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TICK BITE- CARRIERS OF INFECTIOUS DISEASES OF CHILDREN IN TERNOPIL REGION (UKRAINE)

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УКУСЫ КЛЕЩЕЙ - ПЕРЕНОШИКИ ИНФЕКЦИОННЫХ ЗАБОЛЕВАНИЙ У ДЕТЕЙ В ТЕРНОПОЛЬСКОЙ ОБЛАСТИ (УКРАИНА)

Abstract. The results of the monitoring the children s after tick bite during the spring-autumn period in 2018 in Ternopil region are presented Objectives There were cases when the ticks were infected with multiple pathogens (*B. burgdorferi sensu lato* with *A. Phagocytophilum*, *B. miyamotoi* and *A. Phagocytophilum*) simultaneously. Regarding the importance of different stages of *Ixodes ricinus* development in the transmission of infectious agents, it has been shown that a greater number of contaminated individuals were among females and nymphs. From 128 infected ticks-carriers of infectious agents were 36. 7% females, 59.4% nymphs, 0.78% male and 3. 1% larvae. **Material and methods** 128 children (from 1 till 16 year) of Ternopil region (Ukraine) were examined. The participants gave answers to the questions of a unified international questionnaire. Revealing of *Borrelia* the ticks was made by carried PCR technique. For detection of anti - *B. burgdorferi sensu lato* IgM and/or IgG 2 step stages diagnosis was used. **Results** Clinical examination of children affected by ticks have shown that the most common initial clinical manifestation of Lyme borreliosis are typical skin disorders – erythema migrans. A group of children,

which was not subject to preventive treatment was 7 child with neurolyme. **Conclusions** The necessity to prescribe the preventive treatment of Lyme borreliosis in children who have been exposed to ticks has been proved.

Аннотация. Представлены результаты мониторинга укусов детей клещами в весенне-осеннем периоде 2018 года в Тернопольской области, когда клещи были инфицированы множественными патогенами (*B. burgdorferi sensu lato* с *A. Phagocytophilum*, *B. miyamotoi* и *A. Phagocytophilum*) одновременно. Что касается важности различных стадий развития *Ixodes ricinus* при передаче инфекционных агентов, то было показано, что большее число зараженных особей было среди женских особей и нимф. Из 128 инфицированных клещей-носителей инфекционных агентов были 36, 7% женских особей, 59,4% нимф, 0,78% мужских особей и 3,1% личинок. Материалы и методы. Были обследованы 128 детей (от 1 до 16 лет), жителей Тернопольской области (Украина). Участники дали ответы на вопросы единого международного вопросника. Инфицирование клещей *Borrelia* осуществляется методом ПЛР. 2 этапная диагностика была использована для обнаружения анти - *B. burgdorferi sensu lato* IgM и IgG. Результаты клинического обследования детей, пострадавших от укусов клещей, показали, что наиболее распространенным первоначальным клиническим проявлением боррелиоза являются типичные кожные заболевания – мигрирующая эритема. Группа детей, которая не подвергалась профилактическому лечению болезни Лайма, состояла из 7 детей с нейроборрелиозом. Выводы. Необходимость назначать профилактическое лечение боррелиоза Лайма у детей, которые подверглись нападению клещей.

Keywords. *Lyme borreliosis*, *B. burgdorferi*, *A. Phagocytophilum*, *neuroborreliosis*, *B. miyamotoi*, *children*.

Ключевые слова. Лайма боррелиоз, *B. burgdorferi*, *A. Phagocytophilum*, нейроборрелиоз, *B. miyamotoi*, дети.

Introduction. Ixodic ticks clearly occupy a niche of important reservoirs and carriers of pathogens of many infectious diseases not only of animals but also of people. The incidence rate in Ukraine in 2000-2010 increased in 29 times.

Since the 1990s, in Europe, including Ukraine, cases of transmission pathogens to human by various types of boreliosis, causing severe diseases of the musculoskeletal system, the nervous system and the cardiovascular system, are being recorded. In recent years, the advancement in the study of the epidemiology of ticks has made it possible to establish the facts about transmission of dangerous diseases, such as granulocytic anaplasmosis, babesiosis and erlichiosis to humans by ticks.

