



Paper Accepted*

ISSN Online 2406-0895

Original Article / Оригинални рад

Vesna Begović-Kuprešanin[†], Milomir Milanović, Dragan Mikić, Svetlana Popović,
Dejan Hristović, Radmila Rajić-Dimitrijević, Tatjana Takić-Radovanović

The influence of early antibiotic therapy on the clinical manifestations in patients with early lyme disease

Утицај ране примене антибиотика на клиничке манифестације оболелих у раној фази лајмске болести

Military Medical Academy, Clinic for infectious and tropical diseases, Belgrade, Serbia

Received: October 2, 2017

Accepted: October 24, 2017

Online First: October 27, 2017

DOI: <https://doi.org/10.2298/SARH171002190B>

* **Accepted papers** are articles in press that have gone through due peer review process and have been accepted for publication by the Editorial Board of the *Serbian Archives of Medicine*. They have not yet been copy edited and/or formatted in the publication house style, and the text may be changed before the final publication.

Although accepted papers do not yet have all the accompanying bibliographic details available, they can already be cited using the year of online publication and the DOI, as follows: the author's last name and initial of the first name, article title, journal title, online first publication month and year, and the DOI; e.g.: Petrović P, Jovanović J. The title of the article. *Srp Arh Celok Lek*. Online First, February 2017.

When the final article is assigned to volumes/issues of the journal, the Article in Press version will be removed and the final version will appear in the associated published volumes/issues of the journal. The date the article was made available online first will be carried over.

[†] **Correspondence to:**

Vesna BEGOVIĆ KUPREŠANIN

Military Medical Academy, 17 Crnotravska Street, Belgrade, Serbia

E-mail: begovickupresanin@hotmail.com

The influence of early antibiotic therapy on the clinical manifestations in patients with early Lyme disease

Утицај ране примене антибиотика на клиничке манифестације оболелих у раној фази лајмске болести

SUMMARY

Introduction/Objective Lyme borreliosis is a multisystem infectious disease caused by *Borellia burgdorferi* spirochaetes transmitted by the bite of an infected tick. The disease manifestations are very different, with the skin, joints, heart and nervous systems being most often affected.

The aim of this study was to find out whether there are significant differences in the appearance of symptoms and signs of the disease between the subjects who did/did not receive prophylactic, early antibiotic therapy, after the tick bite in patients diagnosed with the early phase of Lyme borreliosis.

Methods The study was carried out on 2070 patients, who were treated or examined at the Clinic for infectious and tropical diseases in the period 1989–2004. The patients were divided into group A (n=591), in which they were given early antibiotic therapy and group B (n=1479), in which they were not. The antibiotic therapy was used within 5 days of a tick bite in patients, with a probable infection, who, at the time, did not have any symptoms or signs. The applied antibiotics included: cephalosporins, macrolides, tetracyclines, semisynthetic penicillins, repeatedly for 7 or 14 days, or benzathine benzylpenicillin once only.

Results The disease developed in a statistically significantly larger number of patients who were not given early antibiotic therapy (537/1479), than in those who received that therapy (10/591), i.e. the ratio was 36.3% versus 1.7%. We concluded that only 2 antibiotics were sufficient for optimal prevention: doxycycline and ampicillin applied for 7 days. The applied antibiotics showed a high statistically significant efficacy, ranging from 93.7% (cephalosporins) to 99.4% (macrolides).

Conclusion The application of early antibiotic therapy after the tick bite was effective in preventing the early phase of Lyme borreliosis, while, in case of infection, it prevented the development of extracutaneous manifestations.

Keywords: Lyme disease; tick; antibiotics; prophylaxis

САЖЕТАК

Увод/Циљ Лајмска борелиоза (ЛБ) је мулти-системско инфективно обољење изазвано спирохетом *Borellia burgdorferi* (ББ) и настаје убудом инфицираног крпеља. Болест се испољава различито, а најчешће су захваћени кожа, зглобови, срце и нервни систем.

Циљ ове студије је био да се код особа код којих је дијагностикована рана фаза ЛБ утврди да ли постоје значајне разлике у испољавању симптома и знакова обољења између оболелих који су примили и оних који нису примили профилактичну, рану антибиотску терапију после убода крпеља.

Метод Испитивање је извршено на 2070 испитаника оба пола са убудом крпеља, лечених или прегледаних у Клиници у периоду 1989–2004. године. Испитаници су подељени у две групе, групу А (n=591) која је примала рану, профилактичну антибиотску терапију и другу, групу Б (n=1479), која ту терапију није примала. Рана антибиотска терапија подразумева примену антибиотика код особа са вероватно насталом инфекцијом до пет дана по убуду крпеља, а нису имали симптоме и знаке болести. Од антибиотика су примењивани цефалоспорини, макролиди, тетрациклини, полусинтетски пеницилини, понављано седам или четрнаест дана, или бензатин безилпеницилин једнократно.

Резултати Болест се развила код статистички значајно већег броја испитаника без профилаксе (537/1479), него код оних који су ту терапију примили (10/591), односно 36,3% :1,7%. Применом пет група антибиотика закључено је да су за оптималну превенцију довољна само два: доксициклин и ампицилин у трајању од седам дана. Примењени антибиотици су испољили високу клиничку ефикасност, која се кретала од 93,7% (цефалоспорини) до 99,4% (макролиди) и била независна од дужине примене.

