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RESEARCH ARTICLE

Geographical and Chronological Analysis of Lyme Borreliosis in Stara Zagora Region

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ABSTRACT

Objectives: Until recently, Lyme Borreliosis (LB) was not commonly seen in Bulgaria. Now, this infectious disease, which is spread in the form of natural foci, is seen in Bulgaria as well as in North America, Europe and Asia. The objective of the present study is to analyze the dynamics and characteristics of the LB epidemic process for Stara Zagora region over two periods, due to the incompleteness of the data available to Regional Health Inspectorate of Stara Zagora (1993-1997 and 2008-2012).

Methods: Complex epidemiological methods, including the epidemiological analysis method, were applied in the data interpretation. Routine methods have been previously applied in studying serological samples from patients diagnosed with LB, including indirect IFA (until 1997) and ELISA (until 2012).

Results: During the first period of our study (1993-1997), the distribution of LB patients by years was irregular, with the highest number of patients in 1993 (32) and 1995(39). During the second period (2008-2012), the highest number of LB patients was recorded in 2008 (93). During both the first and second study periods, we found that LB disease in Stara Zagora Region decreased. However, on a national and world scale, LB disease is increasing, most likely due to the methods of diagnostics, climate change and increased migration.

Conclusions: Because the incidence of LB is increasing worldwide, we believe that the laboratory and clinical diagnostics of LB should be more precise. *J Microbiol Infect Dis 2018; 8(1):13-18*

Keywords: Lyme borreliosis, disease tendency, epidemic process, blood infections

INTRODUCTION

Lyme borreliosis (LB) is widespread in the world, to date, mainly in the northern hemisphere: the United States, almost all countries in Europe, the whole of Asia, etc. The dramatic increase of interest in the disease in recent years is linked to its frequency, which seems to be significant despite the lack of accurate data on the issue. Its promotion in the medical literature enhances the attention to it, to its diagnosing and treatment. The varied multisystem clinical manifestations of LB can mimic a number of other disease units and syndromes in infectious and non-infectious pathology, which is why it very often enters a differential diagnostic aspect [1-3]. The disease is successfully treated with antibacterial agents, especially antibiotics. Moreover, clinically severe, e.g. neurological syndromes are also affected, whereas with different etiology these can be a very difficult therapeutic problem. In a number of cases it is a desirable diagnosis because of a more favorable therapeutic prognosis [4,5]. So far the bacteriological diagnosis of the disease is based mainly on serology. In Bulgaria the first case of LB was discovered and described by Savova et al. in 1988 [6]. In 1994 Tomov managed to isolate the tick causing agent [7].

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LB is a comparatively new disease in Bulgaria. LB incidence has been recorded on a regional level (since 1995 for the town of Stara Zagora) and on a national level (since 1994); these data show unevenness in the course of the epidemic process, which raises numerous questions, some of which we will try to address in the present study. The objective of the present study is to analyze the dynamics and characteristics of the LB epidemic process for the region of Stara Zagora over two periods [4,5,8].

METHODS

Stara Zagora Region, which is located in the territorial center of South Bulgaria, belongs to a natural focal area with so-called "moderate risk" of LB; the altitude is between 200 and 400 meters on average and the causative agent of LB can circulate without resulting in a very high incidence rate. The suspected natural disease-focus areas are concentrated in the regions at the foot of the mountains or in the hilly areas. People of all age groups are susceptible to the disease. Most frequently affected are the people in active age and pensioners engaged in the field of agriculture.