The aim of the study. To investigate:

1. the peculiarities of children affected by ticks species *Ixodes ricinus* bites,
2. at what stage of ticks' development the ticks bite human more often;
3. the level of ticks infected with pathogens of *B. burgdorferi* s.l., *B. miyamotoi* and *Anaplasma phagocytophilum*;
4. the frequency of bites of children by ticks, depending on season in 2018 in the city of Ternopil and Ternopil region ;
5. the influence of tick bites on the health of children.

Objectives. 1. To summarize the results and peculiarities of treatment of the children affected by ticks in the laboratory research conducted in Ternopil Medical University during 2018.

2. To reveal the epidemiological picture of different stages of the tick species *Ixodes ricinus* on the basis of PCR assay, by use of the ROTOR GENE-6000 amplifier in real-time.

3. To highlight the results of examination of children after they have been infected by infected ticks with boreliosis, anaplasmosis .

Material & Methods.

The study was conducted within the framework of scientific research "Research on epidemiology, pathogenesis, clinics and prevention of Borreliosis", which is a part of joint Ukrainian-Polish project under the auspices of the European Union. The tests were performed in the Laboratory of the Center for the Study of Lyme Borreliosis and other ticks infections. The study involved 128 children, residents of Ternopil region, who referred to the Ternopil Regional Children's Hospital, and have been bit by ticks during 2018. The participants gave answers to the questions of a unified international questionnaire, in which they've noted the number and place of ticks bites, described ways how ticks were removed, and marked complaints that bothered them after tick bites. After initial physical examination, the participants were directed to the laboratory examination.

In the first step, IgM and/or IgG antibodies to *B. burgdorferi sensu lato* were determined by the method of immunoassay analysis using the Euroimmun AG test systems (Germany), specific antibodies IgM were detected using Anti-Borrelia Burgdorferi ELISA (IgM), and antibodies IgG – Anti-Borrelia plus VlsE ELISA (IgG). The test was performed within one month after tick bite. According to the manufacturer's recommendations, the result ≥ 22 RU/ml was considered positive, from 16 to 22 RU/ml was considered intermediate, the result ≤ 16 RU/ml was negative. Identification of ticks was carried out with the use of optical-electronic system SEO - IMAGLAB. Determination of pathogens in the ticks was carried out using the PCR method using the "ROTORGene-6000" amplifier in real time.

Results of Research. During 2018, parents of the 376 children asked the researchers of the scientific laboratory of the I. Horbachevsky Ternopil National Medical University to examine the ticks, which bit their children, for the presence of pathogens of infectious diseases (tick born encephalitis). It was discovered that 128 (34, 04 %) ticks were infected with pathogens: *B.*

burgdorferi s.l., *B. miyamotoi* and *A. phagocytophilum*. Pathogens were found in different life stages of ticks such as: imago, nymphs and larvae, both individually and in complex. Particularly combined: *B. burgdorferi*

s.l. with *A. phagocytophilum* and *B. miyamotoi* with *A. phagocytophilum*.

An analysis of the frequency of attacks of infected ticks on children in different months was made. General data are shown in **TABLE 1.** and **FIGURE 1.**

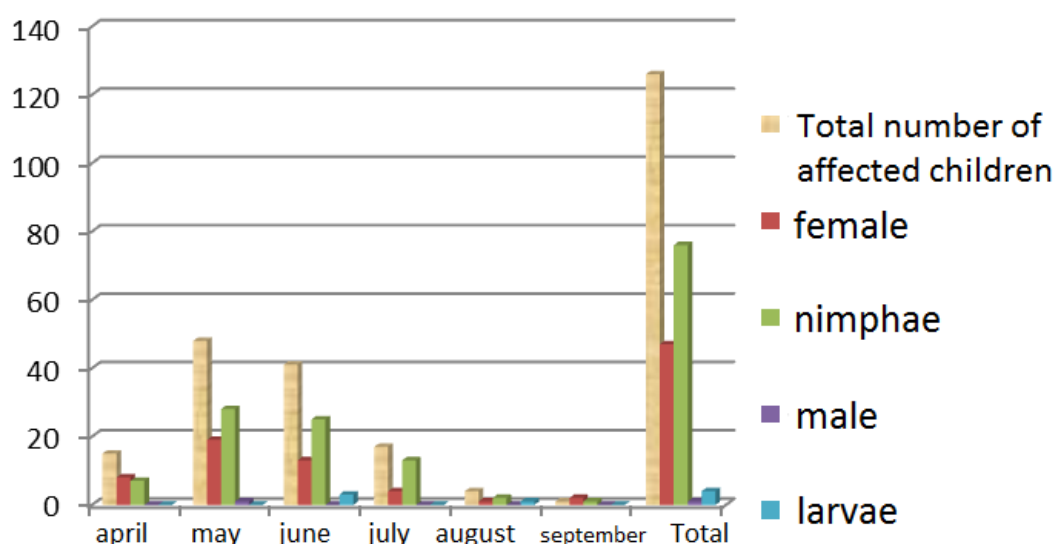
Table 1.

