RĪGAS STRADIŅA UNIVERSITĀTE

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ANALYSIS OF THE LYME BORRELIOSIS EPIDEMIOLOGICAL INDICATORS IN LATVIA AND SEROPOSITIVITY ASSOCIATED FACTORS

Summary of Doctoral Thesis

Speciality – Public Health and Epidemiology

Riga, 2013

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Presentation of the Doctoral Thesis will be held on February 18th, 2013 at 15.00 on the open session of the Promotional Council of Theoretical Medicine disciplines, in the Hippocrates Auditorium, Rīga Stradiņš University, Dzirciema Street 16, Riga.

The thesis is available at RSU library and RSU website: www.rsu.lv

Elaboration of the thesis was supported by ESF project "Support for doctoral study programs and research degrees in Rīga Stradiņš University"

Secretary of the Promotional Council:

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CONTENTS

1. INTRODUCTION

1.1. TOPICALITY

Lyme borreliosis is a transmissive infectious disease with natural foci, the most common tick-borne disease in the temperate climate zone of the Northern hemisphere. Disease is caused by bacteria of the *Borrelia burgdorferi sensu lato* complex transmitted by *Ixodes* ticks in Latvia [1, 2]. Cases of Lyme borreliosis have been registered since 1996 in Latvia, and during the initial registration period of the disease the number of registered cases was very small due to incompleteness of clinical and laboratory diagnostics, however a significant increase in incidence was observed starting 1992 [3].

Calculations demonstrate that Lyme borreliosis affects approximately 85 000 people in Europe every year [4]. During the last few decades, the Lyme borreliosis incidence has grown dramatically in many European countries, partly due to improved diagnosis and reporting. However, there are evidences of a true increase in incidence associated with many nature-derived and socio-economic factors [5, 6], leading to an increased public health risk and burden on national health care systems. In addition, many experts believe that the true number of cases of Lyme borreliosis 2–3 times precedes the number of cases usually reported within epidemiological surveillance systems [7, 8, 9].

During the recent years European Centre for Disease Prevention and Control pays great attention to the tick-borne diseases, including Lyme borreliosis. Studies revealed that Lyme borreliosis risk is associated not only with rick occupations, but to a considerable extent with the recreational activities, resulting in increased public attention to this problem. On the other hand, valuable information on the Lyme borreliosis burden in Europe and particular countries is not available. Therefore, the European Centre for Disease

Prevention and Control defines the following important activities: characterization of various Lyme borreliosis reporting systems, identification of the epidemiological situation, key risk groups and territories in the Member States. Particular attention is paid to Lyme borreliosis serological tests as the role of disease diagnosis is limited in some extent due to a relatively slow antibody production in the early stages of infection, association of antibodies with infective genospecies, as well due to sufficiently high antibody seroprevalence among European citizens. Experts of the European Centre for Disease Prevention and Control Centre believe that improvement of Lyme borreliosis clinical and laboratory diagnosis requires the best practice guidelines for doctors; and promotion of the rational laboratory testing and treatment [10]. Lyme borreliosis is a matter of increasing attention also in the United States of America, regardless sufficiently effective system of the epidemiological surveillance. During the Congress in 2011 a draft law requiring further actualization of the problem of Lyme borreliosis was reviewed and approved [11].

Some research has been performed in the field of Lyme borreliosis in Latvia, focused mainly on the nature of circulating pathogen characterization and prevalence detection in ticks, but so far published results do not allow characterizing the situation in Latvia concerning Lyme borreliosis in all epidemiological aspects. The above mentioned motivates epidemiologists and public health specialists study the Lyme borreliosis epidemiological characteristics and the factors influencing it in Latvia, assess the disease burden, as well analyse the factors related to serological confirmation of diagnosis in order to provide complete information for the monitoring of the disease, data comparison with other countries, for the improvement of epidemiological and clinical guidelines, and complex of the preventing measures.

1.2. The aim of this work

To perform an epidemiological analysis of Lyme borreliosis incidence in Latvia during the period of 2007 to 2011, and identify the factors related to serological approval of the diagnosis.

1.3. Tasks of this work

1. To carry out a systematic selection of the data for Lyme borreliosis cases registered in Latvia during the period of 2007 to 2011 from the State Communicable Disease Surveillance and Monitoring System (VISUMS).
2. To analyze the completeness of reporting, comparing the Lyme borreliosis cases included in the VISUMS data basis with the data from the Management Information System (VIS).
3. To analyse epidemiological features of Lyme borreliosis incidence for 2007 to 2011 in relation to climatic, natural, environmental and social factors, tick activity, the number of tick hosts, as well as epidemiological peculiarities of the tick-borne encephalitis incidence.
4. To analyse the factors related to the serological approval of the Lyme borreliosis diagnosis in 2007 to 2011.

1.3. Hypothesis of this work

1. Influence of the factors affecting Lyme borreliosis incidence in Latvia (demographic, social, entomological, climatic, etc.) is different.
2. Probability of the serological confirmation of Lyme borreliosis diagnosis is related to patient's age, gender, time since infection and disease form.

1.4. Scientific novelty of this work

For the first time a detailed epidemiological analysis of the Lyme borreliosis incidence has been performed in Latvia, demonstrating that the disease has one of the main roles in the natural foci infectious diseases in Latvia; identified the true Lyme borreliosis incidence rate; cleared some of the climatic, environmental and social factors affecting changes in the morbidity; and set some of the factors associated with probability of the serological confirmation.

1.5. Practical significance of this work

The results of this work allow:

1. To develop an epidemiological surveillance system: to introduce the methods developed during the elaboration of the thesis in the epidemiological analysis; to improve guidelines for the epidemiological investigation and the protocol form of the epidemiological investigation in the VISUMS system.
2. To provide preparing of better information for the development and evaluation of the complex of preventive measures.
3. To develop evidence-based guidelines for the algorithm of Lyme borreliosis laboratory investigation, which will allow to optimize the use of financial resources.

1.6. Structure and size of this work

Work is written in Latvian. It has 8 parts: introduction, literature review, materials and methods, results, discussion, conclusions, practical recommendations, references. The thesis is 183 pages long, including 26 figures and 36 tables. Bibliography consists of 223 references. Thesis has nine annexes.

2. MATERIAL AND METHODS

The epidemiological analysis on Lyme borreliosis incidence has been performed for the achievement of the stated objective and for testing of the hypotheses in relation to incidence affected by climatic, natural and social factors, tick and host abundance and activity, pathogen prevalence in ticks, incidence of tick-borne encephalitis; a comparative analysis of the data from the National Health Service's supervised Management Information System (VIS) recordings on episodes of illness in patients with Lyme borreliosis; as well performed investigation of the factors related to Lyme borreliosis patients with serologically confirmed diagnosis in the laboratory of the State Agency "Infectology center of Latvia" from 2007 to 2011.

Performance of the study is consistent with the Rīga Stradiņš University Ethics Committee.

2.1. Data for epidemiological analysis of the dynamics of Lyme borreliosis

For the epidemiological analysis of Lyme borreliosis dynamics the following is used: perennial statistical data on the incidence ("Overview of infectious and parasitic diseases") available in the Department of the Communicable Diseases Risk Analysis and Prevention of the Centre for Disease Prevention and Control of Latvia, the results of the epidemiological analysis carried out in recent years, as well as the State communicable disease surveillance and monitoring system (VISUMS) data for 2007 to 2011 on registered cases of tick-borne diseases.

Reporting on communicable diseases, including tick-borne diseases has been established by the Cabinet Regulation No. 7, adopted 5 January 1999 "Procedures for Registration of Infectious Diseases". The form Nr.058 / u

"Urgent notification on an infectious disease, detection of infectious diseases agents, isolation of resistant microorganisms, and vaccination induced complications (side effects)" is provided for the reporting. Any case of Lyme borreliosis is a subject to registration and further epidemiological investigation in order to reveal territories of infection and other important epidemiological factors. Statistical data on the Lyme borreliosis incidence by age groups and territories, as well as on seasonality are available from 1997. Incidences and their seasonal and geographical trends in perennial dynamics have been statistically analyzed in the work.

2.2. Data for epidemiological analysis of Lyme borreliosis risk factors

A long-term observation results in the *Ixodes* ticks natural foci are obtained from the archived data of the Centre for Disease Prevention and Control. For the statistical analysis of risk factors the data for the period of 1997 to 2011 are used. Because of the role of two vectors in the spread of Lyme borreliosis in Latvia, incidence rates in areas, where *I. ricinus* and *I. persulcatus* tick species were found, analysed separately in the work. Regions of Latvia, located in the east from the taiga tick area borders are named as *I. persulcatus* region in the work, the rest part of Latvia – as *I. ricinus* region.

The previously published studies on *Borrelia* prevalence in ticks in Latvia, results of the laboratory tests performed within EDEN-TBD project, as well as the results of laboratory tests carried out within the annual tick monitoring in 2010–2011 are analysed in this work.

The officially published data on the Lyme borreliosis incidence influencing risk factors (population, employment, forest and agricultural land, number of wild animals, weather conditions, data on environmental pollution) and changes in their dynamics, acquired in the Latvian Central Statistical

Bureau of Latvia and the Latvian Environment, Geology and Meteorology Centre web pages.

2.3. Data for Lyme borreliosis epidemiological information completeness and quality assessment

The records from the National Health Service's supervised Management Information System (VIS) on disease episodes in patients with Lyme borreliosis (diagnosis code A69.2) for the period from 2007 to 2011 are analyzed in order to identify, describe and analyze the possible differences in the State Communicable Disease Surveillance and Monitoring System (VISUMS).

2.4. Data for Lyme borreliosis incidence detailed epidemiological analysis

The data on recorded tick-borne diseases' cases in 2007 to 2011 from the State Communicable Disease Surveillance and Monitoring system (VISUMS) are analysed in this work. The study subjects – persons with approved Lyme borreliosis or tick-borne encephalitis diagnosis during the period from 2007 to 2011, which have been reported according to the procedures, set in the Cabinet Regulation No. 7, adopted 5 January 1999 "Procedures for Registration of Infectious Diseases". Because of the lack of officially adopted case definition for mentioned diseases, the case is considered to be confirmed if the reporting medical practitioner indicated. Altogether 3530 Lyme borreliosis cases and 1606 tick-borne encephalitis cases have been registered during the investigation period and respectively included in this study.

2.5. Data for analysis of the factors associated with serological confirmation of Lyme borreliosis diagnosis

This study used the State Agency's "Infectology center of Latvia" laboratory data about ELISA test results on IgM and IgG class antibodies against *B. burgdorferi* s.l. in the Lyme borreliosis patients registered during the period 2007–2011 from the State Communicable Disease Surveillance and Monitoring system (VISUMS). During the period from 2007 to 2011 the Enzygnost Borreliosis IgM / IgG test-systems were mostly used, produced by the German firm "Dade Behring" until 2009 but starting 2010 – by the German company "Siemens" (the plant owner changed).

2.6. Statistical analysis

The following methods are used in this work: incidence rates (number of cases per 100 000 person-years) and calculation of their 95% confidence intervals by territories, genders and age groups, as well as by employment status, and the relative risk (RR) calculation for comparisons of incidence. The linear single- and multiple regression models are used for the time series analysis. Lyme borreliosis incidence rate time-series data correlation analysis with some climate (average temperature, rainfall), nature (number of wild animals, mean number of ticks in their constant monitoring sites), characterizing the anthropogenic pollution (harmful emissions into the atmosphere from stationary sources) and social (employment rates, the level of vaccination against tick-borne encephalitis) factors have been performed. Pearson and Spearman's rank correlation coefficients have been used for the correlation analysis depending on the specific tasks.