All of these patients have been previously surveyed. A "Specialized Card for Epidemiological Survey of LB" has been completed, which in addition to the passport data of the person contains the answers to a number of questions concerning the disease. The most important of these are: 1) Epidemiologically-oriented questions to the sick person – Has he ever been “bitten” by tick? 2) Clinical picture – presence of *Erythema migrans* (EM)? 3) Positive serological reaction with indirect IFA (indirect fluorescence assay)? The IFA technique assays are based on the "Method Guideline for the Prevention and Control of Lyme Disease, 1990", issued by the National Institute for Infectious and Parasitic Diseases, Central Laboratory for Lyme Disease, Higher Medical Institute Plovdiv, and the Scientific Institute in Dermatology and Venerology. Positive are the following titers: for IgM ≥ 1:64 and for IgG ≥1:128. The reaction antigen was obtained from the American laboratory "Kirkegaard & Perry Lab". The US criteria for diagnosis of "Lyme borreliosis" were used. According to the above instruction, all clinical manifestations without the characteristic *Erythema migrans* require serological verification with "at least two blood samples at an interval of 25-30 days". Practice shows that first serum sampling before 14-20 days from the tick “bite” of the person (tick incident) is pointless. All persons with positive serological result should be reported to the Regional Health Inspectorate for registering and reporting [5] according to Ordinance No. 21/2005 and its amendment and supplement of 2011. It should be borne in mind that the antibody response is slow and in the early stages of the disease the results are often seronegative. The use of whole cell antigens leads to false-positive results in a number of viral, bacterial and autoimmune diseases [9,10]. To reduce the number of these adverse effects, we have been using Sorbent – of Czech origin, a lyophilized Treponema pallidum extract. In more advanced phases of the disease the positive results increased to 70-90% with switching from IgM to IgG [11-14]. Monospecific anti-human IgG and IgM sera used in this method. They are conjugated to fluoresceinisothiocyanate.

For the implementation of ELISA we learn more specifically from the publication of Iva Hristova [8]. Significant antibody titers were obtained 20 days after the beginning of the clinical symptomatology. In indirect IFA, borrelia bodies are used, whereas in ELISA subcellular elements have wider application. ELISA sensitivity is considered to be higher but it does not give any specific advantage. To date, in some US states, indirect IFA has wider application.

This study includes 389 patients from Stara Zagora region; these patients were recorded between 1993 and 1997 or between 2008 and 2012. The diagnosis and methods for studying serological samples were indirect IFA until 1997 and ELISA until 2012.

RESULTS

After discovering the etiological cause of Lyme borreliosis (LB) in 1982, we began testing LB patients from Stara Zagora region in 1988. Blood samples from suspected patients were sent for serological tests in Moscow through the Central LB laboratory in Plovdiv. Three of these samples were positive (4). In 1993, we sent a great number of serum samples from Plovdiv for testing, and 32 of them were positive. At that

time, institutions in Bulgaria were not ready to record and report the new infectious disease (LB). In the following year (1994), LB registration began in Sofia. However, Stara Zagora still had to send blood samples to Plovdiv (in 1994). Of these, 22 were positive [3]. In the next three years (1995-1997), tests were made locally at the section of Epidemiology in Stara Zagora; we provided the relevant apparatus and reagents needed. Fortunately, the epidemiological office in Stara Zagora reacted adequately, and since 1995, LB has been regularly recorded, announced and reported in that region.

First period: 1993-1997

Table 1 shows that for the five-year period (1993-1997) a total of 99 LB patients were recorded and reported.

Table 1. Lyme borreliosis disease in Stara Zagora region.

Year	Number of patients	Incidence rate*
1993	32	8.05
1994	22	5.54
1995	39	9.82
1996	4	1.00
1997	2	0.50
Total	99	4.98

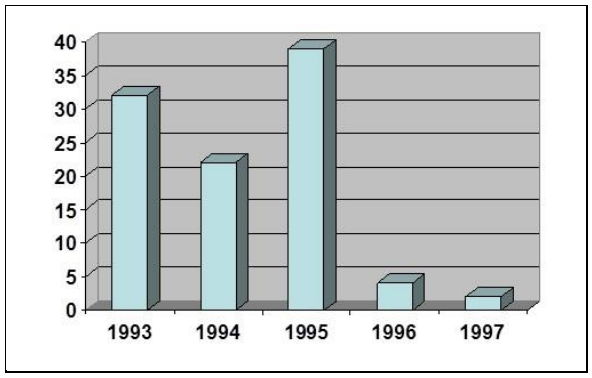

Figure 1. Distribution of LB patients by year

The distribution of LB patients is rather uneven, as the number of patients with LB was highest in 1993 and 1995.

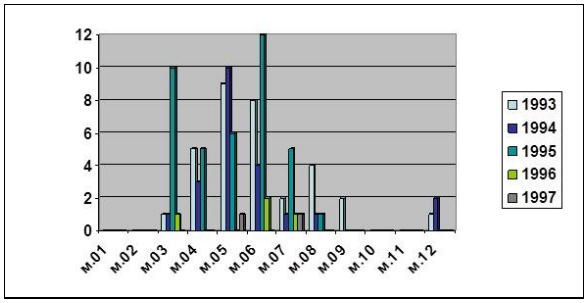

Figure 2. Monthly distribution of LB patients (1993-1997).