Analysis of the frequency of attacks of infected ticks on children in different months.

Month	Total number of affected children	Females	Nymphs	Males	Larvae
April	15	8	7	0	0
May	48	19	28	1	0
June	41	13	25	0	3
July	17	4	13	0	0
August	4	1	2	0	1
September	3	2	1	0	0
total	128	47	76	1	4

According to the results of the analysis (Fig.1), we see that the main transmitters of pathogens of the infections are nymphs and females. Their ratio varies

somewhat depending on the months, reaching close parity in May-June.

Figure. 1. Epidemiological state of different stages of the tick *Ixodes ricinus*.

We have analyzed some patterns of tick infections of children of all ages from 1 month to 17 years

inclusive. The revealed patterns are presented in TABLE 2. and Fig. 2.

Table 2.

Distribution of children by age affected by ticks.

Category	Age categories of children (years)						
	0,1-1	1,1-2	2,1-3	3,1-6	6,1-11	11,1-15	15,1-17
Number of children	5	14	13	42	37	10	7

The children who were affected by ticks were between 3 and 11 years old. This is due to their activity and the lack of due attention from their parents. Infants

are obviously affected by ticks while walking with their parents as they push carts and carriages near the bushes, from which the ticks are falling.

Patterns of affected by ticks of children of different age groups

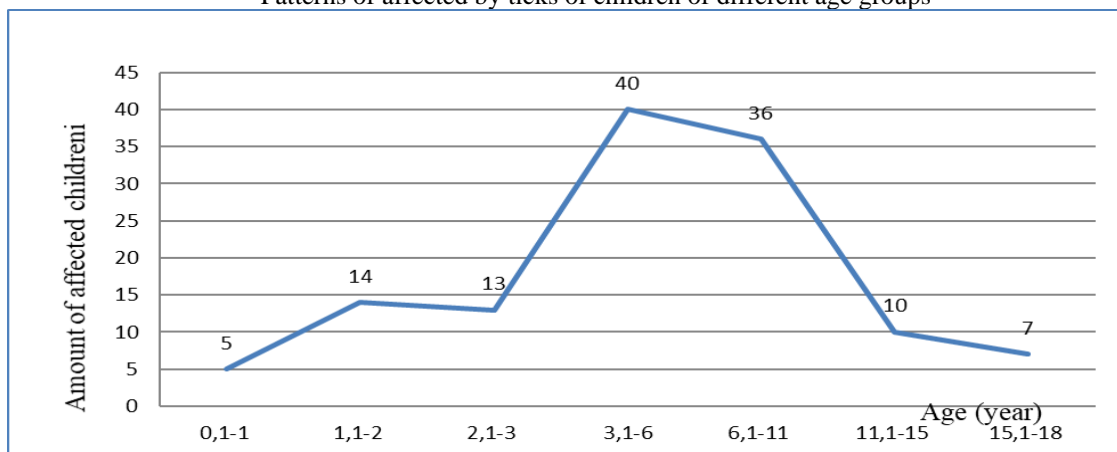


Figure 2. The ages of children affected by ticks.

Table 3.

Analysis of the defeat of the ticks of the species *Ixodes ricinus* by pathogens of infectious diseases in 2018.