For the statistical analysis of individual data on morbidity cases the standard descriptive statistical methods have been used. The data compliance to a normal (Gaussian) distribution is tested by using the Kolmogorov-Smirnov test. The central tendency and dispersion characteristics – standard deviation (SD) and standard error (SE) have been evaluated for the quantitative data. For the quantitative data, inconsistent with the normal distribution, and comparison groups, depending on the task and the data, the nonparametric statistical methods have been used: Mann-Whitney U-test, Kruskal-Wallis H-test. For the quantitative data, consistent with the normal distribution, the analysis of variance – ANOVA has been used. For the evaluation of the categorical signs the non-parametric chi-squared (χ^2) test has been used.

For the determination of the bounded factors the multivariate analysis binary and multinomial logistic regression statistical models have been used with interpretation of the results in the mode of odds ratio.

For all statistical tests used in this work the significance level of 0.05 is chosen, and the result with p-value <0.05 has been evaluated as statistically significant. The 95% confidence interval (CI) is selected for the calculation of the results.

Calculation of the incidence rate and other indicators has been performed in this work by dividing the territory of Latvia in several sub-groups depending on the particular task: for the evaluation of perennial trends a breakdown in 33 administrative areas (7 cities and 26 districts) is used; geographical analysis of the incidence for the period 2007 – 2011 is carried out on the basis of administrative division of towns and counties (119 units), or 6 statistical regions. For the performance of special tasks the division on the basis of the number and density of population in administrative areas have been used, what is explained separately in each particular position.

The analysis of the data has been performed with MS Excel, WinPepi (11.18, 2012), STATISTICA and SPSS 13.0 computer programs.

3. THE RESULTS

3.1. Dynamics of the Lyme borreliosis incidence during the period from 1997 to 2011, and the analysis of influencing factors

Lyme borreliosis incidence rate during the study period (2007 to 2011) was 31.3 per 100,000 person-years (95% CI 30.27–32.35), the average number of cases was 706. Comparing the average Lyme borreliosis incidence with mean incidence in two previous five-year periods (from 1997 to 2001, and from 2002 to 2006), it can be concluded that there has been a relevant statistically significant increase in incidence (Table 3.1). Because of the role of two vectors (*I. ricinus* and *I. persulcatus*) in the spread of Lyme borreliosis in Latvia, the changes in incidence have been separately evaluated in areas where mentioned tick species prevail.

Table 3.1

The average 5-year Lyme borreliosis incidence (per 100 000 inhabitants) changes between 1997 and 2011

Time period	The mean number of cases	The mean number of cases per 100 000 inhabitants (95% CI)	The increase in incidence, times (95% CI)	p
In Latvia, in average				
1997–2001	415	17.3 (16.57 – 18.06)	Ref.	
2002–2006	569.2	24.5 (23.60 – 25.41)	1.42 (1.34–1.50)	<0.0001
2007–2011	706	31.3 (30.27 – 32.35)	1.81 (1.71–1.91)	<0.0001
I. ricinus				
1997–2001	315.6	19.5(18.55 – 20.49)	Ref.	
2002–2006	421.2	26.9 (25.77 – 28.08)	1.37 (1.29–1.47)	<0.0001
2007–2011	502.6	32.1 (30.86 – 33.38)	1.64 (1.54–1.75)	<0.0001
I. persulcatus				
1997.–2001	99.4	12.6 (11.52 – 13.77)	Ref.	
2002–2006	148	19.7 (18.30 – 21.16)	1.56 (1.39–1.75)	<0.0001
2007–2011	203.4	29.4 (27.61 – 31.25)	2.32 (2.09–2.59)	<0.0001

Statistically significant increase in the incidence (p<0.001) during the reference period has been observed in both the areas, where *I. ricinus* tick species are found as well in areas where *I. persulcatus* tick species are observed. Increase in incidence was more evident in areas where *I. persulcatus* ticks species are spread. Analysis of Lyme borreliosis incidence trends led to the conclusion that they significantly fluctuated in some years, however in general during the period from 1997 to 2011 an increasing trend has been observed, more pronounced in *I. persulcatus* tick distribution areas (p <0.05). The number of cases per 100 000 inhabitants in the *I. persulcatus* tick distribution area was lower than in the *I. ricinus* region during all years, except for 2009 and 2010 (Figure 3.1).

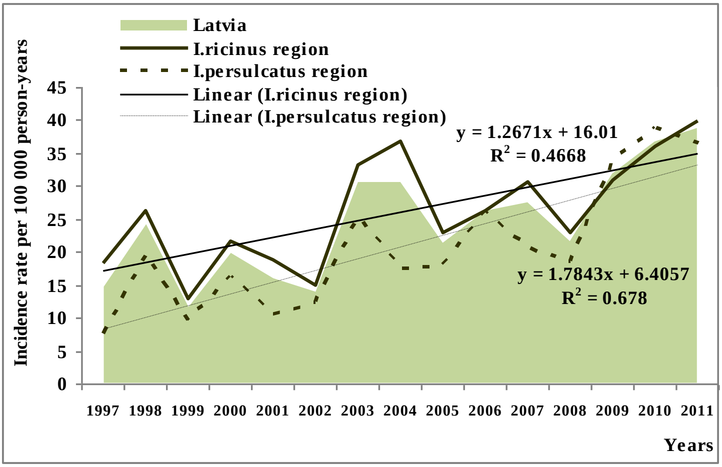

Fig. 4.1. Dynamics of Lyme borreliosis incidence, in total, and in tick distribution areas, in 1997–2011

The dynamics of the disease from 1997 to 2011 has been analysed in this paper, taking into account the characteristics of patient's residence (urban / rural). By 2005 the incidence of Lyme borreliosis in the urban inhabitants was higher than in the rural inhabitants (RR 1.14–1.51), although not in all years these differences are statistically significant; but starting 2007 the incidence among rural inhabitants was higher than in urban inhabitants. Trends of the incidence changes in the described period for rural and urban inhabitants in general are similar (Pearson correlation coefficient 0.796, p <0.01).

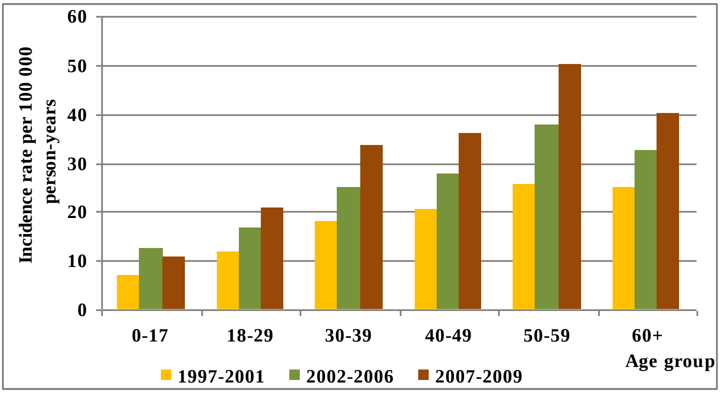

Fig. 3.2.The average Lyme borreliosis 5-year incidence rate (per 100 000 person-years), by age groups, 1997– 2011

During the period from 1997 to 2011 an increase of incidence in all age groups has been found, except in the age group "0–17" years in the period from 2007 to 2011, compared to the previous 5 years (Figure 3.2). In all analysed periods, the maximum incidence observed in the age group "50–59" years.

In order to determine the possible effects of climatic, environmental and social factors on the incidence of Lyme borreliosis from 1997 to 2011, the correlation analysis has been performed. A statistically significant positive correlations between Lyme borreliosis incidence from 1997 to 2011 is found with the number of wild animals (moose (r= 0.7285), red deer (r= 0.788), roe deer (r= 0.5847), wild boar (r= 0.7586), raccoon dogs (r= 0.7911), foxes (0.6311), martens (r= 0.7003), badgers (r= 0.7927) and total (r= 0.6752)), forest cover (r= 0.7866); negative correlations – with the following indicators of pollution: the total emissions of pollutants (r= -0.709), solid particles (r= -0.8203), sulphur dioxide (r= -0.6141) and carbon monoxide (r= -0.7328).

3.2. Assessment of completeness and quality of Lyme borreliosis epidemiological information

During the period from 2007 to 2011 there were 12,436 registered reports about the patients with diagnosis “Lyme borreliosis” (diagnosis code A69.2) in the database of the National Health Service’s supervised Management Information System (VIS), however in the National infectious disease surveillance and monitoring system (VISUMS) – 3530 Lyme borreliosis cases were registered. A significant difference in both databases was due to incomplete reporting of infectious diseases from the side of medical personnel. To ensure an assessment of the database VISUMS representativeness, the comparison has been made for the information included in VIS and VISUMS data bases, using the following criteria: gender, age group, registration year, region, hospitalisation status. A selection of the criteria has been substantiated with the level of data detail obtained from the VIS system.

The proportional distribution of the number of cases by gender in both databases were similar – the proportion of men was 33.24% (95% CI 32.41–34.08) in the VIS database, and 34.99% (95% CI 33.41–36.59) – in VISUMS database; statistically significant differences are not observed ($p>0.05$).

An average age of the patients, registered in the VIS database, was 46.01 years (standard error 0.175), median 48 years, in the VISUMS database – 47.46 (standard error 0.308), median 47.91 years ($p<0.05$). Upon analysis of the data on Lyme borreliosis cases included in the VIS and VISUMS databases it is found that the proportion of age group “0–17 years” in the VIS database is 9.73% (95% CI 9.21–10.26), and in the VISUMS database – 5.98% (95% CI 5.22–6.81). With a higher proportion of children in the VIS database the observed differences in the mean age are explained, as analysing Lyme borreliosis cases registered among adults (18 years and older), statistically significant differences between the two databases are not observed ($p>0.05$).

The analysis of the Lyme borreliosis cases, registered in the VIS and the VISUMS databases during the period from 2007 to 2011 by statistical regions, revealed that the total regional breakdown of the cases registered during the time period of this study differs statistically significantly between databases ($p<0.05$, Figure 3.3). However, due to the possible use of different criteria for determining of the patient's affiliation to the particular statistical region (in the VISUMS database patients have been registered according to the actual place of residence at the time of illness, while in the VIS database – according to the place of residence), and due to significant internal migration the interpretation of the results is difficult.

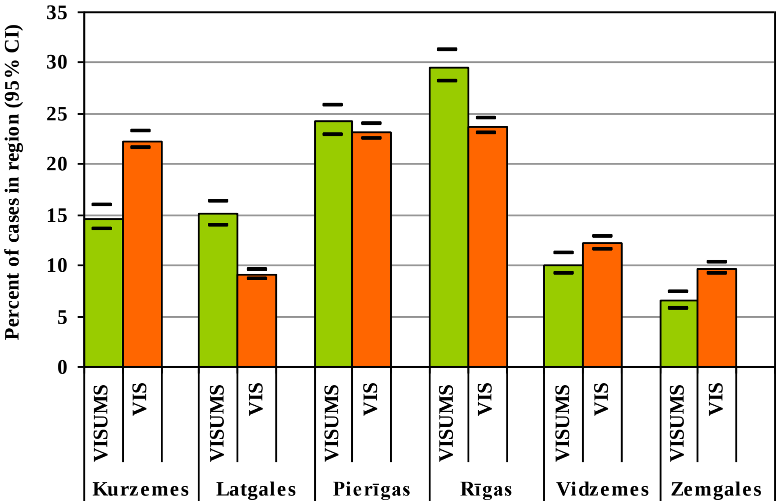

Fig.3.3. Proportion of Lyme borreliosis cases per statistical regions of Latvia from 2007 to 2011, in the VIS and VISUMS databases

Data reconciliation on the hospitalized Lyme borreliosis patients stated that 24.9% (95% CI 23.5–26.4, the variations by region from 9.1% to 59.3%) of the patients included in the VISUMS data basis were hospitalized, the respective ratio for the VIS data base is 7.9% (95% CI 7.4–8.36, the variations by region from 2.5% to 32.9%), the differences are statistically significant ($p<0.05$). The results indicate a higher probability of reporting about the hospitalized patients according to the order stated in the Cabinet Regulation No. 7 of 05/01/1999 "Procedures for Registration of Infectious Diseases", and about a possible under-reporting of non-hospitalized patients. However, comparing the data on distribution of the hospitalized Lyme borreliosis patients by territories, age groups and gender, it can be concluded that a statistically significant differences between the proportion percentages of the two databases are not observed ($p>0.05$, table 3.2).