The months with the most frequent LB cases are March, April, May, and June.

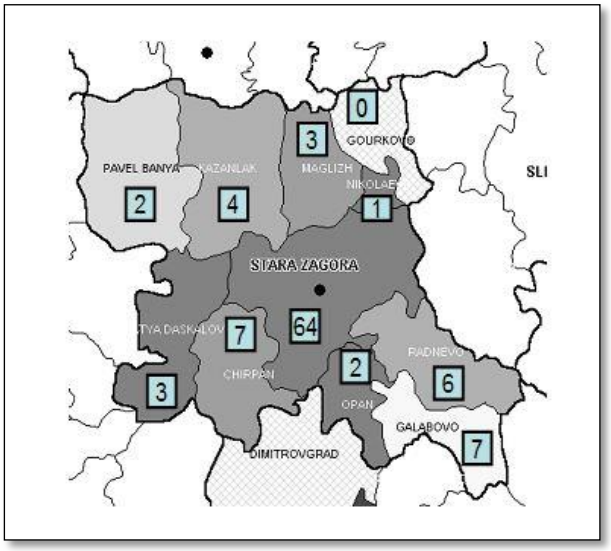

Figure 3. Map of Stara Zagora Region with distribution of LB patients by municipalities (1993-1997).

The greatest number of patients (64) was found in the largest municipality – the town of Stara Zagora.

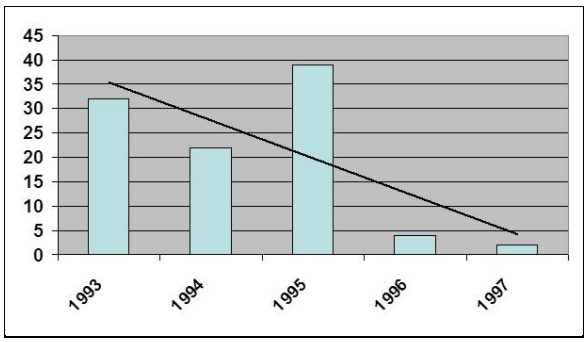

Fig. 4 LB incidence rate by year with a tendency

The LB incidence rate decreased between 1993 and 1997.

Second period: 2008-2012

Table 2 shows that there were 290 recorded LB cases for the period 2008-2012.

Table 2. Incidence rate of Lyme borreliosis in Stara Zagora region

Year	Number of patients	Incidence rate *
2008	93	25.09
2009	87	23.47
2010	55	14.84
2011	34	10.20
2012	21	6.30
Total	290	15.98

* confirmed cases per 100 000 population

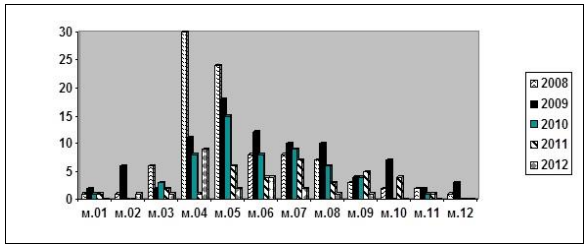

Figure 5. Distribution of LB patients by month for the period 2008-2012.

The most frequent cases were reported in April, May, June and July, but there were newly reported patients year round.

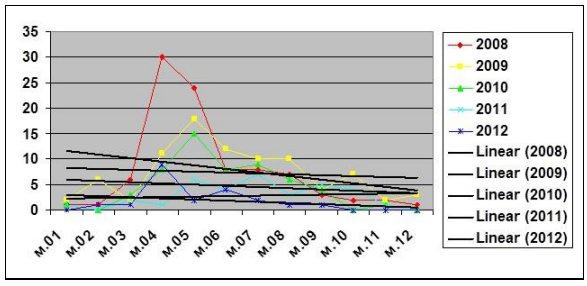

Figure 6. Distribution of LB patients by month 2008-2012 with a tendency.