Life stage of tick	General quantity of affected ticks	<i>B. burgdorferi</i> s.l.	<i>A. phagocytoph.</i>	<i>B. miyamotoi</i>	<i>B. burgdorferi</i> with <i>A. phagocytoph.</i>	<i>B. miyamotoi</i> with <i>A. phagocytoph.</i>
female	47	20	17	1	6	1
lymph	76	33	36	0	6	2
larva	4	1	0	0	2	1
total	127	54	53	1	14	4

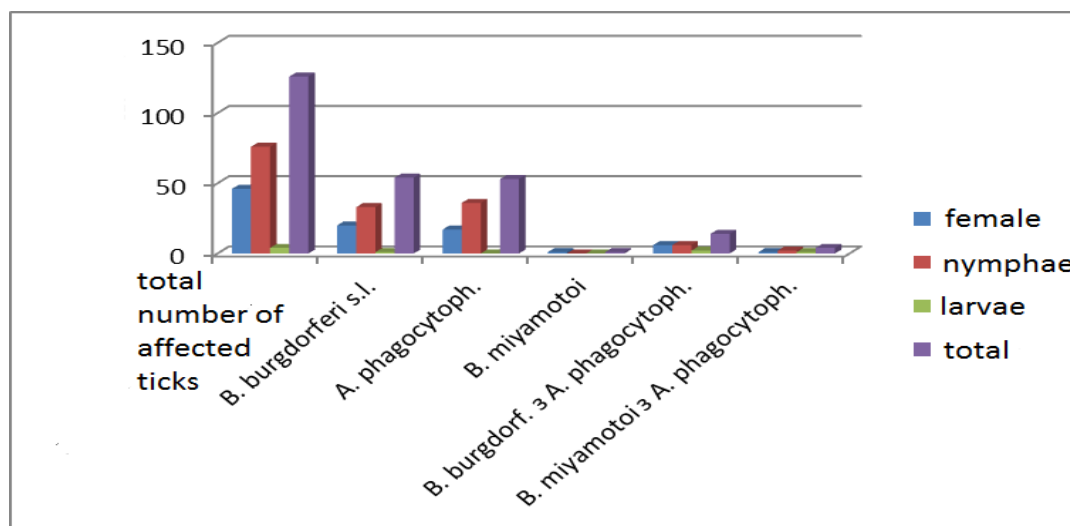


Figure 3. Distribution of infected ticks of the species *Ixodes ricinus*.

Analysis of the distribution of pathogens in different life stages of ticks showed that carriers of causative agents pathogens of infections may be practically all stages of the tick of the species *Ixodes ricinus*. Nymphs are carriers of *A. phagocytophylum* more than females (ratio approximately 2:1). Spirochet *B. burgdorferi* s.l. both stages carry in the same way. Females and nymphs are often carriers of the *B. burgdorferi* complex with *A. phagocytophylum* and *B. miyamotoi* with *A. rhagocytophylum*.

Studies of E. D. Shapiro (2014) showed that the ratio of infestation of *Ixodes scapularis*, *B. burgdorferi* s.l. *spirochetes* in endemic areas of New England is 20-30 % in nymphs and 30-50 % in imago. Ticks are capable of transmitting a pathogen to a person after 72 hours of sucking blood. The less time a tick sucks blood, the lower the probability of transmission of the pathogen. When sucking blood within 48 hours, the probability of transmission of the pathogen is only 1-3 %.

We ask the parents of children affected by ticks to fill in the standard questionnaire. The content of the survey was as follows:

1. Did you visit the doctor for consultation and examination?
2. Have your children taken an antibiotic course on the recommendation of a doctor?
3. Have you observed erythema migrans on the child's body?
4. Have you given blood of the child for specialized laboratory?
5. What are the results of these analysis?
6. Has the repeated course of treatment been applied after the positive results of your child's examination?
7. Did any concomitant disease appear in your child after the treatment was completed?

Research results and discussion.

All 128 children were inspected by the doctor.

After having been examined by a pediatrician, all children were prescribed preventive course of treatment of antibacterial therapy of the Penicillin group. All parents of the children (128) were polled. In 7 (5.4 %) cases, antibiotics were not taken. 16 (12.5 %) of children showed erythema migrans, in all other patients erythema migrans was not observed.

Wormser GP, Dattwyler RJ (2006) Hengge UR, Tannapfel A, Tying SK, et al. (2003) [1,2] indicated the highest time of tick bites between May and October, which coincides with our research.

Risk factors for the onset of Lyme disease in endemic areas include the landscape, climate, habitat near the forest or near fields adjacent to the forest areas (where there are many ticks like vectors), as well as outdoor play of children [2]. Since very few seropositive patients reported about previous symptoms or treatment of Lyme disease, it was thought that *B. burgdorferi* infections may have an asymptomatic course in a large number of children [3].