Table 3.2

Percentage distribution of the hospitalized Lyme borreliosis patients by statistical regions, age groups, gender and data bases, 2007–2011

Data base	**VIS**		**VISUMS**	
	%	**95%CI**	**%**	**95%CI**
Regions:				
Kurzemes	8.99	7.27–10.96	7.96	6.26–9.95
Latgales	37.79	34.78–40.86	35.84	32.66–39.11
Pierīgas	12.87	10.88–15.08	14.9	12.61–17.43
Rīgas	7.56	5.98–9.40	10.81	8.83–13.05
Vidzemes	24.11	21.46–26.91	23.44	20.67–26.38
Zemgales	8.68	6.99–10.62	7.05	5.45–8.95
Age group:				
0–17 years	17.26	14.95–19.78	12.17	10.08–14.52
18–29 years	10.62	8.76–12.72	10.92	8.94–13.17
30–39 years	11.03	9.14–13.16	12.74	10.61–15.13
40–49 years	16.24	13.98–18.70	16.15	13.78–18.76
50–59 years	17.77	15.43–20.31	19.8	17.21–22.59
60–69 years	15.12	12.93–17.52	15.93	13.57–18.52
70 years and older	11.95	9.98–14.15	12.29	10.19–14.64
Gender:				
Men	41.27	38.16–44.42	41.41	38.13–44.75
Women	58.73	55.58–61.84	58.59	55.25–61.87

Based on the performed mutual comparison of the registered Lyme borreliosis cases in VIS and VISUMS databases, analysis of the cases included in VISUMS database in terms of trends, demographics (gender, age) and related indicators, from the point of view of statistical significance is safe enough (representative), including attribution of the results to the overall situation in the country. In turn, the geographical distribution analysis to be carried out with caution, as the study revealed that in some areas (mainly in Kurzeme and Zemgale) a substantial part of the cases may not been reported, so these cases were not included in the VISUMS database.

3.3. Epidemiological analysis of Lyme borreliosis incidence in period from 2007 to 2011

The highest Lyme borreliosis incidence rate in the period from 2007 to 2011 in Latvia has been registered in the age group "60–69 years", together and for women, however for men – in the age group "50–59 years". By contrast, in the age group "0–17 years" the lowest incidence rates has been observed for both genders together, and separately for men and women, the differences are statistically significant (Table 3.3).

Differences in incidence rates by age groups are more expressed in women: peak incidence (in the age group "60–69 years") exceeds the minimum incidence (in the age group "0–17 years") for 5.9 times (95% CI 4.8–7.27, $p<0.001$). Among men, incidence differences are less expressed, and the peak incidence (in the age group "50–59 years") exceeds the minimum incidence (in the age group "0–17 years") for 3.4 times (95% CI 2.7–4.3, $p <0.001$).

The average incidence rate among men is 1.6 times (95% CI 1.5–1.7, $p<0.001$) lower than that for women; the differences are maximal and statistically significant in the age group "60–69 years" (RR = 2.0, 95% CI 1.7–2.4, $p <0.001$), while in the age group "0–17 years" the differences are small and not statistically significant (RR = 1.1, 95% CI 0.9–1.5, $p> 0.05$).

Analysing Lyme borreliosis incidence by gender, age and regions of residence, it can be concluded that the highest incidence rate for women is observed in the age group "60–69 years" in Pierīgas region (108.6 per 100 000 person–years), the lowest – in the age group “0–17 years” in Riga region (7.4 per 100 000 person–years). Among men, the highest incidence rate (50.4 per 100 000 person–years) is found in the age group “30–39 years” in Pierīgas region, the lowest (4.4 per 100 000 person–years) – in the age group “0–17 years” in Zemgales region.

Lower incidence rates have been reported in Zemgales region in all age groups for both genders (except for the age group “70 years and older” – the lowest level found in Vidzemes region), as well separately for men (except for the age group “70 years and older” – the lowest level found in Kurzemes region) and women (except for the age group “0–17 years” – the lowest level found in Riga region).

Table 3.3

Lyme borreliosis incidence rate per 100 000 person–years by gender, age groups and regions, 2007–2011

	0–17 years	**18–29 years**	**30–39 years**	**40–49 years**	**50–59 years**	**60–69 years**	**70 yers and >**	**In total**
Latvia								
In total	10.6	21	33.5	36.2	50.2	53.1	29.2	31.3
Men	9.9	18.8	30.2	27.7	33.9	32.6	23.3	23.7
Women	11.3	23.2	36.8	44.1	63.7	66.8	31.7	37.7
Kurzemes region								
In total	13.1	17.4	39.3	43.1	55.5	57.8	34.6	34.2
Men	15.6	14.4	32.2	28	34.1	37	14.9	23.8
Women	10.6	20.6	46.7	57.6	73.7	72.3	43.6	43.3
Latgales region								
In total	12.7	15.7	29.6	37.6	55.5	49.2	29	30.8
Men	14.8	16.1	29.1	29.2	48	45.4	28.9	27.9
Women	10.5	15.2	30.1	45.8	62.1	51.9	29	33.4
Pierīgas region								
In total	9.6	33.1	54.9	48.4	78.4	74.9	39.2	44.6
Men	7.5	26,1	50.4	33.4	42.5	46.9	34.2	31.6
Women	11.7	40.4	59.6	62.8	108.6	94.5	41.4	56.1
Rīgas regions								
In total	7.1	21.8	29.5	31.9	43.4	54.1	28	29.4
Men	6.7	20.2	27.5	28.1	27.4	25.2	24.2	21.9
Women	7.4	23.4	31.4	35.1	55.4	72	29.7	35.3

Comparing Lyme borreliosis and tick-borne encephalitis incidence rate by gender and age groups, it was found that, unlike the Lyme borreliosis, the incidence of tick-borne encephalitis is higher for men. Comparing the distribution of cases by gender, depending on the diagnosis, a statistically significant differences (Pearson χ^2 = 118.801, p <0.01) has been found. Calculated odds ratio for patients by gender, it is found that women are significantly more likely to develop Lyme borreliosis, both in total (OR = 1.94, 95% CI 1.72–2.19, p <0.01), and in all age groups, except for the age group "0–17 years", where the difference is not statistically significant (p>0.05). Differences in morbidity breakdown by gender and age groups can be partially explained by higher vaccination coverage against tick-borne encephalitis in women (vaccinated women do not have tick-borne encephalitis), a substantial vaccination coverage among children regardless of gender, as well as for other factors.

Comparing Lyme borreliosis and tick-borne encephalitis morbidity odds according to the category of residence[1] together and by gender, a statistically significant difference is found. 59.4% (95% CI 56.9–61.8%) of tick–borne encephalitis cases and 41.7% (95% CI 40.0 to 43.3%) of Lyme borreliosis cases are registered in parishes, however in cities (Riga, and Daugavpils) – 19.6% (95% CI 17.6–21.6%) and 34.6% (95% CI 33.0–36.2%), respectively. Lyme borreliosis odds, compared to tick-borne encephalitis odds are higher in urban areas (maximum – in large cities), both together and separately for men and women. Compared separately odds ratio for Lyme borreliosis patients without erythema migrans with the all tick-borne encephalitis patients, it is found that for the inhabitants of large cities Lyme

[1] large city – city with a population of 100 000 inhabitants and more, city – city with population between 20000 and 99999 inhabitants, town – town with population between 5000 and 19999 inhabitants, parish – administrative unit with less than 5000 inhabitants

borreliosis odds comparing to the tick-borne encephalitis odds are lower than for rural residents (OR = 0.554, 95% CI 0.393–0.779, p <0.05).

Of the registered Lyme borreliosis cases among adults (n=3319), 42.5% (95% CI 40.9– 44.3%) were employed, 48.9% (95% CI 47.2–50.7%) did not work, including 25.9% (95% CI 24.4–27.4%) were retired, others studied (2.2%, 95% CI 1.7 to 2.8%) or did not indicate kind of occupation (6,3%, 95% CI 5.5–7.2%). Occupations of the Lyme borreliosis patients differed also by gender: while a higher proportion of employed was among both men and women (respectively 47.9%, 95% CI 44.9–50.8%, and 39.8%, 95% CI 37.7–41.9%), though it is considerably higher among men. Among women, there is a significantly higher proportion of retired people than among men (30.3%, 95% CI 28.3–32.2% and 17.5%, 95% CI 15.3–19.8%), however this difference can be explained by significant difference between genders in the group of retired people.

Table 3.4

Lyme borreliosis incidence rate and relative risk of employed and unemployed patients, aged 15–64 years, by gender and statistical region, from 2007–2011 (n = 2666)

	Incidence rate per 100 000 person-years		**RR[1]**
By gender	**unemployed**	**employed**	
Men	33.2 (30.07 – 36.47)	21.9 (20.03 – 23.80)	1.62 (1.43–1.84)***
Women	56.1 (52.34 – 60.01)	34.7 (32.36 – 37.07)	1.62 (1.47–1.78)***
By statistical regions:			
Kurzemes	53.5 (46.20 – 61.53)	29.6 (25.48 – 34.15)	1.81 (1.48–2.21)***
Latgales	39.5 (34.11 – 45.57)	29.5 (25.60 – 33.80)	1.34 (1.1–1.63)**
Pierīgas	74.1 (66.41 – 82.49)	40.4 (36.22 – 44.89)	1.84 (1.58–2.13)***
Rīgas	46.2 (41.62 – 51.18)	25.3 (22.94 – 27.89)	1.83 (1.59–2.1)***
Vidzemes	41.5 (34.57 – 49.41)	30.3 (25.57 – 35.73)	1.37 (1.08–1.74)*
Zemgales	20.1 (15.79 – 25.24)	14.7 (11.73 – 18.12)	1.37 (1–1.87)*

[1] – the relative risk of unemployed compared with employed
p-value: *p<0.05, **p<0.01, ***p<0.0001

When calculating the incidence rate per 100 000 person-years[2] for employed and unemployed Lyme borreliosis patients of working-age (15–64 years) (Table 3.4), depending on the kind of occupation, it is found that the incidence rate of unemployed is statistically significantly higher than that of employed, for men and women, in all statistical regions; the highest relative risk of unemployed in comparison with employed is observed in the regions with the highest incidence rate for unemployed: in Pierīgas, Rīgas and Kurzemes regions.

For Lyme borreliosis, as for any of tick-borne diseases, the seasonality is stated by the vector (tick) activity and human activities that result in people coming into contact with disease vectors. Tick activity depends on the environmental conditions, particularly air temperature, relative air humidity and rainfall. Correlations between the mentioned environmental factors and people infection with Lyme borreliosis has been analysed in this work (Table 3.5).

Table 3.5

The correlation between climatic factors and the number of people infected with Lyme borreliosis during tick activity season (April–October) in the 2007–2011 (n=2249)

Region	**Pearson correlation coefficient with:**			
	average monthly air t	**average monthly rainfall in mm**	**average number of days with precipitation per month**	**average monthly relative humidity,%**
Latvia	0.668**	0.472**	0.195 NS	0.009 NS
I. ricinus regions	0.788**	0.667**	0.473*	0.200 NS
I. persulcatus regions	0.663**	0.298 NS	0.090 NS	-0.218 NS

p-value: *p<0.05, **p<0.001, NS – not statistically significant

The calculation results show that during the observation period in Latvia, a statistically significant median close or close correlation has been

[2] based on the data of the Central Statistical Bureau on the employment of inhabitants of Latvia

observed between the number of people infected and: (1) the average monthly temperatures in the whole country or separately in tick distribution regions, (2) the average monthly rainfall in the whole country and in the *I. ricinus* tick distribution region, (3) the average number of days with precipitation in the *I. ricinus* tick distribution region.

