 95% CI 1,69-3,49), picking

mushrooms/ berries (OR_p= 1.63, 95% CI 1,18-2,24), work related to the beeing in nature (OR_p= 1.62, 95% CI 1,03-2,55), incomes less than 500 LTL (OR_p= 0.76, 95% CI 0,59-1,00)(Table 14).

Table 14. Association between risk factors and tick bite in lifetime

Risk factor	General OR	95%. CI	OR_p	95% CI
Picking mushrooms/berries	2.82	1.99–4.01	2.43	1.69-3.49
Fishing	1.61	1.22-2.13	1.63	1.18-2.24
Work with being in nature	1.69	1.08-2.68	1.62	1.03-2.55
Income less than 500 LTL.	0.76	0.59-0.98	0.76	0.59-1.00

OR_p – corrected by controlling gender, education and age

A statistically significant association was found between the tick bites during the year of the survey and the group of risk factors, which included fishing (OR_p=4.90, 95% CI 2.77-8.67), picking mushrooms/berries (OR_p=1.68, 95% CI 1.10-2.55), work related with being in nature (OR_p=2.14, 95% CI 1.29-3.58), life in the settlement with less than 2.000 inhabitants (OR_p=1.78, 95% CI 1.26-2.52) (Table 15).

Table 15. Association between risk factors and tick bite in the year of survey (2006)

Risk factor	General OR	95%. CI	OR_p	95% CI
Mushrooms/bearies	5.76	3.28-10.11	4.90	2.77-8.67
Fishing	1.89	1.32-2.70	1.68	1.10-2.55
Work in nature	2.52	1.51-4.12	2.14	1.29-3.58
Settlement with less than 2000 inhabitants	1.92	1.36-2.70	1.78	1.26-2.52

OR_p – corrected by controlling gender, education and age

The frequence of fishing and gardening affects the frequency of tick bites in the lifetime ($p<0.05$). During the year of survey tick bites frequency was related with the frequency of fishing and taking dog for a walk ($p<0.05$).

Those respondents (n=179), who idicated the number of tick bites during the year of survey, noticed the tick bite on average 2.08 times.

Use of prophylactic measures for tick borne diseases

Although the majority of respondents (69.1%, n=745) claim to have sufficient knowledge about the TBD, but only 6.5% (n=70) of respondents indicated that they were vaccinated against TBE, 41.3% of respondents were using repellents, 64.6% - chose a proper clothing, 41.1% - check their body for possible tick bites while being in nature, 81.4% - after returning home. During the year of the survey 40.4% of respondents used repellents, 63.7% chose the appropriate clothes, 40.3% were checking the body for possible tick bites while being in nature, 81.0% were checking after returning home.

Individuals using preventive measures (vaccination, repellents, appropriate clothing, check-ups while in nature and after returning home) more frequently observed tick bites in the life ($p<0.05$). Respectively during the year of survey persons using repellents and

checking their body for possible tick bites while being in nature or after returning home also were noticing the tick bites more often ($p<0.05$).

Assessing the change in the use of preventive measures, it was found that all of the recommended non-specific preventive measures are applied more often than five years ago.

Relations between tick bite and the prevalence of tick borne diseases

Among all respondents who had a tick bite, 60.6% (n=301) indicated that they suffered from certain symptoms after the tick bite – the redness in the site of tick bite, which occurred immediately after the tick bite (n=184, 61.1%), and the red-spot in bite site that occurred later (n=54, 17.9%) were indicated most frequently.

Among all respondents 1.9% (n=21) were diagnosed with TBD in health care facility (LB - 1.1%, TBE - 0.9%, both diseases - 0.1%).

Prevalence of TBD in population is about 2%. In majority of cases (90.5%) it was claimed there was a tick bite observed, two persons indicated that they had not tick bite (Table 16).

Table 16. Prevalence of tick bite and tick borne diseases

Disease	No of respondents		No of cases (%)	Tick bite in lifetime		OR	95% CI	χ^2 (p)
				Yes No. (%)	No No. (%)			
TBD	1078	Yes	21 (1.95)	19 (90.5)	2 (9.5)	11.51	2.67-49.65	16.97 (*0.00*)
		No	1057 (98.05)	478 (45.2)	579 (54.8)			
LB	1078	Yes	12 (1.1)	11 (91.7)	1 (8.3)	13.13	1.69-102.04	10.14 (*0.00*)
		No	1066 (98.9)	486 (45.6)	580 (54.4)			
TBE	1078	Yes	10 (0.9)	9 (90,0)	1 (10.0)	10.7	1.35-84.73	7.83 (*0.01*)
		No	1068 (99.1)	488 (45.7)	580 (54.3)			

3.4. Abundance of ticks and infectivity with *Borrelia* in different biotopes

Abundance of *Ixodes ricinus* ticks during spring and autumn activity peak

During the study on tick abundance and infectivity 6 ticks collections were performed and totally 9195 *Ixodes ricinus* ticks were collected. 72% (6652 ticks) were collected during the spring collection (May-June), 27% (2543 ticks) - in autumn (August-September). Among all *I. ricinus* ticks collected the adult ticks composed 83% (50% were female (3829 ticks) and 50% (3831 ticks) male), the nymphs – 17%. The distribution of adult ticks by gender was not different in spring and in autumn (Table 17).