According to the results of clinical examination of children, it was found that the respondents who remembered the fact of a bite often pointed to a tick attack in the spring and summer months, for the most part in May – 48 cases, June – 41 cases, and July – 17 cases). Probably for these months there is a cycle of ticks reproduction and a peak of time for people outdoor activities in nature. According to scientific observation, the skin of the lower limbs, buttocks, abdominal region and head in children are the most common zones of ticks bites [4]. We examined the children in dynamics - it was observed an increase of EM within a day by several cm. The most frequent localization of the bite there was in the area of the head (22.7 %) and lower limbs (22.7 %), trunk (15.9 %), neck (11.3 %), upper extremity (11.3%), abdomen (15.9 %), ear section (9 %) in examining group of children, that was confirmed by other studies [12].

In most cases, the tick bite was one-time – 72 cases (75.0 %), twice - in 6 persons 11.4 %), multiple - in 3 children (3.7 %). According to the anamnesis, from the moment of the bite, it lasted up to 12 hours in 35.7 % of children, up to 24 hours - in 34.3 %, and 24-48

hours or more - in 30 % persons. We would like to note some cases when the mother did not pay enough attention to the appearance of erythema migrans after tick bite. In most cases EM was asymptomatic, itchy skin at the site of the tick bite was sometimes observed.

One of the manifestations of Lyme-borreliosis is neuralgic manifestation in the form of peripheral paralysis of the facial nerve. This problem is covered by scientists, as A.L. Belman, M. Ayer, P.K. Kouler, R. Dattwiller [5], L.E. Nigrovich, A.D. Thompson, A.M. et al. [6].

To confirm this problem we present a clinical case that took place in the Ternopil Regional Children's Hospital.

Clinical case 1. A child was admitted to the infectious-diagnostic department of Ternopil clinic with complaints of periodic tightness of the right shin, fever, and pain in the area of the heart. From anamnesis of the disease it is known that 6 months ago the girl had a tick bite in the leg. In the area of the bite there was an erythema migrans. The child did not receive preventive treatment. In November 2018 Immunoblot VlsE (*B. burgdorferi* detected, VlsE (*B. garinii*) boundary result, p41 (detected), OspCb.afzelii-boundary result, Ig G-detected. Lyme disease was diagnosed and treatment for uniodox and azithromycin was prescribed. Antibacterial therapy began one month after the bite and an existing IU lasted 28 days. After 6 months, the child turned to the hospital in the phase of desmination Lyme-borreliosis, seropositive form, of moderate severity. Polyradiculoneuritis.

Erythema migrans is retained for several weeks (2-3), disappearing gradually. It is pale in the center and the clear contour may stay longer. Common symptoms were absent in most cases.

Clinical case 2. 9-year-old girl, was admitted with complaints of left side facial weakness, facial asymmetry. Mother noticed facial asymmetry 4-5 days prior to hospitalization. Anamnesis vitae: a child from the first uncomplicated, full-term pregnancy, timely met all developmental milestones.

Epidemiological anamnesis: six months ago she noticed a tick bite. Mother noted erythema at the site of a bite. They did not seek for treatment.

Physical examination: Gait is normal. AAO X 3. PERRLA. Left side ptosis. Left side facial droop. Tongue is midline. Normal muscle tone. No pathological reflexes. No cerebellar signs.

CBC: Hb 123 g/l, CI 0.9, RBC $4.87 \times 10^{12}/l$, WBC $12.5 \times 10^9/l$, bands 2 %, segmented neutrophils 68 %, eosinophils 2%, lymphocytes 27 %, monocytes 1 %. ECR 4 mm/hr.

Blood biochemistry tests: glucose 5.5 mmol/L, total protein 58 g/l, urea nitrogen 3.4 mmol/L, creatinine 0.048 mmol/L, bilirubin 10.0 mmol/L, Ca 2.0 mmol/L, P 1.2 mmol/L, AST 30 U/L., ALT 20 U/L (normal before 40 U/L), ASL-O normal (normal 150), C-reactive protein (CRP) 0,23 (normal 5.0), Rheumatoid factor (RF) – negative (normal 14), serum glycosides – 3.2.

Immunogram: There were anti- *B. burgdorferi* IgG –98.98 RU/ml (pos. 22), IgM 75.29 RU/ml (pos. 22). Urinalysis: Normal

ECG There are intraventricular conduction disturbances.