69.7% (95% CI 68.2%–71.1%) of Lyme borreliosis patients during the period from 2007 to 2011 indicated the fact of tick bite. Compared to women, men remembered a tick bite more often (respectively 73.3%, 95% CI 70.8–75.7% and 67.8%, 95% CI 65.8–69.7%), the differences between groups are statistically significant (Pearson χ^2=11.596, p=0.001). There were no statistically significant differences between the residents of cities and rural districts, as well as between hospitalized and ambulatory patients (p>0.05), however minimal differences (Pearson χ^2 = 7.806, p = 0.005) are found between Lyme borreliosis patients living in *I. ricinus* and *I. persulcatus* tick distribution regions (respectively 68.4%, 95% CI 66.5–70.2% and 73.2%, 95% CI 70.3–76.0%). Differences are also found depending on the month of patient's illness onset: in total the odds of finding a tick bite fact in patients who became ill during the period from May to August are higher than in patients who became ill during other months of the year (OR=1.795, 95% CI 1.553–2.075, p<0.001).

Analysing of the tick bite sites in Lyme borreliosis patients, it was found that bite in the head or neck is more often observed in the age group “0–17 years” (in this age group, 38.1%, 95% CI 29.6–47.2%) than in adults (3.3, 95% CI 2.6–4.2%); while in adults ticks significantly more likely bited feet (44.8%, 95% CI 42.8–46.9%) (Figure 3.4).

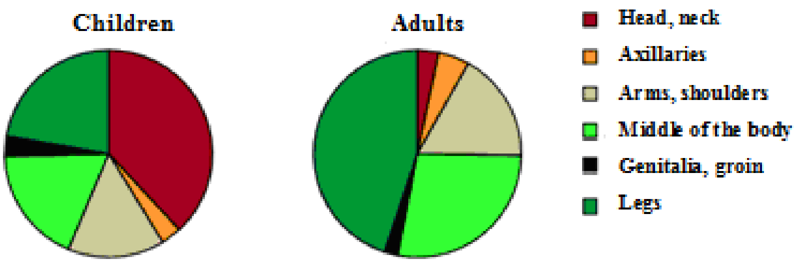

Fig.3.4. Tick bite sites in Lyme borreliosis patients: children and adults, 2007–2011

Odds ratios prove a statistically significant differences for the group of children and adults, in relation to the tick bite probability in the head / neck or legs, and head / neck or other parts of the body together (by the multinomial logistic regression for form of the disease, gender and tick distribution region adjusted odds ratio, respectively OR=21.201, 95% CI 11.860–37.900, $p<0.0001$ and OR=15.596, 95% CI 9.955–24.435, $p<0.0001$).

Tick bite in head or neck, in comparison with other parts of the body, was observed more frequently in *I. persulcatus* tick distribution region (9.9%, 95% CI 7.8–12.5%) than in *I. ricinus* tick distribution region (2.9%, 95% CI 2.2–3.9%), the differences are statistically significant (Pearson χ^2=49.317, $p<0.0001$). In the multinomial logistic regression adjusted for age, gender, registration year and form of the disease OR is 3.116, 95% CI 2.066–4.698, $p<0.0001$.

Comparing the tick bite sites in patients with various forms of Lyme borreliosis, it was found that patients with erythema migrans often indicated a tick bite in feet (45.7%, 95% CI 43.6–47.8%), followed by a tick bite in the middle of the body (27, 0%, 95% CI 25.2–28.9%). Tick bite in the head or neck among patients with erythema migrans is found in 4.2% of cases (95% CI 3.4–5.2%). In patients with other forms of the disease a tick bite is found most often in the middle of the body (29.8%, 95% CI 23.7–36.4) and legs (22.8%, 95% CI

17.4–29.0). The tick bite in the head or neck in this patient group has been observed in 14.4% of cases (95% CI 10.0–19.8%). The odds ratios prove statistically significant differences related to the probability of tick bite in head/neck or other parts of the body in patients with erythema migrans and other forms of the disease (for patients with the other forms of disease odds ratio for tick bite in head/neck was higher: OR=3.799, 95% TI 2.463–5.860, $p<0,0001$).

Statistically significant differences related to the tick bite sites in association with forms of the disease have not been observed in children group: a tick bite in head or neck found in 38.5% (95% CI 27.8–48.7%) of children with erythema migrans, and 37.0% (95% CI 19.4–57.6%) of children with other forms. Statistically significant differences have been found for adults, in turn: a tick bite in head or neck found in 2.6% (95% CI 2.0–3.4%) of patients with erythema migrans, and 11.2% (95% CI 7.0–16, 6%) of patients with other forms, including 12.1% (95% CI 6.2–25.4%) of patients with neuroborreliosis. The odds ratio adjusted for gender, age, statistical year and tick distribution region in the multinomial logistic regression analysis indicates higher odds for a tick bite in head or neck than in other parts of the body in adult patients without erythema migrans, compared to patients with erythema migrans: OR = 3.178, 95% CI 1.815–5.566, $p<0.0001$.

The duration of a tick bite was determined by the anamnesis data. For the most part of the patients – 88.2% (95% CI 86.7–89.4%) tick was removed within 24 hours since the tick bite, for 7.7% (95% CI 6.5–9.0%) – in the range from 24 to 48 hours, while for the remaining 4.1% (95% CI 3.3–5.1%) – after more than 48 hours. The tick removed in the first 24 hours since the bite for 89.9% (95% CI 88.0–91.5%) of women and for 85.4% (95% CI 82.5–87.9%) of men, differences are statistically significant ($p<0.05$). Statistically significant differences have been found according to the seasonal periods: chances of tick removal in the first 24 hours during the period from July to September are higher than at the beginning of the season (April – June): OR = 2.025 (95% CI = 1.515–2.706, p<0.0001).

The methods of the tick removal has been analysed on the basis of a patient anamnesis data, theoretically dividing them into two categories ("right" / "wrong"), and in total 34.1% (95% CI 31.5–36.7%) of patients with Lyme borreliosis ticks were removed properly. Correct tick removal odds are higher among Lyme borreliosis children patients as well as in the adults aged 30 to 39 years and from 50 to 59 years (compared with the age group "70 years and>": $OR_{children}$=2.035, 95% CI 1.127–3.674, p=0.019, OR_{30-39}=1.959, 95% CI 1.214–3.162, p=0.006, OR_{50-59}=1.607, 95% CI 1.026–2.516). Among unemployed retired Lyme borreliosis patients in 72.3% (95% CI 67.2– 77.1%) cases ticks were removed incorrectly, which is significantly more than in the employed population (62.4%, 95% CI 58.1–66. 6%). Compared odds ratios for correct and incorrect removal of ticks in Lyme borreliosis patients in the first and second day since tick bite, it was found that for those persons with a tick removed within 24 hours since bite, the odds ratios for correct removal are lower (in the multinomial logistic regression OR adjusted for age, gender, month of the tick bite and residence category is 0.537, 95% CI 0.338–0.852, p=0.008).

The risk of infection with Lyme borreliosis in the considerable extent is associated with human recreational activities, as stated by the comparison of the data on the number of people with tick bite on weekdays or weekends (including holidays): in total for 60.3% (95% CI 58.3–62.3 %) of the infected people tick bite occurred during weekdays however for 39.7% (95% CI 37.7–41.7%) – during a holiday. Compared with the expected breakdown (71.4% and 28.6%, respectively) using the non-parametric χ^2 test a statistically significant differences were found (χ^2=142.982, p<0.0001), indicating a higher risk of infection during holidays. Such differences in total were found in both genders in Latvia, in all occupations and age groups, except in the age group 70 years and older (χ^2=3.675, p=0.055), and in all statistical regions, except Zemgale region (χ^2=2.480, p=0.115). In the multinomial logistic regression comparing infection odds during holidays and weekdays between residents of large cities and parishes, adjusting for gender, age, occupation and registration year of the case, statistically significant differences have been found: OR = 1.345 (95% CI 1.117–1.619, p=0.002), showing higher odds for infection of city dwellers during holidays.

Comparing incidence rates of certain clinical forms, it was found that during the period from 2007 to 2011 the local skin forms were prevailing (Table 3.6). The incidence rate of Lyme borreliosis local skin forms and arthritis per 100,000 person-years was statistically significantly higher in women compared to men (RR=1.60, 95% CI 1.49–1.73, p<0.0001 and 2.50, 95% CI 1.57–3.97, p<0.001). Comparing age groups, it is found that there are no statistically significant differences in the arthritis incidence rate (p>0.05), although the highest figure recorded in the age group "40–49 years". The highest incidence rate of neuroborreliosis was found in the age group "60 years and>", but statistically significant differences in this age group were found only in comparison to the age group "0–17 years" (RR=2.42, 95% CI 1.29–4.52, p<0.01). Maximum incidence rate for the Lyme borreliosis local skin forms per 100 000 person-years was found in the age group "40–59 years", what was statistically significantly higher than in the age group "18–39 years" (RR=1.69, 95% CI 1.55–1,85, p <0.0001) and "0–17 years" (RR=4.98, 95% CI 4.21–5.89, p<0.0001).

Table 3.6

The incidence rate of Lyme borreliosis individual clinical forms per 100 000 person–years in Latvia, 2007–2011 (total, by age groups, regions, and categories of residence[3])

	Incidence rate per 100 000 person–years (95% CI)		
	local skin form	**neuroborreliosis**	**arthritis**
Average:	27.46 (26.50–28.45)	1.03 (0.85–1.23)	0.83 (0.67–1.02)
by gender:			
Men	20.72 (19.50–21.99)	0,84 (0.61–1.13)	0.46 (0.30–0.69)
Women	33.24 (31.80–34.72)	1.18 (0.93–1.49)	1.15 (0.90–1.45)
By age groups			
0–17 years	7.75 (6.57–9.07)	0.65 (0.35–1.12)	0.65 (0.35–1.12)
18–39 years	22.85 (21.33–24.45)	0.84 (0.57–1.20)	0.57 (0.35–0.87)
40–59 years	38.59 (36.44–40.84)	1.03 (0.71–1.46)	1.19 (0.84–1.65)
60 years and >	36.03 (33.73–38.45)	1.58 (1.13–2.15)	0.91 (0.58–1.36)
By statistical regions			
Kurzemes region	29.99 (27.29–32.88)	1.86 (1.23–2.68)	1.33 (0.81–2.05)
Latgales region	26.14 (23.78–28.67)	1.51 (0.99–2.21)	1.45 (0.94–2.14)
Pierīgas region	41.19 (38.37–44.16)	1.15 (0.72–1.74)	0.94 (0.56–1.48)
Rīgas region	28.54 (26.82–30.35)	0.31 (0.15–0.55)	0.11 (0.03–0.29)
Vidzemes region	16.55 (14.31–19.04)	1.87 (1.17–2.83)	1.95 (1.24–2.93)
Zemgales region	14.07 (12.18–16.17)	0.50 (0.20–1.02)	0.28 (0.08–0.73)
By tick distribution regions			
I.ricinus region	30.60 (29.38 – 31.85)	0.86 (0.66 – 1.09)	0.57 (0.42 – 0.77)
I.persulcatus regions	20.38 (18.91 – 21.94)	1.41 (1.05 – 1.87)	1.41 (1.05 – 1.87)
By categories of residence			
city	25.88 (24.60–27.21)	0.59 (0.41–0.82)	0.49 (0.33–0.70)
district	29.20 (27.77–30.68)	1.50 (1.19–1.87)	1.21 (0.93–1.54)