Table 17. Ixodes ricinus ticks by stage collected during spring and autumn activity peak in 2005

Activity peak	Adult ticks		Nymphs	Total (%)
	male	female		
Spring	2808	2669	1175	6652 (72)
Autumn	1023	1160	360	2543 (28)
Total (%)	3831 (42)	3829 (42)	1535 (17)	9195 (100)

Majority of ticks (26.1%) were collected during 1st collection in spring, the least amount (8.4%) - during 4th collection in autumn. 38.6% of all nymphs were collected during 1st collection. The number of ticks by gender and stage of development is presented in Table No. 18.

Table 18. Number of ticks by number of collection sites and collections.

Collection	No of collection sites*	No. of ticks collected				
		Ixodes ricinus In total (%)	Among them:			
			Adult ticks (%)		Nymphs (%)	
I	38	2406 (26.1)	1814	(19.7)	592	(6.4)
II	35	2167 (23.6)	1834	(19.9)	333	(3.6)
III	36	2079 (22.6)	1829	(19.9)	250	(2.7)
IV	34	910 (9.9)	811	(8.8)	99	(1.1)
V	36	862 (9.4)	699	(7.6)	163	(1,8)
VI	31	771 (8,4)	673	(7,3)	98	(1.1)
Total		**9195 (100)**	7660	(83.3)	1535	(16.7)

* in some collection sites ticks were collected not in all biotopes and not during all collections.

Ticks were collected in 41 collection site. Majority of the ticks were collected in Klaipėda Giruliu forest – 10% (993 ticks) of the total number of ticks collected during the study, the least amount – in Vilnius district Nemenčinė forest - 0.3% (28 ticks). The abundance of ticks among different biotopes was similar with no core difference (t-test, *p>0.05*) in both tick collection periods. The nymphs were about 3-fold more active in spring compared to autumn. 18 adults and 4 nymphs were collected per 1 km route in spring and 8 adults and 1 nymph - in the autumn on average (Table 19).

Table 19. Abundance index of *Ixodes ricinus* ticks collected during the study (no of ticks in 1 km route) by stage and different biotopes in spring and autumn activity peaks in 2005.

Biotope	Abundance index of *Ixodes ricinus* ticks during spring and autumn collection (No of ticks in 1 road)					
	Adult ticks		Nymphs		Total	
	Spring	Autumn	Spring	Autumn	Spring	Autumn
Old forest	17.8	8.6	4.6	1.9	22.4	10.5
Clear-cut	19.5	7.5	4.4	0.9	23.9	8.4
Outer wood	15.7	6.4	2.4	0.8	18.1	7.2
Total	17.6	7.5	3.8	1.2	21.4	8.7

p>0.05

Prevalence of *Borrelia* in *Ixodes ricinus* nymphs

To determine whether the emerging tick habitats are conducive to the enzootic maintenance of LB pathogens, the prevalence of *Borrelia* infection of *I. ricinus* nymphs was next measured. Multiplex qPCR was used in order to detect and differentiate between the

LB group spirochetes and relapsing fever *Borrelia* species, including *Borrelia miyamotoi*, a relapsing fever species with global distribution, which is transmitted by *Ixodes* ticks.

156 (13%) of 1172 nymphs from the spring collection were found infected with LB group *Borrelia* spp., and 17 (1.5%) nymphs had *B. miyamotoi* infection (Table 20).
The prevalence of the infection with LB group *Borrelia* spp. or *B. miyamotoi* was similar for the nymphs from different habitats (p >0.20).

Table 20. Infection rate with *Borrelia* in *Ixodes ricinus* nymphs after qPCR analysis.

Borrelia group	No of investi-gated ticks*	No of positive samples (%)	No of positive samples by biotopes (%)		
			Old forest (n=501)	Clear-cut (n=430)	Outer wood (n=241)
Lyme borreliosis[a]	1172	156 (13.3)	70 (14.0)	48 (11.2)	38 (15.8)
Relapsing fever[b]	1172	17 (1.4)	10 (2.0)	3 (0.7)	4 (1.7)
Total	1172	173** (14.8)	80 (16.0)	51 (11.9)	42 (17.4)

* only spring collection was investigated
** 1 mix infection
[a] $\chi^2 = 3.111$, $df=2$, $p=0.211$
[b] $\chi^2 = 2.819$, $df=2$, $p=0.244$

The structure of *Borrelia* population

61 qPCR-positive samples were subject to sequencing of partial *rrs-rrl* IGS region for identification of *Borrelia* species and genetic variant in order to compare the structure of *Borrelia* population in *I. ricinus* nymphs collected in different biotopes and the population of LB reservoir hosts in the known and in the newly established biotopes. 52 samples were positive for LB group *Borrelia* species: 42 (81%) were *B. afzelii*, 9 (17%) were *B. garinii*, and 1 (2%) was *Borrelia valaisiana* for which medical importance is not known (Table 21).