Закључак Примена ране антибиотске терапије после убода крпеља је ефикасна у превенцији ране фазе ЛБ, а када је болест била испољена није било екстракутаних манифестација.

Кључне речи: Лајмска болест; крпељ; антибиотици; профилакса

INTRODUCTION

Lyme borreliosis (Lb) is a multisystem infectious zoonotic disease caused by the *Borellia burgdorferi* (Bb) spirochaetes transmitted by the bite of an infected tick of the genus *Ixodes*. The disease manifestations are very different, with the skin, joints, heart and nervous systems being most often affected.

The tick bite is the primary mode of infection, and the infection can occur via the conjunctiva or micro trauma that occurs after the tick destruction and skin irritation by its contents [1].

Ixodes ricinus has been proved to be vector in our country. The infection occurs after 1-3 days, and rarely within 24 hours, due to inappropriate tick removal [2,3]. The risk of developing Lyme disease after the bite of an infected tick is 1- 4%, while asymptomatic infections range up to 26% in the endemic area [4,5]. There is a greater risk of the occurrence of manifest illness in children, as well as of the late stage development in people with an asymptomatic infection.

Depending on the stage and extent of the disease, the duration of antibiotic therapy for skin manifestations at the early stage is 4-6 weeks, while for extracutaneous manifestations it is about 2 months. At the late stage, antibiotic treatment lasts up to 16 weeks, i.e. until the loss of subjective symptoms and clinical signs of the disease [1,6-8].

The fact that it affects multiple organs and systems, the development of the late stage of the disease with definite tissue and organ damage, the onset of autoimmune diseases, recurrence and disability, and a possible death outcome were the subject of research in many clinical studies in terms of disease prevention and recommendations for the use of antibiotic therapy after the tick bite, and before the manifestation of the disease [9,10].

Most authors consider that neither the routine use of antibiotics nor serological testing is necessary right after the tick bite [11,12]. However, the aforementioned therapy is justified in endemic areas in cases of repeated bites and of serious suspicion concerning the possibility of an infection [9,13].

One study involving 600 patients showed that the use of antibiotic therapy was unnecessary due to a small number of infected (1.4%) in patients who did not receive an antibiotic after the tick bite, but the same authors recommended carrying out additional studies concerning this subject. [14].

Due to the early dissemination of the cause of the disease, the difficulty in diagnosis and major problems in the treatment of infected patients, particularly in the late stage of the disease, there are opinions that, if there is a suspicion of an infection after the bite, it is justified to apply antibiotics for up to two weeks [6, 8].

The aim of this paper is to determine the effect of prophylactic, i.e. early application of antibiotic therapy on the clinical manifestations in the early phase of Lb. For the purpose of this study, prophylactic antibiotic therapy was defined as the use of antibiotics up to five days after the tick bite in individuals with suspected Bb infection who did not show clinical symptoms and signs of the disease at the time of the application of antibiotics.

METHODS

The study was designed as a retrospective-prospective cohort study. It initially included 2470 patients who were treated at the MMA Clinic for Infectious and Tropical Diseases or examined at the outpatient clinic in the period January 1st, 1989 - December 31st 2004. Four hundred patients were

excluded; out of whom 207 did not show up for follow-up monitoring and 193 received early antibiotic therapy. The study did not include patients who had a positive serological reaction to the Bb antigens at the first examination, i.e. those who had previously developed an infection.

All patients were classified into two categories. The first category consisted of people who came to the Clinic for Infectious and Tropical Diseases because of a tick bite. The presence of probable Bb infection was based on the existence of at least one of the following criteria: the presence of Bb in the removed tick; the tick's presence in the skin for more than 48 hours; data on the inappropriate tick removal and the presence of the antibodies to Bb antigens in the serum after 4-6 weeks. In some patients, based on the clinician's assessment, early antibiotic therapy was applied, while in others, it was not.

The second category included patients who came with some of the symptoms and were diagnosed with the early phase of Lyme disease. The diagnosis was based on a characteristic change in the skin, i.e. erythema migrans (EM), clinical picture and the course of the disease, an increase in antibody titer against Bb antigens in two consecutive serum samples within 2-4 weeks (IIF) or on the positive result after 4-6 weeks (ELISA), response to antibiotic therapy, exclusion of other related diseases, data concerning the tick bite and stay in the endemic area.

The total of 2070 patients was divided into group A, in which early antibiotic therapy was applied, and group B, in which it was not.

The disease symptoms included: headaches, myalgia, arthralgia, loss of concentration, sleep and mood disorders, paraesthesia, palpitations and pruritus. The observed signs of the disease included: high body temperature, EM and multiple EM (MEM), heart rhythm disorders, myocarditis, pericarditis, encephalopathy, meningitis, encephalitis, cranial neuritis, radiculoneuritis, arthritis, myositis, and lymphadenopathy.

Based on the type of antibiotic and duration of its application, all patients who received antibiotic therapy, were divided into three groups: those who were given benzathine benzylpenicillin once parenterally, those who were given antibiotics for seven days orally and those who were given antibiotics for fourteen days orally (macrolides, tetracyclines, cephalosporins, semisynthetic penicillins). In each of these groups, those who suffered and those who did not suffer from the early stage of Lyme disease were singled out.