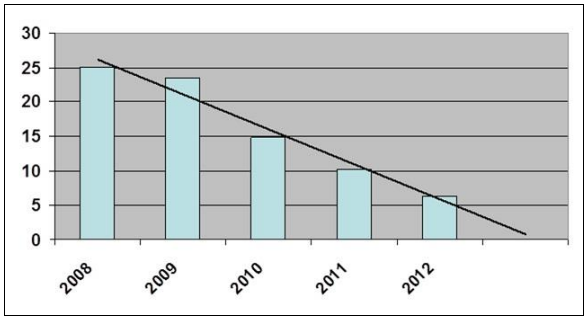

Figure 7. LB incidence rate by year with a tendency.

During that period of time, the disease decreased. The working hypothesis is supported with great probability (P>0.05) according to the data from Tables 1 and 2. Over the years specified, no significant changes occurred in the LB causative agents (*Borrelia*), LB transmitters (ticks), or climate conditions in Stara Zagora region. Therefore, we found no statistical difference in the LB incidence rate for the studied periods. However, there was a decrease in the LB incidence rate in Stara Zagora region over both study periods.

Over 19 years of registration and reporting, the average incidence rate for the country [1] is 6.63 (confirmed cases per 100,000 population) (126,06 patients over 19 years).

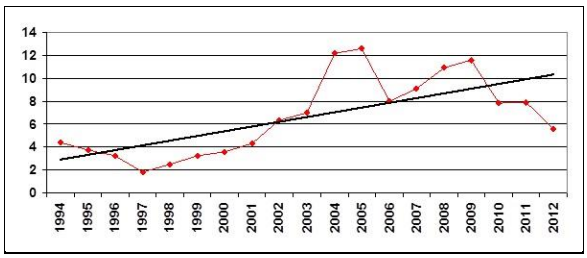

Figure 8. LB incidence rate in Bulgaria.

DISCUSSION

The human body is a "destination" in the parasitic system of the agent, so the patient is not infectious to others, and therefore, isolation is not necessary. The dynamics of incidence depends on the biological activity of the transmitter. The population dynamics of ticks (larvae, nymphs, imago) among humans are complex, so it is hard to forecast [15]. On the other hand, it is known that VlsE lipoprotein is

one of the new antigens it's the diagnostics of LB. Its major immunodominant epitope is located within the IR6 region. The C6 peptide that reproduces the IR6 epitope is now used as a simple executable commercially available diagnostic test [9]. During infection, VlsE undergoes antigenic variation [12]. Its variable and non-variable regions become the basis for the formation of an astronomical number of different variants that can lead to immune deceptions [13].

This Ordinance № 21 expressly states that the diagnosis of "probable" for LB is inapplicable. Hence, only laboratory-confirmed cases are included when calculating the incidence rate. In its 2007 report, Stara Zagora Regional Health Inspectorate included patients classified as "probable", and therefore, the results of that report may be inaccurate.

In the interest of fairness, we should note that since 2008, the US has reported [11] "probable" cases, but these are not included when calculating the incidence rate. On the other hand, the "Law on protection of personal data" (in force as of 2002) in Bulgaria has led to a serious restriction of information about epidemiological analyses. Re-infection and further disease development are possible. In addition, it should be noted that asymptomatic forms of LB are not uncommon.

CONCLUSION

The blood infections (including LB) have complex epidemiology; because of this, they cannot be eradicated. The epidemic process has changed over the years due to many factors, including various causative agents, numerous transmitters, various sources of infection, specific mechanisms for infection transmission, and various endemicity types, etc. These factors have caused an increase in the incidence rate over time. Having described the first three cases of LB in Stara Zagora region, the tendency for the region is a decrease in the incidence rate. However, on a national and global level, the incidence of LB is increasing due to improvements in diagnosis, climate change, and the increased movement of people and animals. More specifically for Bulgaria, the undoubted impact on the incidence rate has the inclusion of Lyme borreliosis as a clinical path.

Negative serological results do not necessarily lead to a negative diagnosis. As a phenomenon, hyperdiagnostics is quite real. Here, the responsibility of the physician-clinician in the assessment of laboratory tests shall be emphasized, as he/she should exclude any possible false-positive results in epidemiological and clinical data [16]. In a diagnostic aspect, we believe that it is better to report 2-3 more cases than to fail to register missed and untreated LB patients.

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Conflict of interest: The authors declare no personal or financial conflict of interest.

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