Table 21. The distribution of *Borrelia* species in *Ixodes ricinus* nymphs from different biotopes.

Species	Biotopes			Total No of LB group (%)
	Old forest	Clear-cut	Outer wood	
B. afzelii	17	14	11	42 (81)
B. garinii	6	1	2	9 (17)
B. valaisiana	1	0	0	1 (2)
B. miyamotoi	4	3	2	n/d

All 9 randomly selected samples positive for the relapsing fever species were positive for *B. miyamotoi*. The identified LB group *Borrelia* species were distributed similarly in the nymphs from diverse biotopes (p >0.45) allowing to make an assumption that LB reservoir host are the same in differente biotopes.

The structure *of B. afzelii* population

B. afzelii was typed in order to determine the population structure of this specie. The *rrs-rrl* IGS sequences obtained were aligned with previously detected and described *rrs-rrl*

IGS typing matrix of *B. afzelii* and that allows to identify the cases of known or novel polymorphisms.

The *rrs-rrl* IGS locus was sequenced for 42 random samples of *B. afzelii* from infected nymphs. 29 (69%) of these ticks were infected with a single variant of *B. afzelii*, whereas the remaining 13 (31%) ticks showed evidence of infection with multiple types of spirochete's. Totally there were 57 *rrs-rrl* IGS sequences (single or in combination) detected, matching 9 genetic variants. According to the previously detected *B. afzelii rrs-rrl* IGS types most prevalent variants were the following: type 1 - 10 (18%), type 2 - 12 (21%), type 3 - 11 (19%) and type 7 - 10 (18%). Type 4, 8, 9 and NT11 variants were found in 1, 3, 6, and 3 cases respectively. Besides in one nymph a novel *rrs-rrl* IGS variant of *B. afzelii* was identified.

Variety of other *Borrelia* species

B.garinii detected in 9 samples from 8 Lithuanian areas. The fact that in collected ticks the *B. garinii* circulates along with *B. afzelii* was detected in at least three areas - Klaipėda county (Liverių forest, Ankštakių forest) and Panevėžys district (Žalioji Forest), with *B. miyamotoi* it circulates in Druskininkai Drapaliu forest. *B. afzelii, B. garinii* and *B. miyamotoi* were detected in Kėdainių district Babėnų forest,

8 different alleles were detected after the sequencing of *B.garinii*. A comparison of gene sequences with the previously described sequences showed that at least 4 of them are identical to those, which were identified in LB patients in Sweden.

Borrelia belonging to Relapsing fever *Borrelia* group was detected in 17 samples out of 8 places in Lithuania. These samples were specifically selected and tested for possible infectivity with *B.miyamotoi*. Studies have confirmed that this type of *Borrelia* is widespread in Lithuania. In our study it was found in the forests of Druskininkai, Prienai, Kėdainiai, Klaipėda, Šilutė and Joniškis districts.

9 *B.miyamotoi* positive samples were identical to each other and completely matched the variant found in Sweden.

4. CONCLUSIONS

1. There were no statistically significant changes in overall Lyme disease incidence dynamics during the period 1995-2006t, but Lyme borreliosis natural habitat has the property to expand at the expense of areas where the incidence rate was previously low.

2. The climatic (average air temperature and snow cover) risk factors are the most important ones influencing the incidence of Lyme borreliosis in Lithuania. Although the social and ecological factors may be relate to LB spread, but they are less important.

3. Tick bites was observed by 46.1% of Lithuanian population during the lifetime, 16.6% tick bite was observed during the year of survey, 2.6% of population observed a tick bite the first time during the year of survey. Significant risk factors influencing tick bites were fishing, picking mushroom / berry, work or being outdoor, low-income and living in small settlements.

4. Nymps' infectivity in Lithuania is 14.8%. *B. afzelii* (81%) is dominating among *B. burgdorferi* s.l. transmitted by the *Ixodes ricinus* nymphs. *B. afzelii* together with *B. garinii* (17%) includes variants that are pathogenic to humans.

5. There is no difference in infectivity of ticks form new or old biotopes and this indicates that the natural cycle of LB's quickly recovers and may lead to the spread of Lyme borreliosis in Lithuania. Similar composition of species in ticks from different biotopes might be caused by common reservuar of infection both in known and re-emerging enzootic sites (new biotopes).

5. Prevalence of *B. miyamotoi* detected in Lithuania indicates that the habitat of this type of *Borrelia* is not limited only to Scandinavia but reaches the region of Easten Europe.

LIST OF PUBLICATIONS

– L. Ašoklienė, K. Žagminas, J. Bunikis, B. Morkūnas. Sergamumo Laimo liga pokyčiai Lietuvoje 1995-2006 metais // Visuomenės sveikata. 2007, Nr. 2, p. 56-60.