Data sources and examinations included: biographical data from medical and health records, tick bite data, physical, radiological and electrophysiological examinations, serological tests concerning Bb antigens (reaction of indirect immunofluorescence IIF or ELISA tests done at the MMA Institute of Microbiology) and specialist examinations according to indications.

The tick was removed either appropriately by a surgeon or epidemiologist at the MMA, or inappropriately by the patients themselves and/or other adults when a child was bitten. Tick gut contents were tested for presence of Bb by an epidemiologist at the MMA Epidemiology Institute, in a

dark field, using phase-contrast microscopy. All patients were monitored clinically, laboratorically and serologically within the period of 1 to 15 years.

Antibiotics registered for administration in our country and recommended for the treatment of Lyme disease were applied in the early antibiotic therapy.

Continuous variables were summarized as means (M) and standard deviations (SD), the significance between the groups was determined by the Student's T test for the validity of data distribution. All variables were presented as frequency of certain categories, while statistical significance of differences was tested by the Chi-square test. Statistical significance was accepted at a minimum level of $p < 0.05$.

Principles of ICH Good Clinical Practice were strictly followed and ethical approval from the Ethics Committee was obtained on September 15th, 2017.

RESULTS

In total, there were 2,070 patients. Group A consisted of 591 patients who received early antibiotic therapy, while Group B, which had 1,479 patients, did not receive any antibiotic therapy. Comparing the number of patients in the groups, a statistically significant difference was found due to the fact that the group without antibiotic therapy was significantly larger than the other one (Table 1). Group A was significantly smaller than group B, because early antibiotic therapy was applied only to those with a probable infection, according to the assessment of the responsible physician.

Table 1. Basic demographic and relevant clinical data.

Parameters	Groups				Probability
	Early antibiotic treatment		Without antibiotic treatment		
	A	B	A	B	
	n	%	n	%	
Age (years), $x \pm SD$	35.2 \pm 19.4		39.4 \pm 20.7		$t=4.24$; $p<0.001$
Patients (n=2070)	591	24.06	1479	75.94	$\chi^2=112.39$; $p<0.001$
Gender					
Male	457	77.3	1115	75.4	$\chi^2=0.76$; $p=0.38$
Female	134	22.7	364	24.6	
Tick presence					
Yes	260	44.0	652	44.1	$\chi^2=0.00$; $p=0.99$
No	331	56.0	827	55.9	
Tick removing					
Appropriately	342	57.9	652	45.0	$\chi^2=27.34$; $p<0.001$
Inappropriately	249	42.1	798	55.0	
Duration of tick presence*					
1–12 h	74	12.52	146	9.87	$\chi^2=2.31$; $p=0.69$
13–23 h	228	38.58	562	38.00	
24–48 h	111	18.78	245	16.57	
> 48 h	63	10.66	157	10.62	
Unknown	115	19.79	369	24.95	

*Skin, based on anamnestic data.

In both groups, there were more male patients, a total of 1572 (75.94%), while there were only 498 females (24.06%) and there was no statistically significant difference in the distribution of sexes

in the groups. The average age of patients in group A was 35.2 ± 19.4 years, while in group B it was 39.4 ± 20.7 . The patients in group B were statistically significantly older than those in group A. The average age of women was 46.47 ± 20.26 , while of men was 33.89 ± 19.54 , with women being statistically significantly older ($p = 0.001$).

When the presence of tick during the examination is concerned, it was found that the distribution of the subjects was practically identical, as there was no statistically significant difference between these groups concerning the presence or absence of ticks, and the use of antibiotics ($p = 0.99$) as well. The analysis of a large number of patients (2041) with appropriately and inappropriately removed tick showed a uniform ratio of these categories 994 (48.7%): 1047 (51.3%) (Table 1).

The applied treatment was analyzed concerning the method of removing ticks. It was found that in group B that did not receive early antibiotic therapy, the tick was appropriately removed in 652 patients (45%), inappropriately removed in 798 patients (55%), while 29 patients did not know about the tick bite and they came to the doctor's in the early phase of Lb. In group A, the tick was appropriately removed in 342 patients (57.9%) and inappropriately removed in 249 patients (42.1%). There were statistically significantly more patients in the group that received the antibiotic, and in which the tick was previously appropriately removed ($p < 0.001$).

The duration of tick presence ranged from one hour to more than 48 hours. Analyzing the duration of tick presence in the skin, we can conclude that there was no statistically significant difference between the groups (Table 1).

After the tick bite in infected patients, the signs of Lb were most frequently present on the skin (Table 2).

Table 2. Clinical signs due to Bb exposure.

Parameters	Groups				Probability
	Early antibiotic treatment		Without antibiotic treatment		
	n	%	n	%	
Systems					
Skin	9	1.52	466	31.51	$\chi^2=213.03$; $p<0.001$
Nervous	0	0.00	79	5.34	Not shown due to extremely low frequencies in early antibiotic treatment group
Other	1	0.17	51	3.49	
Joints	0	0.00	42	2.84	
Heart	0	0.00	41	2.77	
Total	10	1.69	679	45.91	

The results show that the clinical signs on the skin were found in 31.51% of patients who did not receive early antibiotic therapy, as opposed to those who did (1.52%) ($p < 0.001$).

The total number of signs of the disease was 679, and the number of patients was 537, which means that in some patients there were several affected systems. Table 2 shows the frequency of signs on a particular organ system in relation to the total number of recorded signs in group B (679). We observed that the nervous system was affected in 5.34%, other organs were affected in 3.49%, locomotor system in 2.84% and cardiovascular system in 2.77% of patients.