The incidence rate of Lyme borreliosis local skin forms, neuroborreliosis and arthritis was different in statistical regions, although not in all cases the differences are statistically significant. In Pieriga region the highest incidence rates for local skin form are registered, but in Zemgale – the lowest incidence rates (RR = 2.93, 95% CI 2.51–3.42, p <0.0001). In Vidzeme region the highest but in Riga region – the lowest incidence rates for

[3] City – a city with a population of 20,000 and more, district – administrative area with a population of up to 19,999

neuroborreliosis are detected (RR = 6.04, 95% CI 2.93 to 12.46, p <0.0001) and arthritis (RR = 17.37, 95% CI 6.01 to 50.22, p <0.0001). In *I. persulcatus* tick region (in the east of the country), in comparison with *I. ricinus* region (the central and west part of the country), statistically significantly higher incidence rates of neuroborreliosis and arthritis were observed (RR respectively, 1.65, 95% CI 1.14–2.39, p <0.01, and 2.46, 95% CI 1.64–3.69, p <0.0001) and lower – erythema migrans incidence (RR=0.67, 95% CI 0.61 to 0.72, p <0.0001). Comparison of incidence rates for clinical forms by patients' residence category (independently from statistics or tick distribution region) revealed a higher incidence rates for all analysed forms in rural than in the urban inhabitants. The relative risk of the disease for rural residents compared to the urban residents in patients with erythema migrans is RR=1.13 (95% CI 1.05–1.21, p<0.001), with neuroborreliosis RR=2.54 (95% CI 1.71–3.77, p<0.0001), with arthritis RR=2.48 (95% CI 1.59–3.81, p<0.0001).

3.4. Analysis of the factors related to the approval of Lyme borreliosis diagnosis serological confirmation

Cases of Lyme borreliosis included in VISUMS database, which were tested in the laboratory of the State Agency "Infectology Center of Latvia" during the period from 2007 to 2011, with the interval from onset of the disease until the investigation between 0 and 365 days (as indicated in the protocol of the epidemiological investigation according to the date of the disease onset), were included in the analysis On the cases investigated several times, the results of chronologically first investigation were analysed. The analysis includes data on 1118 patients who were investigated by ELISA against *B. burgdorferi* s.l. in determining both IgM and IgG class antibodies, 114 patients were investigated only for IgM antibodies and 109 patients were investigated only for IgG class antibodies. The total number of cases included in the selection is 1341. The selection included 466 men (34.8%) and 875 (65.2%) women, average age for men 42.25 (± 0.864), median 42 years, women 49.64 (± 0.613), median – 52 years, for both genders – 47.07 (± 0.509) years, median 49 years. Selection included Lyme borreliosis patients from all regions of Latvia, the majority – from Riga and Riga region (48.6%, 95% CI 45.8–51.3%). The number of investigated cases varied over the years: the minimum number of patients was investigated in 2008 (13.5%, 95% CI 11.7–15.4%), maximum – in 2007 and 2011: 24.2% (95% CI 21.9–26.5) and 24.1% (95% CI 21.8–26.5%), respectively. Cases, which test results for the particular test were "borderline", were excluded from the analysis.

Analysis of the investigation results of Lyme borreliosis patients (n=1144) with ELISA test for the presence of IgM antibodies, revealed that the positive test result was 58.8% (95% CI 55.9–61.7%) of investigated, 53.3% (95% CI 48.2–58.3%) of men and 61.8% (95% CI 58.2–65.3%) women. The positive test result was found in 56.9% (95% CI 53.7–60.1%) of the investigated patients with erythema migrans, while in patients with other forms – in 68.8% (95% CI 61.6 to 75.4%) of cases. Among the hospitalized patients for 56.1% (95% CI 51.6 to 60.4%) of cases IgM antibodies were found as a result of investigation, while among patients who were treated in an outpatient setting – for 61.0% (95% CI 57.1 to 64.9%) of cases. Compared the mentioned groups with the χ^2 method, statistically significant differences in the set of positive samples has been found between men and women (χ^2=7.691, p=0.006), as well between patients with erythema migrans and other forms (χ^2=9.149, p=0.002). Among patients who had a history of tick bite, a positive result was in 53.5% (95% CI 50.0–57.0%) of cases, while among other patients – in 71.9% (95% CI 66.7–76.7%) of cases (χ^2=32.873, p<0.0001).

The proportion of the positive results among persons investigated during the first month of illness were 55.6% (95% CI 51.7–59.5%) in patients with erythema migrans, and 76.4% (95% CI 68.5–83.2%) – in patients with other forms, differences are statistically significant (χ^2=20.700, p<0.0001). In the second month of illness IgM class antibodies were detected in 58.9% (95% CI 50.5–67.0%) of investigated patients with erythema migrans and in 52.9% (95% CI 27.8–77.0) of patients with the other disease forms, the differences are not statistically significant (χ^2=0.223, p= 0.627). IgM antibodies were detected in patients with a known tick bite date and with laboratory investigations carried out in the range of 15 to 60 days after a tick bite, in 56.3% (95% CI 51.0–61.6%) of cases of erythema migrans and in 77.2% (95% CI 64.2– 87.3%) of cases with other forms, the differences are statistically significant (χ^2=8.845, p=0.003).

The average interval from infection to the investigation was 35.8 (±1.69) days, (median 13 days), slightly longer in patients with erythema migrans (36.76 ± 1.89, median 14 days) than in patients with other forms of the disease (31.17 ± 3.69, median 8 days), statistically significant differences are not observed (p> 0.05, by Mann-Whitney U-test). Hospitalized Lyme borreliosis patients were laboratory investigated much earlier than outpatients (mean interval from disease onset to the investigation respectively 22.57 ± 1.89, median 8 days and 46.53 ± 2.57, median 21 days), and a statistically significant differences are stated between groups (p <0.0001, by Mann-Whitney U-test. Among the groups of patients with positive and negative test results statistically significant differences are not observed in relation to the interval from the disease onset to the investigation (the average length of the interval respectively 34.78 ± 2.06, median 14 days and 37.37 ± 2.88, median 11 days, p>0.05, by Mann-Whitney U-test).

The relation between number of possible factors and results of ELISA test for the IgM class antibodies against *B. burgdorferi* s.l. was tested by binary logistic regression and significance of gender, age, registration year, clinical form (erythema migrans / other) and hospitalization status was proven (Table 3.7).

Table 3.7

Odds ratio[1] for positive result of determining ELISA IgM antibodies against *B. burgdorferi* s.l. in relation to some factors in patients with Lyme borreliosis diagnosis set in 2007–2011 (n = 1144)

Factor	OR (95% CI), both genders (1st model)	OR (95% CI), men (2nd model)	OR (95% CI), women (3rd model)
Gender (woman)	1.502 (1.158–1.946)*	–	–
Form (other)	1.872 (1.309–2.679)*	3.363 (1.816–6.228)**	1.323 (0.849–2.061)^NS
Hospitalisa-tion (yes)	0.,696 (0.535–0.905)*	0.841 (0.542–1.303)^NS	0,632 (0.455–0.878)*
Registration year	1.167 (1.077–1.265)**	1.189 (1.036–1.364)*	1.157 (1.047–1.279)*
Age	0.992 (0.985–0.998)*	0.993 (0.983–1.004)^NS	0.990 (0.982–0.998)*

[1] odds ratio for the positive test result, adjusted by the interval from disease onset to the investigation and place of residence

*p<0.05, **p<0.001, NS – not statistically significant

The table 3.7 illustrates results that testify that women, patients with non-skin form of the disease, and non-hospitalized Lyme borreliosis patients have significantly higher odds for positive result related to IgM antibody detection in ELISA test. Among men, odds of a positive test result in patients with non-skin form was 3.363 times higher than in patients with erythema migrans, however for women this difference was not statistically significant. Hospitalized women had lower odds of obtaining a positive laboratory test result than outpatients, and unlike men odds ratio was statistically significant.

With increasing patient’s age, the odds for a positive result decrease for both genders together and for women. Due to the possible association of age with the probability of the serological confirmation, patients' odds ratios has been analyzed separately, dividing them into different age categories; statistically significant results were obtained only for women with a breakdown into two groups: younger than 40 years and 40 years and older. In the age group “0–39 years” the proportion of positive results among women was 68.7% (95%

CI 61.7– 75.0%), while in the age group “40 years and older” – 59.2% (95% CI 55.0– 63.4%), χ^2=5.528, p=0.019. Odds of a positive result in the older (40 years and over) women were lower than in those under the age of 39 years (odds ratio was matched with a statistical year, place of residence, clinical form, interval from the onset of the disease till investigation, and hospitalization status OR=0.656, 95% CI 0.463–0.930, p<0.05).

Figure 3.5 illustrates rate of the positive results by gender and age groups (0–17 years, 18–29 years, 30–39 years, 40–49 years, 50–59 years, 60–69 years, 70 years and more, labelled as 1–7 in the figure respectively).

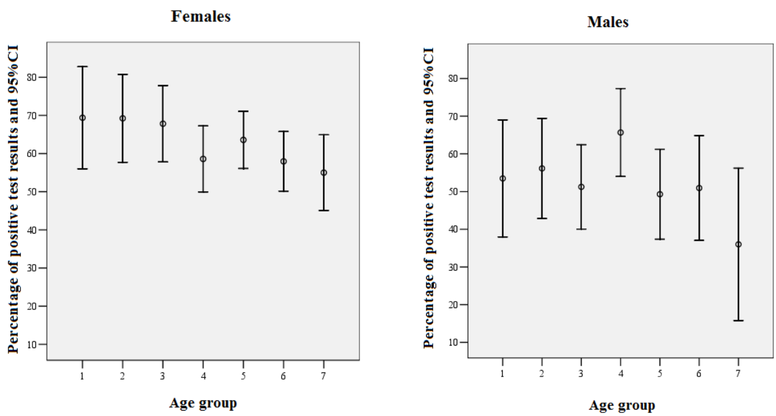

Fig.3.5. Detection of IgM antibodies against *B. burgdorferi* s.l. by ELISA: proportion of the positive results in age groups, men and women, 2007–2011 (n = 1144)

The binary logistic regression did not prove statistically significant association between the interval from the onset of the disease and investigation, and positive test result (odds ratio 0.999, 95% CI 0.996–1.001, p>0.05), as well did not prove the correlation between the interval from the tick bite, and the positive test result (odds ratio 1.000, 95% CI 0.997–1.002, p>0.05).

During the first 10 weeks of the disease the prevalence of positive samples was as follows: in the first week – 54.4% (95% CI 49.4–59.3%), in the 10th week – 57.9% (95% CI 33.5–79, 8%), maximum – in the 4th disease week, 73.0% (95% CI 61.4–82.7%).

The separate analysis of the test results in patients with a known tick bite date during first 14 weeks from tick bite indicated an increasing trend of proportion of positive samples from 29.7% (95% CI 15.9– 47.0%) in the first week to 72.7% (95 % CI 49.8– 89.3%) in the 9th week, after that the prevalence of positive samples decreased (Figure 3.6).