– L. Ašoklienė, K. Žagminas, J. Bunikis. Erkių įkandimų paplitimas ir juos sąlygojantys veiksniai Lietuvoje // Visuomenės sveikata. 2008, Nr. 3, p. 70-79

– P. Comstedt, L. Asokliene, I. Eliasson, B. Olsen, A. Wallensten, J. Bunikis, S. Bergstrom. Complex population structure of Lyme borreliosis group spirochete *Borrelia* garinii in subarctic Eurasia. PLoS One**.** 2009, 4(6): e5841

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REZIUME

Laimo boreliozė (LB) yra dažniausia pernešėjų platinama infekcija ES, kur kasmet registruojama apie 85000 šios ligos atvejų. Vidutiniškai Lietuvoje kasmet registruojama apie 1500 susirgimų LB, 1991-2006 metais vidutinis sergamumo rodiklis buvo 34/100 000 gyventojų.

LB plitimą lemia erkių gausa ir jų infekuotumas LB sukėlėjais – borelijomis. Europoje *Ixodes ricinus* erkės, pernešančios LB sukėlėjus didžiojoje Europos dalyje, taip pat ir Lietuvoje, paplitę visuose šalies rajonuose. Suaugusiųjų erkių infekuotumas Europoje 3-58 proc. (vidutiniškai 17,4 proc., nimfų 2-43 proc. (vidutiniškai 10,8 proc.), lervų 0-8 proc. (vidutiniškai 1,9 proc.). Lietuvoje suaugusių erkių užkrėstumas borelijomis vidutiniškai siekia nuo 6,9 iki 13,7 proc. Nimfų infekuotumo duomenys Lietuvoje yra nepakankami.

Tiek klinikiniu, tiek ekologiniu aspektu svarbu, kokie borelijų genotipai paplitę konkrečioje teritorijoje. Nerimą kelia naujai atrastų erkių platinamų organizmų, kurių medicininė reikšmė yra žinoma arba galima, pvz., įvairių *Borrelia*, *Rickettsia*, *Anaplasma*, *Babesia* rūšių, paplitimo didėjimas, tačiau šių organizmų paplitimo išaiškinimas Lietuvoje yra nepakankamas.

Europoje didelis dėmesys skiriamas erkių gausą, o kartų ir LB sergamumą, įtakojančių veiksnių nustatymui. Lietuvoje LB ir erkių platinamų ligų (EPL) rizika nėra išsamiai apibūdinta, neįvertinta, kurie veiksniai, klimatiniai-gamtiniai ar socialiniai yra labiausiai susiję su LB ir EPL.

Darbo tikslas – įvertinti Laimo boreliozės epidemiologinius dėsningumus ir juos sąlygojančius svarbiausius veiksnius (gamtinius-klimatinius ir socialinius) Lietuvoje 1995–2006 m.

Darbo uždaviniai

1. Aprašyti bendrus epidemiologinius sergamumo Laimo borelioze dėsningumus: daugiametę dinamiką, sezoniškumą, teritorinį pasiskirstymą, sergamumą įvairiose amžiaus ir socialinėse grupėse.
2. Įvertinti gamtinių-klimatinių ir socialinių veiksnių įtaką sergamumui Laimo borelioze Lietuvoje.
3. Nustatyti erkių įkandimų ir kitų erkių platinamų ligų rizikos veiksnių paplitimą bei pokyčius per 5 metus tarp Lietuvos gyventojų ir įvertinti, kokie veiksniai yra labiausiai susiję su erkių įkandimais.
4. Įvertinti erkių paplitimą ir nimfų infekuotumą borelijomis naujuose, anksčiau netirtuose biotopuose, nustatyti borelijų genotipinę struktūrą.

Darbo aktualumas

Lietuvoje LB srityje atlikti tyrimai daugiausia skirti erkių gausos ir infekuotumo borelijomis nustatymui, didžiausią dėmesį skiriant suaugusių erkių infekuotumui ir jose aptiktų borelijų genotipavimui. Svarbu tai, kad LB sukėlėjus žmogui gali perduoti ir suaugusios erkės, ir nimfos, tačiau pastarųjų vaidmuo LB epidemiologijoje gali būti kur kas reikšmingesnis, kadangi nimfos yra mažesnės nei suaugusios erkės ir maitinasi trumpiau,

todėl dažnai lieka nepastebėtos, o tai užtikrina ne tik pasimaitinimo, bet ir sukėlėjų perdavimo ir jų plitimo galimybę . Iki šiol Lietuvoje atlikti šios stadijos erkių infekuotumo tyrimai buvo tik epizodiniai, nedidelės apimties, neleidžiantys daryti išvadų apie nimfų infekuotumą LB borelijomis ir paplitusius borelijų genotipus. Taip pat naujų, kitose šalyse aptiktų erkių platinamų borelijų paplitimo nustatymas gali būti svarbus klinikine prasme, pavyzdžiui, ieškant neaiškios etiologijos karščiavimų priežasties.