The frequency of changes on the skin, in relation to the total number of patients (547), was 86.84%, the changes in the nervous system were registered in 14.44%, on other organs in 9.50%, on the locomotor system in 7.88% and in the cardiovascular system in 7.49% of patients.

At the same time, we observed that in patients who received early antibiotic therapy, there were no signs of disease in the nervous, cardiovascular, and bone-muscular systems.

Table 3. Efficacy of early applied antibiotic therapy.

Antibiotics class	Lyme disease absent		Lyme disease present		Total	
	n	%	n	%	n	%
Macrolides	182	99.4	1	0.6	183	100.0
Semisynth. penicillins	121	98.4	2	1.6	123	100.0
Benzathine benzillpenicillin	165	98.2	3	1.8	168	100.0
Tetracyclines	98	97.0	3	3.0	101	100.0
Cefalosporines	15	93.7*	1	6.3	16	100.0
Total	581	98.3	10	1.7	591	100.0

* Significantly different ($p < 0.05$) from macrolides

After applying early antibiotic therapy in 591 patients, 581 (98.3%) of them did not develop the early phase of Lyme disease. Macrolides (99.4%) showed the highest efficacy, followed by semi-synthetic penicillins (98.4%), benzathine benzylpenicillin (98.2%) and tetracyclines (97.0%). Cefalosporins (93.7%) showed the lowest efficacy, which was statistically significantly lower in relation to other groups of antibiotics (Table 3).

In group A that received early antibiotic treatment, only 10 (1.7%) out of 591 patients were infected, while 581 of them were not infected (98.3%). In contrast, in group B that did not receive early antibiotic therapy, 537 (36.3%) out of 1479 patients were infected, while 942 (63.7%) were not (Table 4). The difference found between these two groups of patients was highly statistically significant in favor of the group that received early antibiotic therapy ($p < 0.001$).

Table 4. The frequency of early phase of Lyme disease in patients regarding type of treatment.

Treatment	Lyme disease present		Lyme disease absent		Total	
	n	%	n	%	n	%
Early antibiotic treatment	10	1.7	581	98.3	591	100.0
Without antibiotic treatment	537	36.3	942	63.7	1479	100.0
Total	547		1523		2070	100.0
Probability	$\chi^2=258.40; p < 0.001$					

DISCUSSION

Today Lb is one of the greatest imitators in medicine, because it is a multisystem disorder involving many organs and systems, with the occurrence of a chronic disease form, with the development of disabilities, autoimmune diseases, and sometimes lethal outcome [15].

The disease occurs in all parts of the world. In the United States, the incidence is 12-39 patients per 100,000, since the probable cases have also been reported since 2012 [16,17]. In Europe, the incidence ranges from 35 patients in Germany to 206/100 000 infected in Slovenia [18].

In Serbia, the number of the infected is decreasing (6.83/100.000), since only 487 cases were reported in 2015 [19]. In the period from 1986 to 2000, Lb was represented in the zoonotic group with 16.34% in Belgrade, while from 1991 to 2000 in Serbia 4768 people developed the disease [20].

In our study, there were significantly more males (75%), aged 11-30, as most of them were members of the army who had been outdoors on the terrain (younger ages were members of their families). In Europe, about 60% of women with the tick bite have been registered, which is similar to the findings in Serbia (57%), aged 20-60 and over [19].

In the early 1990s, the infected ticks were frequent on the territory of Belgrade, ranging from 20 to 67%, with a tendency to decrease. In 2007, this number amounted to 30% [2,21]. The situation was similar everywhere. In Europe the infection of *Ixodes ricinus* with Bb ranged from 15-40%, in endemic areas of America up to 50%, while *Ixodes pacificus* was infected with Bb in 1-2% of cases [22]. The risk of the occurrence of manifest Lb after the bite of the infected tick in Europe ranges from 1-4%, in hyperendemic areas 4.7-5% [4, 23], but there are also data on a significantly higher risk (up to 27%) [24].

At the time of examination, 44% of our patients in both groups, had the tick in the skin, while others came with a removed tick or not knowing about the bite. With the largest number of subjects, 48.31%, the tick stayed in the skin for up to 24 hours before the first examination.

In our study, there were 21 early-phase Lb patients with the tick staying in the skin for only 10 hours or up to one day, who developed changes in the central and peripheral nervous systems, which is in agreement with some authors [25, 26], but also in contradiction with some others [2,3].

Given the large number of subjects in this study, i.e. 994 who had the tick removed appropriately (48.7%) and 1047 who had it removed inappropriately (51.3%), it was possible to assess the significance of these two methods of tick removing for the occurrence of the disease. In the case of appropriate tick removing, the disease was reported in 22% of patients, while in the case of inappropriate removing, in 78%, which was statistically significantly different. The ratio of patients with the tick inappropriately removed who were given early antibiotic therapy was 1.01% versus 28.7% of patients in the group without antibiotic therapy. There was a significantly higher number of subjects in the group receiving the antibiotic and in whom the tick was previously appropriately removed ($p < 0.001$).