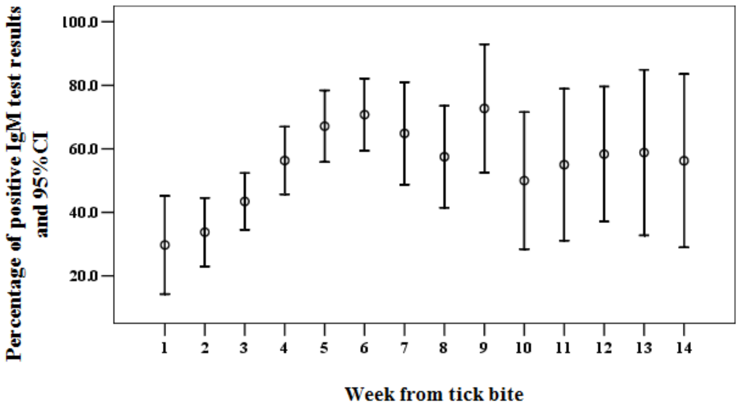

Figure 3.6. Detection of IgM antibodies against *B. burgdorferi* s.l. by ELISA in the first 14 weeks after a tick bite: the prevalence of positive results (%), 2007–2011 by weeks (n = 658)

Analysis of the investigation results of Lyme borreliosis patients (n=1170) tested with ELISA for the presence of IgG antibodies stated that the positive test result was 43.9% (95% CI 41.1–46.8%) of investigated, 42.4% (95% CI 37.5–47.5%) of men and 44.7% (95% CI 41.2–48.3%) of women. Among the investigated patients with erythema migrans the positive test result was found in 42.6% (95% CI 39.5–45.7%) of cases, while in the patients with other forms – in 51.4% (95% CI 43.8–59.0%) of cases. Among the hospitalized patients in 41.9% (95% CI 37.4–46.6%) of cases as a result of the investigation the IgG class antibodies were found, while in patients who were treated in an outpatient setting – in 45.2% (95% CI 41.5– 49.0%) of cases. When comparing the mentioned groups with the χ^2 method, statistically significant differences in the proportion of the positive samples have been detected among patients with erythema migrans and other forms (χ^2=4.738, p=0.03). Statistically significant differences in the proportion of positive samples (prevalence of the positive samples, respectively 40.6% and 53.2%, χ^2=6.863, p=0.009) have been detected among the groups of patients with erythema migrans and other forms, investigated in the first month of illness. When comparing groups of patients with erythema migrans and other forms of the disease investigated in the second month, a statistically significant differences in the prevalence of positive samples have not been detected (prevalence of positive samples, respectively 45.3% and 44.4%, χ^2=0.004, p=0.948). Amongst patients with known tick bite date and laboratory investigations carried out in the range of 15 to 60 days after a tick bite, in 34.2% (95% CI 29.2– 39.4%) of cases with erythema migrans and 54.30% (95% CI 39.0– 69.1%) of cases with other forms the IgG class antibodies were found, the differences are statistically significant (χ^2 =7.115, p = 0.008).

The average interval from the onset of the disease to the investigation was 39.51 (± 1.78) days (median 16 days), slightly longer in patients with erythema migrans (39.90 ± 1.95, median 17 days) than in patients with other forms of the disease (37.38 ± 4.39, median 10 days), statistically significant differences are not observed (p>0.05, by Mann-Whitney U-test). Hospitalized patients were laboratory investigated much earlier than outpatients (median interval from onset of the disease till the investigation respectively 26.17 ± 2.30, median 9 days, and 48.32 ± 2.48, median 23 days), a statistically significant difference has been observed between groups (p<0.0001 by Mann-Whitney U-test). Between the groups of patients with positive and negative test a statistically significant differences in the interval from the onset of the disease to the investigation are not observed (the average length of the interval 38.83 ± 2.48, median 16 days and 40.05 ± 2.51, median 15 days, p>0.05 by Mann-Whitney U-test).

The relation between number of possible factors and results of ELISA test for the IgG class antibodies against *B. burgdorferi* s.l. was tested by binary logistic regression and significance of clinical form (erythema migrans / other), registration year, age, place of residence in one of the tick distribution regions (*I. ricinus* or *I. persulcatu*s) and type of residence place (large city, city, town, parish[4]) was proven (Table 3.8).

Table 3.8

Odds ratios[1] for determination of IgG class antibodies to *B. burgdorferi* s.l. in the positive result of ELISA test in relation to a certain factors in patients with Lyme borreliosis diagnosis, 2007–2011 (n = 1170)

Factor	OR (95% CI), genders (1st model)	OR (95% CI), men (2nd model)	OR (95% CI), women (3rd model)
Form (other)	1.798 (1.253–2.580)*	1.945 (1.029–3.680)*	1.740 (1.121–2.701)*
Registration year	1.274 (1.176–1.380)**	1.259 (1.093–1.451)*	1.282 (1.163–1.413)**
Age	1.023 (1.016–1.030)**	1.024 (1.011–1.036)**	1.023 (1.014–1.031)**
Tick distribution region (*I. persulcatus*)	0.640 (0.486–0.842)*	0.661 (0.410–1.064)^{NS}	0.618 (0.441–0.868)*
Inhabited place[4]	1.100 (1.003–1.206)*	1.137 (0.966–1.339)^{NS}	1,083 (0.968–1.212)^{NS}

[1] odds ratio for the positive result, adjusted by gender, hospitalization status and the interval from onset of the disease to the investigation

*p<0.05, **p<0.001, ^{NS} – not statistically significant

The binary logistic regression did not prove a statistically significant association of the interval from onset of the disease to the investigation with positive result (odds ratio 1.000, 95% CI 0.998–1.002, p>0.05), however the subgroup with the known tick bite date (n=766) showed a statistically

[4] large city – city with a population of 100 000 inhabitants and more, city – city with population between 20000 and 99999 inhabitants, town – town with population between 5000 and 19999 inhabitants, parish – administrative unit with less than 5000 inhabitants

significant relationship between the duration of the interval from the tick bite and the investigation of a positive outcome probability (odds ratio 1.009, 95% CI 1.005– 1.013, p<0.001).

Results reported in the table 3.8 suggest that patients with non-skin form of the disease, living in *I. ricinus* distribution regions (total and female) and in the smaller settlements (only together) have statistically significantly higher odds of the positive result for IgG class antibodies detection in the ELISA test. During the period from 2007 to 2011, there was a positive result probability upward trend from year to year. With increasing age, increases the proportion of positive results (%) in both genders in total, and separately for men and women (Figure 3.6).

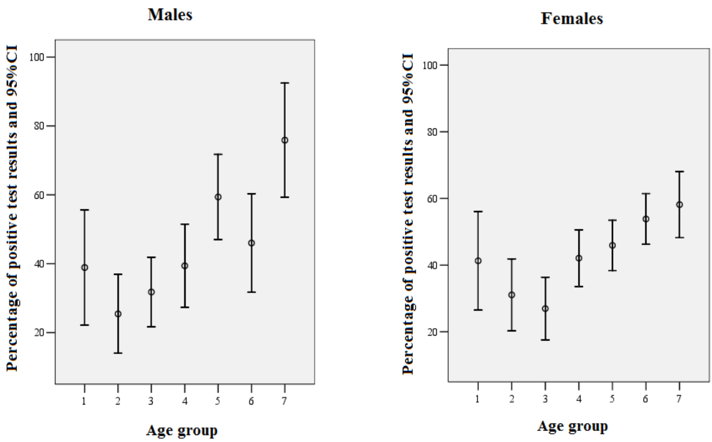

Figure 3.6. Detection of IgG class antibodies against *B. burgdorferi* s.l. by ELISA test: proportion of the positive results in the age groups of men and women, 2007–2011 (n = 1170)

The odds of a positive test result increase with age for both genders together and separately for men and women. In general, for younger people (aged 0–39 years) odds for positive result are lower than for the older age group (positive result odds ratio adjusted by year of statistics, form of the disease, hospitalization status, gender, tick distribution regions and type of residence place, OR = 0.412, 95% CI 0.314–0.542, $p < 0.05$).

The prevalence of positive test results in Lyme borreliosis patients tested within the first 10 weeks were as follows: in the 1st week – 43.6% (95% CI 38,6,4–48,7%), in the 10th week – 54.2% (95% CI 32.8 –74.5%) however statistically significant trend in the proportion of positive samples has not been established. Separate analysis of the test results in patients with a known tick bite date 14 weeks after tick bite, revealed an upward trend in the proportion of the positive samples from 29.8% (95% CI 20.8– 40.1%) in the first two weeks till 54.7% (95% CI 44.2–65.0%) during the period from the 9th to 12th week, after then the prevalence of positive samples decreased (Figure 3.7).

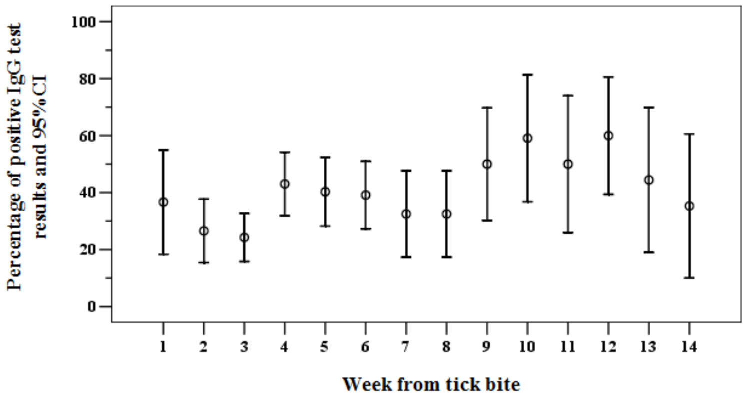

Fig. 3.7. Detection of IgG class antibodies against *B. burgdorferi* s.l. by ELISA test in the first 14 weeks after a tick bite: the prevalence of positive results (%), 2007–2011 by weeks (n = 622)

Analysis of the cases where a patient was tested by ELISA IgM and IgG antibodies against *B. burgdorferi* s.l. in one day, and the test result was not rated as “borderline” (the number of the selected cases 978), discovered that in general the odds of a positive test was higher for persons who had a positive second test (OR=2.140, 95% CI 1.642–2.789, $p<0.001$). For patients tested for both IgM and IgG antibodies time intervals between laboratory investigation and disease onset (n = 978) or tick bite (n = 644) were analysed by groups of test results (Table 3.9).

Table 3.9

The time intervals between laboratory investigation with ELISA test for detection of antibody response against *B. burgdorferi* s.l. and onset of the disease or tick bite, by groups of the test results, 2007–2011 (n=978/644)

Interval	IgM+IgG+	IgM+IgG-	IgM-IgG+	IgM-IgG-
The mean interval from the onset of the disease (95% CI)	35,83 (29.89–41.78)	34,60 (28.30–40.89)	40,13 (29.70–50.56)	39,42 (31.20–47.64)
1.quartile	4	5	2	3
2.quartile	15	14	15	11
3.quartile	44.7	35	57	35.7
The mean interval from the tick bite (95% CI)	66.26 (56.03–74.49)	60.53 (51.27–69.79)	83.73 (66.19–101.27)	51.88 (43.14–60.62)
1.quartile	24	23	24.5	14
2.quartile	41.5	38	63	26
3.quartile	82	70.75	118	61

Statistically significant differences between results' groups were not found ($p>0.05$) when compared the average interval from disease onset to the laboratory investigation, while significant differences were found in relation to the duration of the interval from the tick bite until investigation ($p <0.0001$). Differences in medians and distribution in quartiles can also partially explain different test results in groups (e.g., higher proportion of patients with negative results in both tests was laboratory investigated earlier than the rest of patients). A significant proportion of patients with test results "IgM-IgG+" has been investigated within 2 days since onset of the disease, and a positive IgG test for a part of these cases could be explained by inaccurate indication of the date of the onset of the disease, as well as, possibly with antibodies against *B. burgdorferi*, which for the particular patient may be related to previous illness episode.