Lietuvoje sergamumą LB ir kitomis EPL gali įtakoti ne tik bendri visai Europai būdingi rizikos veiksniai, bet ir kai kurie specifiniai, būdingi tik Lietuvai ar Baltijos šalims veiksniai. Šie veiksniai gali tiesiogiai ar netiesiogiai sąlygoti erkių gausos pokyčius, tačiau pasikeitusių ar naujų biotopų atsiradimo įtaka EPL plitimo atžvilgiu Lietuvoje iki šiol nebuvo nagrinėta. Taip pat nenagrinėta įvairių rizikos veiksnių koreliacija su sergamumu LB bei erkių gausa.

Mokslinis darbo naujumas

Šiame darbe išnagrinėti ir aprašyti LB sergamumo dėsningumai, aprašyti pagrindiniai LB epidemiologiniai požymiai, įvertintos ir statistiškai pagrįstos sergamumo tendencijos. Taip pat atlikta LB rizikos veiksnių analizė, nustatyti labiausiai LB plitimą Lietuvoje įtakojantys gamtiniai-klimatiniai, ir socialiniai rizikos veiksniai, nustatytos ir įvertintos įvairių rizikos veiksnių sąsajos (koreliacijos) su LB sergamumu ir erkių gausa. Atlikus gyventojų apklausą, nustatytas erkių įkandimų ir erkių platinamų ligų rizikos veiksnių paplitimas tarp Lietuvos gyventojų bei įvertintas šių veiksnių pokytis per penkerius metus.

Darbe pirmą kartą įvertinta naujai susiformavusių biotopų galima ekologinė reikšmė LB plitimui. Taip pat atlikus molekulinius tyrimus, nustatytas borelijų paplitimas ir genotipai *Ixodes ricinus* nimfose, surinktose skirtinguose biotopuose bei išaiškinta nauja, iki šiol Lietuvoje neaptikta grįžtamosios karštinės borelijų grupei priklausanti *Borrelia miyamotoi*.

Ginamieji disertacijos teiginiai

- Gamtiniai – klimatiniai, ekologiniai ir socialiniai veiksniai įtakoja erkių arealo plitimą ir sergamumą LB Lietuvoje.
- Landšafto natūralizacija ir susiformavę nauji biotopai sąlygoja LB židinių atsiradimą.
- Lietuvoje nimfų infekuotumas borelijomis nesiskiria nuo suaugusiųjų erkių.
- *Borrelia miyamotoi* arelas neapsiriboja Skandinavijos šalimis ir yra didesnis, nei iki šiol nustatyta.

Tyrimo metodika

Siekiant darbo tikslo ir uždavinių atlikta LB sergamumo aprašomoji analizė, jį įtakojančių rizikos veiksnių aprašomoji analizė, EPL rizikos veiksnių paplitimo tarp gyventojų tyrimas, erkių gausos ir infekuotumo borelijomis paplitimo skirtinguose biotopuose tyrimas.

LB ir kitų EPL sergamumo analizei atlikti panaudoti oficialūs Užkrečiamųjų ligų profilaktikos ir kontrolės centro ir Valstybės užkrečiamųjų ligų registro duomenys. Surinkti

sergamumo duomenys analizuoti dinamikoje, nustatant ir statistiškai įvertinant sergamumo tendencijas, sergamumą pagal amžių, lytį, sezoniškumą, geografinį pasiskirstymą.

LB sergamumą įtakojančių veiksnių analizei panaudoti įvairių institucijų oficialiai skelbiami arba iš šių institucijų gauti duomenys (klimatiniai, socialiniai, ekologiniai)

Rizikos veiksnių paplitimui tarp Lietuvos gyventojų nustatyti atlikta gyventojų apklausa interviu būdu pagal specialiai parengtą klausimyną.

Erkių gausos ir infekuotumo tyrimui atlikti 41 Lietuvos vietovėje 3 skirtinguose biotopuose buvo surinkta 9195 *Ixodes ricinus* erkių. Pavasarinio rinkimo nimfos buvo tiriamos molekuliniais tyrimais infekuotumui borelijomis nustatyti.

Rezultatai

Laimo boreliozės sergamumo analizė

1995-2006 metų laikotarpiu vidutinis LB sergamumo rodiklis buvo 42,3 100000 gyv. (vidutiniškai kasmet užregistruota 1502,4 LB atvejai), šiuo laikotarpiu stebima LB sergamumo didėjimo tendencija nėra statistikai reikšminga (netiesinės regresijos koeficientas (*b)* lygus 0,068, p=0,083).

Kadangi dėl nepakankamos LB diagnostikos į sergamumo tendencijos vertinimą neįtrauktas 1991-1994 metų laikotarpis, šis laikotarpis įvertintas nustačius kitos erkių platinamos ligos – erkinio encefalito – sergamumo tendenciją. Įvertintus daugiametę EE sergamumo dinamiką (1968-2006 m.) galima daryti prielaidą, kad 1992-1994 metais galėjo įvykti realus tiek EE, tiek LB sergamumo padidėjimas.