In patients with manifestations in the nervous and cardiovascular systems in our study, in most cases the tick was inappropriately removed, which is in line with the data given by Southern (2009) [27]. According to other authors' data concerning appropriate tick removal, the number of patients was statistically significantly lower in relation to the group where it was inappropriately removed (6%: 46%), but this study included a small number of subjects ($n = 52$) [28]. According to the results of our authors, the early phase of Lb occurred in 0.61% of patients with appropriately removed tick, and in 2.5% of patients with inappropriately removed tick [29], while others claim that it occurred in 0.20% of patients with appropriately removed tick and 1.44% of patients with inappropriately

removed tick [21]. The aforementioned numerical indicators are lower in relation to the data from our research due to the longer monitoring of our patients and the timely started antibiotic therapy. The results of other studies suggest that 96% of patients with an appropriately removed tick did not suffer from Lb in the highly endemic region [30], thus emphasizing the importance of appropriate forceps tick removal even without the antibiotic prophylaxis [31].

In our study, we observed the frequency of symptoms and signs of Lb and registered 12 different symptoms and 24 signs, which we grouped according to the affected organ systems. The disease was manifested on the skin in the form of EM and other skin changes in 86.84%, in the nervous system in 14.44%, in other organs (lymphadenopathy, liver damage, high body temperature, hypothyroidism, multiple-systemic infectious disease syndrome – MSIDS) in 9.50%, on the locomotor system in 7.88% and in the cardiovascular system in 7.49% of patients compared to the total number of patients ($n = 547$). In patients who received early antibiotic therapy, there were no signs of disease in the nervous, cardiovascular, and bone-muscular systems. In one patient (0.17%) who received early antibiotic therapy, we registered a high body temperature without other signs of the early phase of Lb, as opposed to those who did not receive antibiotic therapy, in whom we registered the following: 3.49% MSIDS (in 29 patients), lymphadenopathy, liver damage and hypothyroidism.

In patients who received early antibiotic therapy after the tick bite, Russian authors observed the infected (1.1%) without EM and with MSIDS, and in those without antibiotic therapy (12.3%), with MSIDS, sleep disorder, EM and MEM, lymphadenopathy and cardiac disorders [32]. Other authors paid less attention to this category of patients and did not include them in the analysis when they evaluated the efficacy of prophylactic post-bite therapy [9,14,30,33]. Our results show that in the group with antibiotic therapy only EM and MSIDS were observed, while all other signs were observed in the group without early antibiotic therapy. The difference between these two groups in relation to the number of signs was also statistically highly significant.

EM and MEM were significantly more frequently present (31.51%) in the group without early antibiotic therapy compared to patients with the treatment (1.52%). MEM was observed in 2.3% of patients without early antibiotic therapy, which was found in the USA in a higher percentage (20%) due to the early dissemination of Bb in that area [33]. Today, this can also be accounted for by new strains with high spirochetemia [34]. Compared to the total number of patients ($n = 547$), the incidence of skin changes was 86.84%. Similar results were also reported worldwide, ranging from 70% to 90% in the USA [35], and in Slovenia 48%. Some patients with Lb without early antibiotic therapy had several organic systems affected, while 5.34% had changes in the nervous system, 2.47% in the locomotor system and 2.77% in the cardiovascular system. Other studies state the same signs of disease with more or less equal frequency [31], which we also observed in our patients.

Unlike in our study, other authors followed their patients for a shorter period of time and observed fewer symptoms and signs. Among our patients there were more neurological manifestations

observed because we paid special attention to the damage of the peripheral nervous system due to Lb [36]. The incidence of cardiac manifestations in Lb was lower in relation to the data of other authors where they stated a frequency of up to 10% [37].

Opinions regarding the type and duration of antibiotic therapy in the treatment of Lb have not yet been harmonized. The general recommendation is that doxycycline, amoxicycline and cefuroxime should be administered at an early phase for 2-3 weeks, while intravenous ceftriaxone, cefotaxime or benzylpenicillin for 2-4 weeks in the case of extracutaneous manifestations. [38-40] The previous recommendation for treating the late phase was 2-4 weeks' therapy and even longer therapy until the disappearance of the symptoms [7]. New studies did not justify the extended, 12-week antibiotic treatment in late-stage patients with prolonged Lb symptoms [41]. Based on our experience, a longer 4-6 weeks' treatment prevents the dissemination of Bb and its affecting other organs and systems [1,6,8]. On the other hand, other authors consider that 10 days of doxycycline and amoxicillin therapy is efficient in the treatment of the early phase of Lb [42].

There is a significant difference in the recommendations for the treatment of early phase of Lb, symptoms associated with Lb and prophylaxis between the Infectious Diseases Society of America (IDSA) and the International Lyme And Associated Diseases Society (ILADS) which advocates extended antibiotic treatment in all categories [8]. IDSA, along with several other medical associations, is in the process of developing new guidelines for the prevention, diagnosis and treatment of Lb [43].

There are two opposing standpoints in the world regarding prophylaxis after a tick bite. The authors who are against the early application of antibiotic therapy base their opinion on a small number of infected after the tick bite 2-3% [31], costs and possible side effects of antibiotics [11,12]. Other authors, including us, advocate the use of early antibiotic therapy, regardless of the small number of the infected after the tick bite. Among the infected, there are often patients with a severe clinical picture with extracutaneous manifestations and a possible fatal outcome [15].