4. DISCUSSION

Lyme borreliosis incidence in Latvia is characterized by a continuous increasing trend since the beginning of the registration of the disease (1986), particularly noticeable during the last 15 years (1997 to 2011). In many other European countries, for example, England and Wales, Bulgaria, the Netherlands, Norway, Poland, Finland, Germany, in recent years Lyme borreliosis incidence increase was observed as well [7]. However, the literature sources indicate that these increase trends could be assessed with caution, as they may be related to the reporting and diagnostic improvements, as well with increased public awareness. Scientific studies prove that as a result of interaction of the climatic, environmental and social factors the human infection risk with tick-borne diseases is changing over the years. In general, since fifties of the twentieth century in the northern hemisphere a significant increase in minimum daily temperatures, and winter temperatures has been registered, especially in northern Europe; during recent years the spring starts on average about two weeks earlier than it was before 1980, thus the vegetation and tick activity period duration lengthened [12], which is favourable for enlargement of tick populations, for increase of pathogen circulation, as well as extending humans active contact period with the natural foci. Compared to other European countries, during the period from 1997 to 2004 in Latvia in general the similar disease risk associations have been reported, depending on the place of residence (urban or rural area) [7, 13]. However, Lyme borreliosis relative risk changes discovered among the urban and rural inhabitants (in the period from 1997 to 2011, the incidence rate increasing trend was more manifested in rural population, and after 2007 the incidence rate among urban inhabitants is lower) may indicate to Lyme borreliosis diagnosis and reporting improvement in the rural population over time.

Analysis of the rate of changes in incidence by age groups was stated that it has increased in all age groups, but most of all – in the age group “50–59 years”, which could be partly explained by relevant changes in the employment structure of the Latvian inhabitants, increasing of the number of unemployed persons during recent years, especially in the pre-retirement age group. According to the survey results performed in Latvia in 2001, unemployed persons indicated collection of mushrooms and berries as most frequent reason to visit forest [14], thereby exposing themselves to a higher risk of infection with tick-borne diseases [15], including Lyme borreliosis.

The correlation analysis carried out in in this work for finding the association of Lyme borreliosis incidence with the possible climatic, environmental and social factors from 1997 to 2011, refers to the factors described in the literature – the possible role of forest animals in increase of the Lyme borreliosis incidence [16, 17, 18]. The study of long-term dynamics did not discover statistically significant strong correlations with average temperatures, rainfall and other factors characterising weather conditions, however it could be explained by the quality of the analysed meteorological data, the level of details and relatively short observation period. The study found a statistically significant negative correlation with the following indicators of pollution: the total emissions of pollutants, solid particles, sulphur dioxide and carbon monoxide. The author has no data of published studies, which would explain such a phenomenon from an ecological point of view. However, other researchers point out that the decline in pollution of the environment is one of the indicators of the changes in the structure of economies of countries, and therefore, may show indirectly the change in population behavioural patterns, providing a higher infection risk due to the frequent contacts with natural foci [19, 20].

The statistics show that the Lyme borreliosis incidence rate in Lithuania and Estonia are significantly higher than in Latvia: in the period from

2007 to 2011 the incidence rate in Lithuania ranged from 34 to 106.6, while in Estonia – from 53.7 to 171.8 per 100 000 person-years [21, 22], which could indicate possible deficiencies in the reporting of Lyme borreliosis cases in Latvia. Comparing the data base of the officially registered Lyme borreliosis cases in the period from 2007 to 2011 (VISUMS) with the National Health Service database (VIS) on Lyme borreliosis cases during the respective period, it is found that the number of Lyme borreliosis cases for which medical practitioners have submitted report to the National Health Service, is 3.5 times higher than that reported according to the prescribed procedures in the Cabinet Regulation No. 7, adopted 5 January 1999 "Procedures for Registration of Infectious Diseases". This indicates to a significantly higher incidence of Lyme borreliosis in Latvia. According to literature data, a significantly lower incidence of infectious diseases than diagnosed has been usually reported in the passive reporting framework, e.g. the studies carried out in the United States studies indicate that the actual number of Lyme borreliosis cases is 2.8 to 2.9 times higher than the reported to the monitoring systems [8, 9]. However, it is important that the part of cases reported to be representative of the general population of patients, as observed during the development of the thesis, in VISUMS and VIS data comparison.

In Latvia during the study period, women significantly more often suffered from Lyme borreliosis. Compared with both the United States data [23] and the results of the study conducted in Europe [7], incidence rate in women is significantly higher. The highest incidence rate among women can be partly explained by Swedish study [24] results, which show that women over the age of 40 years have for 48% higher risk of tick bite compared to men over the age of 40 years and for a 96% higher risk compared to younger men. This study revealed a statistically significant differences in Lyme borreliosis incidence rates by gender; researchers believe that the observed morbidity differences cannot be explained only with exposure variations and are going to continue research on biological, sociological, and immunological mechanisms that could explain the results described.

Lyme borreliosis incidence rate per 100 000 person-years among unemployed working-age inhabitants is statistically significantly higher than among employed in whole Latvia, for both genders, separately in statistical regions ($RR>1$, $p<0.05$), which may indicate a higher risk of infection due to leisure activities in nature [14].

Performed analysis of the climatic factors influencing Lyme borreliosis incidence in the 2007–2011 tick activity seasons gives evidence that Lyme borreliosis incidence increase is being facilitated by a warm and wet seasons, which is beneficial to the survival of ticks and promotes human contact with the natural foci [25, 26].

Differences in the frequency of tick bites to various parts of body revealed in the thesis may be explained by differences in *I. ricinus* and *I. persulcatus* tick activity levels and a maximum height where during the activity phase the mentioned tick species can be found: the literature has shown that *I. persulcatus* have been found up to 80 cm from the ground, but *I ricinus* ticks – usually in 15–20 cm height [27, 28]. However, in areas with extremely high vegetation *I. ricinus* ticks can be found up to 80 cm above the ground [29]. Tick bite rate to different parts of the body for Lyme borreliosis patients generally corresponds to a Swedish study concerning the data on the distribution by age (children / adults) [30]. The observation results carried out in the Republic of Karelia of the Russian Federation regarding the tick bite topographical locations on the human body indicates the highest probability of a tick bite in head and neck – up to 14.3% in adults and up to 70.9% in children under 5 years of age [31], which according to the results of this thesis, is more typical for inhabitants of Latvia living in *I. persulcatus* region. The hypothesis of a possible anatomical location of a tick bite in relation to Lyme borreliosis clinical forms proposed by Swedish researchers, indicating that among patients with neuroborreliosis there is a statistically significantly higher proportion of persons with tick bite in head or neck. From the point of their view, probably the highest neuroborreliosis incidence rate among children has also been associated with a higher incidence of tick bite in the head or neck [30]. This thesis found statistically significant differences between the tick bite for patients with different forms of the disease, the causes of which require additional research. However, making the interpretation of results, possible selection bias must be taken into account because for a significant proportion of patients the fact of tick bite is not recognized.

Duration of tick bite as a key factor for the probability of Lyme borreliosis development has been emphasized in many studies, indicating that infection transmission risk is significantly lower in the first day since the tick bite than later [32, 33]. This thesis shows that ticks were removed within the first 24 hours after the bite for the majority of Lyme borreliosis patients. Due to the fact that the analysed tick bite duration was determined by anamnesis data the probability of recall bias cannot be ruled out. The study finding that persons, to whom a tick was removed within 24 hours after bite, had lower odds for its proper removal could partly explain the observed discrepancy with the literature data. Higher odds for quick tick removal observed during tick season point on more conscientious attitude of inhabitants towards the tick-borne diseases' risk at this time. The study found that for the majority of Lyme borreliosis patients (two-thirds) ticks were removed by choosing inappropriate techniques (with hands, knife, oil, etc.), which increases the risk of infection transmission [34]. Differences found in the rate of the proper tick removal were observed between age and occupation groups, as well as according to the residential category give evidence about different awareness of recommendations for proper behaviour in the case of tick bite, a variety of readiness and willing to follow them, indicating the direction for improvement of the complex of preventive measures.

Data on incidence rate of the Lyme borreliosis individual clinical forms in the literature are difficultly comparable due to the fact that the disease is a subject to reporting only in certain countries, as well as for a variety of case definitions. Most publications are referred to the distribution percentage of the individual forms in Europe: erythema migrans is present in 60–80% of registered cases, neuroborreliosis – 10–16%, arthritis – 7–8% [35, 36, 37, 30]. The relative rate of individual forms found in this thesis was different from that described in the literature: cases with erythema migrans make up to 93.7% of all cases with a clearly defined clinical form, neuroborreliosis found in 3.5%, and arthritis – 2.8% of cases. The largest proportion of cases of erythema migrans in Latvia would indicate a better account of this clinical form compared to other countries. Comparing the incidence rate of certain clinical forms by gender, it is found that women compared to men, have a slightly higher incidence of arthritis and significantly higher incidence of erythema migrans (the differences are statistically significant). One can put forward the hypotheses that (1) the presence of a higher rate of women with mentioned disease forms may be related to different attitude in the assessment of Lyme borreliosis symptoms, as a result, women more often apply for help or (2) women have a higher risk of acquiring mentioned disease forms due to different immune response mechanism on Lyme borreliosis agent. Swedish study found that the immune response to *B. burgdorferi* of women in climacteric and postmenopausal period significantly differs from men's response, increasing the odds of Lyme borreliosis development in women [24, 38]. Due to the fact that women have a higher incidence than men in all adult age groups, it can be concluded that differences by gender is set by two mentioned, and possibly other factors. The thesis has shown that children have a statistically significant lower incidence rate of erythema migrans, however neuroborreliosis and arthritis incidence is comparable to that of the adult group rates. This could indirectly point to the shortcomings of reporting of erythema migrans cases among children. Dissertation revealed differences in the distribution of individual clinical forms by statistical regions. These differences may be real (related to different prevalence of *Borrelia* genospecies in ticks and other biological factors), or they can be explained by reporting failures. For example, in Riga region to compare with other regions, incidence rate of erythema migrans cases were in average level, while that of neuroborreliosis and arthritis – in the lowest level. One of the possible explanations is a greater diagnostic capabilities and better reporting of cases in metropolitan residents. The additional evidence of this interpretation – comparison of incidence rate for some clinical forms, selected the category of residence (city or parish) for grouping criteria. It is found in the thesis that the inhabitants of districts (regardless of the statistical region) has the greatest risk of exposure to all forms of Lyme borreliosis, an addition the risk is at least two times higher ($p<0.05$) with more severe forms of the disease (neuroborreliosis, arthritis). Differences in disease incidence rates by territories can be explained by unequal levels of the prevalence of Lyme borreliosis pathogens in ticks. The studies carried in Latvia related to *B. burgdorferi* s.l. presence in the ticks collected in nature indicate that the percentage of the pathogen prevalence in ticks in the territory of Latvia in recent years were not equal. During an extensive study, conducted in 1998 to 2002, ticks collected in four Latvian regions were investigated, and the highest prevalence of *Borrelia* were found in ticks from the eastern part of Latvia, what borders with Russia and Belarus (36%), as well as in the north-eastern part what borders with Estonia (33%). 11% and 22% of infected ticks were from Zemgale and Kurzeme. *B. afzelii* was found in 76% of the infected tick samples, in 18% – *B. garinii*, 2%– *B. burgdorferi* s.s. and 2% – *B. valaisiana.* This study did not find differences in the prevalence of *Borrelia* genospecies by territories of Latvia [39]. In 2006 to 2009, during the European Commission-funded international Sixth Framework Programme “Global Change and Ecosystems” EDEN (*Emerging Diseases in a* *changing European eNvironment*) project framework performed laboratory tests in order to detect the presence and genospecies of *B. burgdorferi* s.l. revealed that in *I. ricinus* tick distribution area 17.8% of infected ticks identified, in *I.persulcatus* area – 25.5%; *B. afzelii* was found in 82% of the samples, *B. garinii* – in 16%, but statistically significant differences were not detected for the prevalence of particular *Borrelia* genospecies by territories [40]. Based on this study and the results of this thesis, it can be concluded that the differences in the Lyme borreliosis incidence level in the regions of Latvia is largely determined by the uneven *Borrelia* prevalence level in ticks in the regions of the country. For example, in Zemgale region, where a low incidence rate with all Lyme borreliosis forms have been detected, according to the both studies, a small percentage of infected ticks (11% and 14.4%) was also found. At the same time, available data do not give a full explanation of the individual differences in the incidence rate of particular clinical forms, and points on the need for the further research in order to ascertain the prevalence of Lyme borreliosis pathogens and genospecies, as well as their relationship to the disease incidence.