Sergamumas LB koreliuoja su amžiumi – tai reiškia, kad didėjant amžiui didėja sergamumas (b = 9,911, $p = 0,011$), t.y. LB dažniau serga suaugę žmonės. 1999-2006 m. laikotarpiu vaikai sudarė 9,4 proc. visų susirgusiųjų. 1999-2004 m. susirgimų vaikų, jaunesnių kaip 1 metų amžiaus grupėje nebuvo. Dažnesnis sergamumas LB stebimas tarp vyresnio amžiaus žmonių: didžiausias 50-59 m. amžiaus grupėje ir 1999-2006 m. laikotarpiu vidutiniškai siekia 85,6/100.000 gyv., mažiausias sergamumas jauniausioje – 0-9 m. amžiaus grupėje.

Analizuojant sergamumo LB duomenis pagal lytį, nustatyta, kad nors 1999-2006 metų laikotarpiu kasmet moterų sergamumo LB rodikliai buvo didesni nei vyrų, tačiau statistiškai reikšmingas skirtumas tarp šių grupių nenustatytas (χ^2=3,17, $p=0,08$).

LB sergamumo analizė pagal užsiėmimą/profesiją yra nepakankama ir labai ribota, kadangi oficialiai registruojamas tik keleto rizikos grupių ar profesijų sergamumas LB. Pagal analizuotus duomenis, didelę dalį susirgusiųjų LB sudaro niekur nedirbantys žmonės (pensininkai ir bedarbiai). 1999-2006 metais vidutiniškai 24,3 proc. (vidutiniškai 391 atvejis/metus) tarp visų susirgusiųjų buvo pensininkai, 2004-2006 metais 12,4 proc. – bedarbiai (vidutiniškai 197,3 atvejai/metus).

Palyginus gyventojų sergamumą pagal gyvenamąją vietą, matyti, kad miesto gyventojai dažniau nei kaimo gyventojai serga LB. Sergamumas LB tarp miesto gyventojų yra beveik du kartus didesnis nei tarp kaimo ($p<0,05$).

Laimo boreliozės susirgimai Lietuvoje registruojami ištisus metus, tačiau šiai ligai būdingas aiškiai išreikštas sezoniškumas. 1999-2006 metais sezoninis LB sergamumo

pakilimas stebėtas liepos-spalio mėnesį. Šio pakilimo metu Laimo liga vidutiniškai susirgo 71,7 proc. visų per metus susirgusiųjų asmenų.

Palyginus sergamumą apskrityse 1995-2000 m. ir 2001-2006 m. laikotarpiais, matyti, kad visose apskrityse jis padidėjo. Daugiau kaip keturis kartus sergamumas išaugo Telšių ir daugiau kaip du Alytaus apskrityse. Vidutinis metinis Laimo ligos sergamumo pokytis apskrityse 1995-2006 metų laikotarpyje svyravo nuo -3,16 iki 24,78 proc.

Alytaus, Telšių ir Vilniaus apskrityse 1995-2006 metų laikotarpyje stebima bendra statistiškai reikšminga netiesinė sergamumo didėjimo tendencija. Tačiau šiame laikotarpyje nenustatyta sergamumo tendencijos statistiškai reikšmingų pokyčių skirtingais periodais (Alytaus apskrityje $p = 0,644$, $p = 0,748$, $p = 0,438$; Telšių apskrityje $p = 0,715$, $p = 0,486$, $p = 0,747$; Vilniaus apskrityje $p = 0,936$, $p = 0,852$, $p = 0,746$).

Rizikos veiksnių aprašomoji analizė

Erkių platinamų ligų, taip pat ir Laimo ligos, sergamumas susijęs su erkių aktyvumu. Statistinė analizė parodė stiprų ryšį tarp Laimo ligos sergamumo ir erkių gausos (Kendall koreliacijos koeficientas lygus 0,49, $p = 0,028$). Tai patvirtina prielaidą, kad sergamumas LB tiesiogiai priklauso nuo erkių aktyvumo.

Erkių aktyvumo ir sergamumo Laimo liga priklausomybė nuo klimatinių veiksnių, vertinta analizuojant vidutinę oro temperatūrą, kritulių kiekį, santykinį drėgnumą, sniego dangą. Šių veiksnių analizė Vilniaus mieste, Kauno ir Klaipėdos miestuose bei rajonuose parodė stiprų, statistiškai reikšmingą koreliacinį ryšį tarp sergamumo Laimo liga ir vidutinės oro temperatūros, neigiamą, statistiškai reikšmingą koreliaciją tarp sergamumo ir sniego dangos ir silpną, bet statistiškai reikšmingą ryšį tarp sergamumo ir kritulių kiekio. Statistiškai reikšmingas ryšys tarp erkių gausos ir oro temperatūros nustatytas Vilniaus mieste, tarp erkių gausos ir kritulių kiekio Klaipėdos mieste, Vilniaus mieste ir Kauno rajone nustatytas statistiškai patikimas ryšys tarp erkių gausos ir santykinio drėgnumo, silpnai neigiama koreliacija tarp erkių gausos ir sniego dangos Kauno rajone.