In the prophylaxis of Lyme disease, the most commonly used drugs were cefotaxime, ampicillin, doxycycline, penicillin [22,33]. The results of a multicentre study in Germany and Austria show that the local application of 10% azithromycin at the site of the bite of the infected tick reduces the occurrence of EM [44]. We consider that the recommendation for local therapy is not justified because EM does not occur in 20-30% of infected patients, and patients later develop extracutaneous manifestations. It was previously described in experimental studies that the damage to the hematoencephalic barrier occurs as soon as 12 hours after spirochaet inoculation [45], which does not support the use of local antibiotic therapy at the tick bite site.

In our study, we administered 4 groups of antibiotics orally and benzathine benzylpenicillin once parenterally in order to prevent the disease after the tick bite. The results of our study show that early antibiotic therapy after the tick bite was very effective in the prevention of Lb. This was documented by a statistically significant decrease in the number of patients who received early

antibiotic therapy (1.7%) compared to the group that did not receive this treatment (36.3%). Besides, the symptoms and signs of the disease were also significantly less pronounced in the group with early antibiotic therapy compared to the group without. Extracutaneous manifestations as an indicator of the severity of the clinical picture, in the group that received early antibiotic therapy, were not present at all. In contrast, in the group that did not receive early antibiotic therapy, symptoms and signs were observed in cardiovascular, nervous and bone muscular systems.

The high incidence of early phase of Lb during the conducted trial at the MMA Clinic for Infectious and Tropical Diseases [10] correlates with the highest number of Lb cases reported in Serbia in the period from 1991 to 2000, which totaled 4768, and a high percentage of infected ticks [20]. The aforementioned findings coincided with the great migration of the population due to sanctions and wars on the territory of ex-Yugoslavia. In addition, in our patients we observed a greater number of symptoms and signs than in other studies, as we followed them for more than one year [10].

A study by Russian authors showed that the use of doxycycline reduces the occurrence of the early phase of Lb in patients with the infected tick bite, as 11 times more patients were found in the group without prophylactic antibiotic therapy [32].

Several studies showed, according to the results of the meta analysis, that the disease did not develop in people who received prophylaxis 72 hours after the tick bite, and that the risk of developing the infection was eleven times lower in the group that received antibiotics (doxycycline or amoxicillin), compared to the placebo group (0.2% : 2.22%) [14,30].

The administration of a single dose of 200 mg doxycycline after the tick bite showed that early antibiotic therapy was very effective in reducing morbidity of the early phase of Lb, as the disease developed in 8 times fewer patients compared to those who did not receive antibiotics (0.4% : 3.2%) [9]. In this study, subjects were monitored for 6 weeks and only EM was described, which is insufficient to evaluate the efficacy of the drug, because symptoms and signs of the disease can be subsequently exhibited up to one year after the bite. EM does not occur in 20% to 40% of patients with early phase of Lb. Following the publication of the results of this study, a single dose of 200 mg doxycycline after the tick bite was recommended in certain individuals within the given treatment criteria [33].

Contradictory opinions concerning the justification of the application of antibiotics have existed since the discovery of Lb and its treatment [11,12] up to the present [31]. According to some authors, a small number of patients after the tick bite (on average 1.1% - 3.4%) does not require the use of prophylactic therapy [11,12, 31,39]. In hyperendemic areas in the USA, Lb was developed in 3% of patients with a tick bite without antibiotic therapy, so the use of prophylaxis in less endemic areas would have an even smaller effect [31]. In Europe the tick infection rate is lower than in the USA, the risk of developing Lb is low (up to 4%), so prophylaxis is not recommended after the bite [4,31].

This study showed that the early antibiotic therapy was extremely effective in preventing the disease compared to the control group (1.7% : 36.3%). Our study followed the patients for 1-15 years, and therefore the percentage of patients who were infected at the early stage of Lb without early antibiotic therapy was greater [10]. Secondly, all the five antibiotic groups were found to be clinically highly effective. The best results were achieved with roxithromycin (99.4%), then with half-synthetic penicillins (98.4%), benzathine benzylpenicillin (98.2%), and doxycycline (97%), while cefalosporins (93.7%) showed the lowest statistically significant efficacy. As far as the efficacy of antibiotics and the duration of their use are concerned, (single-dose parenterally, 7 or 14 days orally) there were no statistically significant differences.

Previously, the efficacy of short-term administration of amoxicillin as prophylaxis was not known and was, therefore, not recommended [33]. After a meta-analysis of 4 studies [30], it was concluded that the ten-day administration of amoxicillin may be as effective as 200 mg of doxycycline. Our results showed that amoxicillin was very effective, even when given for only 7 days. In addition, benzathine benzylpenicillin, not having been considered so far, also showed good efficacy. Our study showed that, out of the 13 antibiotics we used, roxithromycin was the most efficacious, but at the same time it was, by far, the most expensive drug. In previous studies, it was the second line-treatment for the early phase of Lb [33]. According to the principles of the World Health Organization (WHO) that in the case of equal efficacy and good tolerance of more drugs, low-cost drugs should be used for treatment, in the past years we have decided to use ampicillin and doxycycline for a seven-day period. In case of need, i.e. in allergic patients (whom we did not register in our study), clarithromycin, azithromycin, (as suggested by BNF, 2010) or roxithromycin could be used [39].

In our study, we did not detect side effects of the applied drugs. There was no evidence of photosensitivity or gastrointestinal disturbances in soldiers who were exposed to the sun as part of their regular activities and diets that were not fully aligned with their usual family eating habits.