The results of the analysis of the serological confirmation indices of Lyme borreliosis patients investigated in the laboratory of the State Agency "Infectology Center of Latvia" points to an association of a number of factors with the serological confirmation of the diagnosis with ELISA tests for detection of IgM or IgG antibodies against *B. burgdorferi* s.l.

The proportion of the positive samples in ELISA test for detection of IgM antibodies in patients with erythema migrans revealed in this thesis was 56.9% (for patients investigated in the first month – 55.6%), which fits the limits specified by *P.Oschmann et al.* (up to 90% [35]), however is slightly higher than in other studies [30, 41, 42, 43]. The relationship between age of the patient and probability of IgM antibody detection (with increasing age decreases the probability of a positive result) determined by logistic regression is generally in accordance with the immune response and corresponds to the results of other studies [44]. Detected percentage differences in positive samples by gender (most likely probability of positive result in women) is not described in detail in the studies analysed by the author of this thesis, and could be explained by influence of biological, gender-related factors, although the possibility of selection biases is not ruled out also. Differences in the proportion of positive samples in women of the age group "30–39 years" (67.8%), "40–49 years" (58.6%) and "50–59 years" (63, 6%) indirectly indicate to the possible role of hormonal factors. Some researchers point out that women have a higher probability of false-positive result in ELISA test [45], however findings of the study carried out in the United States are controversial, pointed on higher odds of IgM antibody detection in Lyme borreliosis patients for men [46]. Due to the fact that only ELISA test results were evaluated in this work, a possible non-specific cross-reactivity with other pathogens should be taken into account (e.g. *Treponema pallidum* [47]), as well as the peculiarities of the immune system (women have a higher rate of systemic, autoimmune diseases). The largest percentage of the positive samples and the highest probability of the positive result for non-hospitalized patients could be explained by the differences in periods of investigation of outpatients and hospitalized patients (hospitalized patients in the study group were investigated on average faster than outpatients). Statistically significant differences found in prevalence of positive samples in patients with erythema migrans and other Lyme borreliosis forms are associated with disease duration and correspond to the literature data [35, 48]. Dynamics of the positive samples by weeks since illness / tick bite is generally consistent with results observed in other studies (the maximum percentage of positive samples of investigated was identified in the 4th disease week, although also in the 6th and 9th week from a tick bite) [42, 49]. Also, in studies by other authors [49], like in this thesis, in part of the patients in the first week of the tick bite have been detected IgM antibodies, which may indicate the early antibody response, as well as previous infection with Lyme borreliosis.

In the framework of this thesis performed analysis of the ELISA test results for IgG class antibodies against *B. burgdorferi* s.l. indicates an association of a positive result with a patient's age, and, furthermore, the opposite direction of this association is observed in the IgM antibody response, that is, with increasing age the probability of a positive outcome increasing. Similar results have been described in a study in Poland [44]. The highest proportion of positive samples in the elderly patients could be partly explained by the higher antibody seroprevalence against Lyme borreliosis pathogens in this age group. Studies on the prevalence of antibodies against *B. burgdorferi* s.l. among healthy individuals have not been carried out in Latvia to date; as a result the comparative analysis of the results is not possible. In studies of the other countries a higher prevalence of IgG class antibodies in the elderly population was found [50, 51], as well as in those exposed to the risk of infection due to work commitments [52] or hobbies [53]. Increasing odds of the positive IgG test result to persons who live in rural areas, found in the thesis, as well a significant prevalence of positive samples in the first two weeks since a tick bite in investigated patients (29.8%), imply to a possible previous infection. The results of this thesis shows that the persons who reside in the *I. persulcatus* regions were less odds for the positive IgG test results, which could be due to the selection biases, as well as due to the impact of circulating in the region *Borrelia* genospecies on test results.

The total percentage of positive results in the assay for detection of IgG-class antibodies response is lower than that described in other data sources [35, 48, 54], and could be associated to a relatively quick investigation of a patient since the onset of the disease. The thesis revealed an upward trend in the percentage of the positive samples in terms of interval since tick bite that meets the data from scientific literature [41, 48].

An increased probability of the positive result in the observation period can be explained by both the purposive patient investigation, as well as by modernization of the laboratory tests, enhanced the test-system with VlsE antigen, which improves sensitivity [55, 56].

5. CONCLUSIONS

1. Lyme borreliosis incidence in Latvia is increasing since the start of registration. During the study period, i.e. in the period from 2007 to 2011, the incidence rate increased by 41%, and the increase of the incidence rate was more noticeable (an increase for 75%) in *I. persulcatus* tick distribution region.
2. Changes in Lyme borreliosis incidence are due to impact of a natural (the number of tick hosts, forest areas), environmental (pollution) and climate (temperature, rainfall during the tick activity season) factors. Incidence increases with increased number of ticks hosts and forest areas, and with decrease of the environmental pollution. Seasonal changes in the incidence are largely determined by climatic factors.
3. Incidence of Lyme borreliosis varied in different gender and age groups. Women fall ill 1.59 times more frequently than men. Adults fall ill 3.36 times more frequently than children, and relatively more often than representatives of the respective groups in other countries.
4. Lyme borreliosis incidence rate among unemployed persons is 1.62 times higher than for employed, pointing to strong relation of an employment factor related to the risk of disease.
5. Patients with different Lyme borreliosis clinical forms, children and adults, living in *I. ricinus* and *I. persulcatus* tick distribution regions have different tick bite anatomic sites: a tick bite in head or neck is found more often in children, in persons living in *I. persulcatus* tick distribution area, and in patients who had not Lyme borreliosis erythema migrans form.
6. Differences in the frequency of Lyme borreliosis clinical forms in the statistical and tick species distribution regions are due to differences in the level of *Borrelia* prevalence in ticks and disease reporting deficiencies in certain territories.

7. Reporting of Lyme borreliosis in Latvia is incomplete (about 1/3 of cases has been reported), but reported cases are representative in relation to all cases.

8. Women, younger patients and patients who had not Lyme borreliosis erythema migrans form have elevated IgM antibody odds in ELISA test.

9. Elderly patients, patients who had Lyme borreliosis erythema migrans form, persons living in *I.ricinus* tick distribution area and rural inhabitants have increased odds for IgG class antibodies in ELISA test.

10. Greater odds for IgG class antibodies in elderly patients and rural inhabitants indirectly indicates a relatively great antibody prevalence against *B. burgdorferi* s.l., pointing to the incidence of undiagnosed Lyme borreliosis cases in population of Latvia.

6. PRACTICAL RECOMMENDATIONS

1. Improve the protocol form of the epidemiological investigation of Lyme borreliosis cases VISUMS system, and develop new guidelines for the epidemiological investigation.
2. On a regular basis manage the data comparisons in VIS and VISUMS databases for registered Lyme borreliosis cases. Evaluate the possibility of foreseeing of automatic reporting functionality in the framework of an e-health program development.
3. Develop mechanisms to encourage medical staff reporting on the diagnosed Lyme borreliosis cases (e.g., as inclusion of the bonus points for doctors who provide full reporting in the family doctors work quality evaluation system).
4. Improve the treatment guidelines for Lyme borreliosis patients determining the criteria for hospitalization.
5. Improve the laboratory investigation guidelines for patients with suspicions of Lyme borreliosis: perform laboratory tests for patients with erythema migrans only in cases of doubt about the diagnosis, but the confirmatory test (Western Blot) perform for all samples for which the positive or borderline result in ELISA test has been found. Develop a differentiated approach to the payment for laboratory tests, taking into account the reasons for their prescription.
6. Improve the prevention guidelines for patients who apply for medical treatment due to a tick bite taking into account an experience of the other countries (United States, Russian Federation).
7. Conduct a seroepidemiological study of antibodies against *B. burgdorferi* s.l. to find the prevalence in population of Latvia.

7. APPROBATION OF THE WORK

The approbation of the thesis "Analysis of the Lyme borreliosis epidemiological indicators in Latvia and seropositivity associated factors" – 2 July, 2012 at 10:00, oral report in the interdepartmental meeting of the Department of Public Health and Epidemiology, and the Department of Infectology and Dermatology of the Riga Stradins University with the participation of the members of the Preventive medicine association of Latvia.

8. LIST OF PUBLICATIONS ON THE THESIS TOPIC, AND REPORTS ON THE RESULTS OF THIS WORK

8.1. Scientific publications

1. Bormane, A., Ranka, R., Lucenko, I., Lavrinoviča, M., Kloviņš, J., Kalniņa, V., Puzuka, A., Vetce-Vecmane, K., Baumanis, V. Borrelia burgdorferi-infected ticks and incidence of Lyme borreliosis in Latvia. *Proceedings of the Latvian Academy of Sciences. Section B, Natural, exact and applied sciences,* 2001, 55 (4), 164–170. ISSN 1407-009X.

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8.3. Oral reports on the results of the work

1. Lucenko, I. Tick-borne encephalitis and Lyme borreliosis. Epidemiological situation in Latvia (1993.–2002.). 10th Baltic-Nordic Conference on Tick-borne Zoonozes, Tartu, Estonia, 05.04.2003.

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8.4. Poster presentations on the results of the work

1. Lucenko, I., Arša, F., Bormane, A., Krūmiņa, A.Perevoščikovs, J., Rjazanceva, G., Rohlina, L., Rozentāle, B., Storoženko, J., Trofimova, J., Vīksna, L., Zamjatina, N., Briģis, Ģ. Tick-borne encephalitis and Lyme borreliosis in Latvia: epidemiological situation in 2007–2009. European Scientific Conference on Applied Infectious Disease Epidemiology (ESCAIDE), Lisbon, Portugal, 13.11.2010.

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ACKNOWLEDGMENTS

I sincerely thank my supervisors Dr.med., Professor *Ģirts Briģis* and Dr.med., Associate Professor *Angelika Krūmiņa* for inspiring, responsive, constructive and valuable recommendations.

I wish to express my gratitude to Dr.habil.med., Professor *Ludmila Vīksna* for support, valuable advices and responsiveness.

Invaluable assistance provided by my colleagues – epidemiologists, especially thanks to *Jurijs Perevoščikovs*, Dr.biol. *Antra Bormane* and Dr.med. *Anita Brila*.

Many ideas would remain unrealized without support from Head of stationary "Infectology Center of Latvia" of the "Riga East Clinical University hospital" Dr.med., Professor *Baiba Rozentāle* and Head of the Laboratory, Dr.med. *Jeļena Storoženko*.

I would like to thank to the laboratory specialists of "Infectology Center of Latvia" *Jūlija Trofimova*, *Ļubova Rohlina* and *Frīda Arša* for help and advices.

I would like to thank also the RSU Public Health and Epidemiology Department team for friendly support and assistance.

I would like to thank *Andrejs Ivanovs* and *Mihails Savrasovs* for help in statistical issues.

Many thanks for the assistance in designing to *Jūlija Kurzemniece - Solovjova*.

I would like to thank RSU Vice-Rector Dr.habil.med., Professor *Iveta Ozolanta* and scientific secretary *Ingrīda Kreile* for support in the development of this work.

Sincere thanks to my friends and family, especially my husband, for a deep emotional support, understanding, love and encouragement.

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