Analizuojant stambiųjų laukinių raguočių gausos svyravimų įtaka Laimo ligos sergamumui ir erkių gausai, nustatyti silpni, statistiškai nepatikimi koreliaciniai ryšiai tarp stambiųjų raguočių gausos (briedžių, tauriųjų elnių ir stirnų) ir LB sergamumo bei erkių gausos.

Kaip vienas iš socialinių veiksnių, galinčių įtakoti Laimo ligos sergamumą, analizuotas bedarbių skaičius, bedarbių dalis tarp darbo jėgos ir nedarbo lygis, tačiau statistiškai reikšmingos koreliacijos tarp sergamumo Laimo liga ir šių veiksnių nenustatytos.

Analizuojant oficialius žemės naudmenų plotus matyti, kad 1991-2004 metais Lietuvoje žemės ūkio paskirties žemių plotais sumažėjo 15 proc. 1995-2005 metais nustatyta silpnai neigiama statistiškai nepatikima koreliacija tarp žemės ūkio paskirties plotų ir sergamumo Laimo liga

Erkių platinamų ligų rizikos veiksnių paplitimas tarp Lietuvos gyventojų

Vertinant erkių įkandimų paplitimą 46,1 proc. (95% PI 45,51; 46,69) (n=497) respondentų nurodė, kad jiems kažkada gyvenime buvo įsisiurbusi erkė. 16,6 proc. (95% PI 15,18; 18,02) (n=179) teigė, kad erkė jiems buvo įsisiurbusi apklausos metais, 2,6 proc. (95% PI 1,18; 4,02) (n=28) apklausos metais erkė įsisiurbė pirmą kartą. 58,3 proc. asmenų

(n=627) mano, kad erkės pastaraisiais metais įsisiurbdavo dažniau, nei prieš penkerius metus.

Nustatyta, kad gyvenime dažnesnį sąlytį su erkėmis sąlygoja žvejyba (χ^2=11,447, *p<0,05*), grybavimas/uogavimas (χ^2=35,321, *p<0,05*), poilsiavimas ar darbas sode/sodyboje (χ^2=7,054, *p<0,05*), šuns vedžiojimas lauke (χ^2=3,935, *p<0,05*).

Apklausos metais pagrindiniai rizikos veiksniai, sąlygoję dažnesnius erkių įkandimus buvo žvejyba (χ^2=12,343, *p<0,05*), grybavimas/uogavimas (χ^2=45,602, *p<0,05*), poilsiavimas ar darbas sode/sodyboje (χ^2=10,513, *p<0,05*) ir poilsiavimas gamtoje su nakvyne (χ^2=5,298, *p<0,05*).

Nustatyta statistiškai reikšminga asociacija tarp erkės įkandimų gyvenime ir rizikos veiksnių grupės, kuri apima žvejybą (OR_p=2,43, 95 proc. 95% PI 1,69-3,49), grybavimą/uogavimą (OR_p=1,63, 95 proc. 95% PI 1,18-2,24), darbą, susijusį su būvimu gamtoje (OR_p=1,62, 95 proc. 95% PI 1,03-2,55), pajamas, mažesnes kaip 500 Lt (OR_p=0,76, 95 proc. 95% PI 0,59-1,00).

Per apklausos metus stebėtas erkės įkandimas reikšmingiausiai susijęs su rizikos veiksnių grupe, kuri apima žvejybą (OR_p=4,90, 95 proc. 95% PI 2,77-8,67), grybavimą/uogavimą (OR_p=1,68, 95 proc. 95% PI 1,10-2,55), darbą, susijusį su būvimu gamtoje (OR_p=2,14, 95 proc. 95% PI 1,29-3,58), gyvenimą gyvenvietėje, kurioje yra mažiau kaip 2000 gyventojų (OR_p=1,78, 95 proc. 95% PI 1,26-2,52).

Nors dauguma respondentų (69,1 proc., n=745) teigia, kad jiems pakanka žinių apie erkių platinamas ligas, tačiau tik 6,5 proc. (n=70) apklaustųjų nurodė, kad yra pasiskiepiję nuo erkinio encefalito, 41,3 proc. – naudoja repelentus, 64,6 proc. – pasirenka tinkamą aprangą, 41,1 proc. – apsižiūri savo kūną dėl galimų erkių įkandimų būdami gamtoje, 81,4 – grįžę namo. Apklausos metais repelentus naudojo 40,4 proc. respondentų, rinkosi tinkamą aprangą – 63,7 proc., apsižiūrėdavo savo kūną dėl galimų erkių įkandimų būdami gamtoje – 40,3 proc., apsižiūrėdavo grįžę namo – 81,0 proc.