CONCLUSION

Taking into account our extensive experience in this field and results of the application of prophylactic therapies, we join the authors who are in favour of early antibiotic therapy after a tick bite. It is clinically justified, prevents the development of extracutaneous manifestations of Lb, is well tolerated, and the proposed antibiotics (doxycycline and ampicillin) are inexpensive.

REFERENCES

1. Bojić I. Evropska borelijoza (Lajmska bolest) i druge bolesti čiji se uzročnici prenose ubodom krpelja. Loznica: Naš dom; 2000. p. 29.
2. Drndarević D, Lako B, Stojanović R, Stajković N, Obradović M, Živanović B, et al. Ixodes ricinus dokazan vektor Lajm borelijoze i u Jugoslaviji. Vojnosanit Pregl. 1992; 49(1): 8–11.
3. Bağcı IS, Ruzicka T. Ticks, Borrelia Burgdorferi and Lyme Disease. Turk J Dermatol. 2016; 10: 116–21.
4. Guy N. Lyme disease: Basis for treatment strategy, primary preventive care and secondary preventive care. Med Mal Infect. 2007; 37: 381–93. (In French)

5. Fahrner H, van der Linden MS, Sauvain JM, Gern L, Zhioua E, Aeschlimann A. The prevalence and incidence of clinical and asymptomatic Lyme borreliosis in a population at risk. *J Infect Dis.* 1991; 163: 305–10.
6. Bojić I. Principi lečenja inficiranih Borreliom burgdorferi i obolelih od od lajmske bolesti. *Vojnosanit Pregl.* 1998; 55(1): 37–40.
7. Burascano JJ. Advanced Topics in Lyme Disease. Diagnostic hints and treatment guidelines for Lyme and other tick borne illnesses. International Lyme and associated disease Society. 2008, 16th Edition. (Accessed: September 2017) Available from: <http://www.lymenet.org/BurrGuide200810.pdf>
8. Cameron DJ, Johnson LB, Maloney EL. Evidence assessments and guideline recommendations in Lyme disease: the clinical management of known tick bites, erythema migrans rashes and persistent disease. *Expert Rev Anti Infect Ther.* 2014; 12(9): 1103–35.
9. Nadelman BR, Nowakowski J, Fisch D, Falco CR, Freeman K, McKenna D, et al. Prophylaxis with Single – Dose Doxycycline for the Prevention of Lyme Disease after an Ixodes scapularis Tick Bite. *N Engl J Med.* 2001; 345: 79–84.
10. Begović Kuprešanin V. Rana primena antibiotika i njen uticaj na kliničke manifestacije u ranoj fazi Lajmske bolesti. (Dissertation). Kragujevac: Medicinski fakultet, 2011.
11. Costello CM, Steere AC, Pinkerton RE, Feder HM Jr. A prospective study of tick bites in and endemic area for Lyme disease. *Conn Med.* 1989; 53: 338–40.
12. Shapiro ED, Gerber MA, Holabird NB, Berg AT, Feder HM Jr, Bell GL, et al. A controlled trial of antimicrobial prophylaxis for Lyme disease after deer-tick bites. *N Engl J Med.* 1992; 327: 1769–73.
13. Fix AD, Strickland GT, Grant J. Tick bites and Lyme disease in an endemic setting: problematic use of serologic testing and prophylactic antibiotic therapy. *JAMA.* 1998; 279(3): 206–10.
14. Warshafsky S, Nowakowski J, Nadelman RB, Komer RS, Peterson SJ, Wormser GP. Efficacy of antibiotic prophylaxis for prevention of Lyme disease: a meta- analysis. *J Gen Intern Med.* 1996; 11: 329–33.
15. Kugeler KJ, Griffith KS, Gould LH, Kochanek K, Delorey MJ, Biggerstaff BJ, et al. A review of death certificates listing lyme disease as a cause of death in the United States. *Clin Infect Dis.* 2011; 52(3): 364–7.
16. Adams D, Fullerton K, Jajosky R, Sharp P, Onweh D, Schley A, et al. Summary of Notifiable Infectious Diseases and Conditions - United States, 2013. *MMWR Morb Mortal Wkly Rep.* 2015 Oct 23. 62 (53): 1–122.
17. Centers for Disease Control and Prevention. Lyme Disease Data. CDC. [Accessed: March 14, 2016.] Available at: http://www.cdc.gov/lyme/stats/index.html?s_cid=cs_281
18. Wilking H, Stark K. Trends in surveillance data of human Lyme borreliosis from six federal states in eastern Germany, 2009–2012. *Ticks Tick Borne Dis.* 2014; 5: 219–24.
19. Izveštaj o zaraznim bolestima u Republici Srbiji za 2014 godinu: Institut za javno zdravlje Srbije “Dr Milan Jovanović Batut”; 2015.
20. Čekanac R, Stajković N, Krstić G. Epidemiološke karakteristike lajmske bolesti u Jugoslaviji. *Acta Medica Semendrica.* 2002; 45–48, 202.
21. Mladenović J. Epidemiološki aspekt lajmske bolesti na teritoriji grada Beograda. (Dissertatio) Niš: Medicinski fakultet; 2014.
22. Edlow AJ. Tick – Borne Diseases, Lyme: Treatment & Medication. eMedicine. Available from: <http://www.emedicine.com/derm/topic536.htm>. Retrieved 2007-08-21.
23. Nahimana I, Gern L, Blanc DS, Praz G, Francioli P, Peter O. Risk of *Borrelia burgdorferi* infection in western Switzerland following a tick bite. *Eur J Clin Microbiol Infect Dis.* 2004; 23: 603–8.
24. Maiwald M, Oehme R, March O, Petney TN, Kimmig P, Naser K, et al. Transmission risk of *Borrelia burgdorferi* sensu lato from *Ixodes ricinus* ticks to humans in southwest Germany. *Epidemiol Infect.* 1998; 121: 103–8.
25. Stanek G. Tick-transmitted diseases in central Europe. *Wien Clin Woch.* 2002; 114 (13–14): 471–2.
26. Meiners T, Hammer B, Gobel UB, Kahl O. Determining the ticks cutal index allows assessment of tick feeding duration and estimation of infection risk with *Borrelia burgdorferi* sensu lato in a person bitten by an *Ixodes ricinus* nymph. *Int J Med Microbiol.* 2006; 296(suppl 40): 103–7.
27. Southern R. Tick Bite Dangers and Symptoms. 2009. Available from: <http://www.edubook.com/tick-bite-dangers-and-symptoms/17304/>
28. Oteo JA, Martinez de Artola, Gomez-Cadinanos R, Casas JM, Blanco JR, Rosel L. Evaluation of methods of tick removal in human ixodidiasis. *Rev Clin Esp.* 1996; 196 (9): 584–7.
29. Mladenović J, Čekanac R, Stajković N, Krstić M. Rizik od pojave Lajmske bolesti nakon uboda krpelja. *Vojnosanit Pregl.* 2010; 67(5): 369–75.
30. Warshafsky S, Lee HD, Francois KL, Nowakowski J, NadelmanBR, Wormser PG. Efficacy of antibiotic prophylaxis for the prevention of lyme disease: an updated systematic review and meta-analysis. *J Antimicrob Chemother.* 2010; 65: 1137–44.
31. Stanek G, Wormser GP, Gray J, Strle F. Lyme borreliosis. *Lancet.* 2012; 379(9814): 461–73.