Tarp visų respondentų, kuriems buvo įsisiurbusi erkė, 60,6 proc. (n=301) nurodė, kad jautė kokius nors ligos požymius po erkės įkandimo - dažniausiai buvo nurodomas paraudimas erkės įkandimo vietoje, atsiradęs iškart po erkės įkandimo (n=184, 61,1 proc.) ir paraudimas-dėmė erkės įkandimo vietoje, atsiradęs vėliau (n=54, 17,9 proc.).

Tarp visų respondentų 1,9 proc. (n=21) gydymo įstaigoje buvo nustatyta erkių platinama liga (Laimo liga – 1,1 proc., erkinis encefalitas – 0,9 proc.).

Erkių gausa ir infekuotumas borelijomis skirtinguose biotopuose

Erkių gausos ir infekuotumo tyrimo metu atlikti 6 erkių rinkimai, kurių metu iš viso surinkta 9195 *Ixodes ricinus* erkės. Pavasarinių rinkimų (gegužės-birželio mėn.) metu buvo surinkta 72 proc. (6652 erkės), rudeninių (rugpjūčio- rugsėjo) – 27 proc. (2543 erkės). Tarp visų surinktų *I. ricinus* erkių suaugusios erkės sudarė 83 proc. (50 proc. patelės (3829 erkės) ir 50 proc. (3831 erkė) patinėliai), nimfos – 17 proc. Vidutiniškai, 18 suaugusių erkių ir 4 nimfos surinktos 1 km maršrute pavasarį ir 8 suaugusios ir 1 nimfa rudenį.

Siekiant nustatyti ar pavojinga erkių buveinė yra esminiai svarbi LB patogenams, buvo nustatytas ir įvertintas borelijų infekcijos paplitimas *Ixodes ricinus* nimfose. 159 (13 proc.) pavasarinės kolekcijos nimfoms buvo nustatytas infekuotumės *Borrelia* spp., 16 (1,5 proc.) nimfų buvo infekuotos *B. miyamotoi*. Nimfų infekuotumas LB grupės

Borrelia spp. ar *B. miyamotoi* buvo panašus skirtinguose biotopuose surinktose erkėse ($p > 0,20$).

61 sekų nustatymui tinkamas mėginys buvo tiriamas lyginant *rrs-rrl* IGS regioną, siekiant nustatyti Borelijų rūšies genetinį variantą. 52 mėginiai buvo teigiami LB grupės *Borrelia* rūšiai: 42 (81 proc.) buvo *B. afzelii*, 9 (17 proc.) *B. garinii* ir 1 (2 proc.) *Borrelia valaisiana*, kurios medicininė svarba nėra žinoma.

9 pasirinkti teigiami grįžtamosios karštligės mėginiai buvo teigiami *B. miyamotoi*. Nustatytos LB grupės boreliojos rūšys buvo panašiai pasiskirsčiusios skirtinguose biotopuose (p>0,45), tai leidžia daryti prielaidą, kad LB rezervuariniai šeimininkai skirtinguose biotopuose yra tie patys.

Išvados

1. Lietuvoje 1995–2006 metais bendroje LB sergamumo dinamikoje neįvyko statistiškai reikšmingų pokyčių, tačiau Laimo boreliozės gamtinis arealas turi savybę plėstis rajonų, kuriuose sergamumas anksčiau buvo žemas, sąskaita.
2. Svarbiausi rizikos veiksniai, sąlygojantys sergamumą Laimo borelioze Lietuvoje, yra klimatiniai (vidutinė oro temperatūra bei sniego danga). Nors socialiniai ir kiti ekologiniai veiksniai gali būti susiję su LB plitimu, jie yra mažiau reikšmingi.
3. Erkių įkandimus gyvenime buvo pastebėję 46,1 proc. Lietuvos gyventojų, 16,6 proc. erkė buvo įsisiurbusi apklausos metais, 2,6 proc. apklausos metais erkė įsisiurbė pirmą kartą. Lietuvoje reikšmingiausi erkių įkandimus sąlygojantys rizikos veiksniai yra žvejyba, grybavimas / uogavimas, darbas, susijęs su buvimu gamtoje ir gyvenimas nedidelėse gyvenvietėse.
4. Erkių nimfų infekuotumas Lietuvoje yra 14,8 proc. Tarp *Ixodes ricinus* nimfų pernešamų *B. burgdorferi* s.l. borelijų dominuoja *B. afzelii* (81 proc.). Ji kartu su *B. garinii* (17 proc.) apima žmonėms patogeniškus borelijų variantus.
5. Erkių nimfų infekuotumas nesiskiria nei naujuose, nei senuose biotopuose, kas rodo, kad natūralus LB ciklas greitai atsistato ir tai gali sąlygoti Laimo boreliozės plitimą Lietuvoje. Panašią borelijų rūšinę sudėtį erkėse skirtinguose biotopuose gali lemti bendras infekcijos rezervuaras tiek žinomose, tiek pavojingose enzootinėse vietose (naujuose biotopuose).
6. Lietuvoje nustatytas *B. miyamotoi* paplitimas rodo, kad šios borelijos arealas neapsiriboja Skandinavija, bet siekia ir Rytų Europos regioną.