32. Vorobjova NN, Korenberg JI, Volegova GM, Moskvitina IG, Rasinkaja TK. Klinicheskie aspekti rezultatov antibiotikoprofilaktiki iksodovih kleshtevih boreliozov. *Med Parazitol (Mosk)* 1996; 2: 8–11.
33. Wormser GP, Dattwyler RJ, Shapiro ED, Halperin JJ, Steere AC, Klempner MS, et al. The clinical assessment, treatment, and prevention of Lyme disease, human granulocytic anaplasmosis, and babesiosis: clinical practice guidelines by the Infectious Diseases Society of America. *Clin Infect Dis*. 2006; 43(9): 1089–134.
34. Pritt BS, Mead PS, Johnson DKH, Neitzel DF, Respcio-Kingry LB, Davis JP, et al. Identification of a novel pathogenic *Borrelia* species causing Lyme borreliosis with unusually high spirochaetemia: a descriptive study. *Lancet Infect Dis*. 2016; 16(5): 556–64.
35. Dandache P, Nadelman RB. Erythema migrans. *Infect Dis Clin North Am*. 2008; 22(2): 235–60.
36. Martić V, Begović Kuprešanin V. Neuropatije u sklopu lajmske bolesti. In: *Infekcije perifernog nervnog sistema*. Beograd: Medija centra „Obrana“; p. 37–70.
37. Arroja JD, Fassa AA, Zimmermann M. Lyme carditis. *Rev Med Suisse*. 2015; 11(464): 563–6, 568.
38. BNF Lyme disease. U: Martin J, Ed. *British National Formulary*. London: BMJ Group and Pharmaceutical Press; 2010; 60: 328.
39. Shapiro ED. Lyme disease. *N Engl J Med*. 2014; 370: 1724–31.
40. Jen C, Dorado V, Lu B, Nguyen S. Lyme Disease: The Pharmacist's Role in Treatment and Prevention. *US Pharm*. 2016; 41(4):22–6.
41. Berende A, ter Hofstede HJ, Vos FJ, van Middendorp H, Vogelaar ML, Tromp M, et al. Randomized Trial of Longer-Term Therapy for Symptoms Attributed to Lyme Disease. *N Engl J Med*. 2016; 374(13): 1209–20.
42. Kowalski TJ, Tata S, Berth W, Mathiason MA, Agger WA. Antibiotic treatment duration and long-term outcomes of patients with early Lyme disease from a Lyme disease-hyperendemic area. *Clin Infect Dis*. 2010; 50(4): 512–20.
43. Infectious Diseases Society of America. Project Plan: Guidelines for the Prevention, Diagnosis, and Treatment of Lyme Disease by the Infectious Disease s Society of America, the American Academy of Neurology, and the American College of Rheumatology. (Accessed: March 14, 2016) Available at http://www.idsociety.org/uploadedFiles/IDSA/Guidelines-Patient_Care/PDF_Library/LD%20Project%20Plan%20March%202015%282%29.pdf
44. Shapiro DE, Wormser PG. Prophylaxis with topical azitromycin against Lyme borreliosis. *Lancet*; 246–8.
45. Garcia-Monco JC, Villar BF, Alen JC, Benach JL. *Borrelia burgdorferi* in the central nervous system: Experimental and clinical evidence for early invasion. *J Infect Dis*. 1990; 161: 1187–93.