Transthoracic Echocardiography of the heart valves, the mitral valve prolapse of the the 1 degree with minimal regurgitation.

Diagnosis: Lyme disease, early disseminated phase, left facial palsy.

Of the 81 children, who had been attacked by ticks, 20 children had manifestation of erythema migrans, the rest of the children had asymptomatic course. All children had a general clinical trial (general blood test, general urine test), but tests did not show any signs of inflammation. In the serological test one month after the bite, an increase in IgM was detected in only 2 patients from this group. The rest of the screening tests showed a variant of the norm.

In the hospital, 9 children (6 girls and 3 boys) were diagnosed with Lyme Arthritis (LA). Anamnesis study showed that in half of children with arthritis, erythema migrans (ME) was diagnosed on the eve of the disease. In one child, EM developed in the place of the bite, in 4 - in remote areas, 4 of them were not observed. In 3 children, the EM occurred within the first 24 hours after the tick bite, after 7 days-2 or more people. In 3 children, tick attacks occurred in the park area, and one in the countryside, with 3 people in the forest, the rest did not remember the terrain.

The results of modern scientific research on Lyme borreliosis are controversial, since these violations often coexist in the clinical aspect and, both independently and collectively, are considered both in determining the factors of disturbance in the health of people who have suffered as a result of an attack of ticks (Rizzoli A., Haufler H., 2011). In clinical practice cases of masking for other diseases are described, for example, hypersensitivity reaction to the tick bite (occurs within 48 hours after the bite of the tick, rapidly disappears for 24-48 h), multivariate erythema - allergic reaction, dermatomycosis, ring-shaped granuloma (Aucott J., Morrison B., et al.) [7].

The proportion of patients with systemic symptoms is higher in the US than in Europe. In 30-50% of cases, the lesions are increased due to the early spread of *B. burgdorferi* [6]. All signs and symptoms of the disease, including skin lesions, usually disappear spontaneously in a month [5]. Epidemiological studies reported positive IgM and / or IgG antibodies against the pathogen in 5-37 % of patients with a single EM and 22-89 % with multiple EM [6,7]. According to T. Balmelli, JC Piffaretti [10], all three genotypes of borrelia species can cause the entire spectrum of clinical manifestations of LB. There is evidence that *B. afzelii* is more often associated with skin manifestations, *B. garinii* - with neurological symptoms, and *B. burgdorferi* is mainly associated with arthritis [7].

Discussion of this study requires solving several important issues. Firstly, this presentation emphasizes the necessity of identification of the ticks like vector of

transmission of infectious disease. Children who have been infected by ticks have been screened for a general blood test. A short 5-day course of antibiotic therapy with the antibiotics of penicillin group were prescribed for children with EM (19.7 %) It was Amoxicillin 50 mg / kg / day for 3 admissions (max 1.5 g). But for children more than 8 years old: doxycycline 100 x 2 g was prescribed (from 10 to 21 days) . The administration of antibiotics is suggested by all health authorities when EM is highly suspected [9].

The study of Lyme-borreliosis in children (Eugene D. Shapiro [14] showed that in 58 % of children after tick bite manifested erythema migrans. Among other symptoms of the disease was recorded headache – 16 %, arthralgia – 11 %, increase regional lymph nodes – 5.0 %, atony - 2.5 %. Antibacterial therapy for 3-5 days was not sufficient, so further blood tests showed the presence of Ig G to borrelia in the titre 1:64. Similar results were also demonstrated by the study of Lyme borreliosis in children in Connecticut in 1996 (Gerber M.A. [13], Shapiro E.D., Burke G.S., Parcells V.J., Bell G.L. [11,12]. According to these data, single erythema migrans was present in 66 % of children, and in 23 % there were multiple lesions with migrating erythema, 6 % of persons showed presence of arthritis, 3 % - paralysis of the facial nerve, 2 % aseptic meningitis and 0.5 % - carditis. The prescription of a two-week antimicrobial therapy gave a positive result. After treatment there were no complications.

Conclusions.

1. An analysis of the examination of infected children showed that the highest frequency of their lesions was observed in May (48), with the number of nymphs somewhat predominant number of females (28: 19 and 25: 13).

2. Investigation of some patterns of ticks infestation of children aged 1 month to 17 years inclusive showed that among the most frequently affected people by ticks are children aged 3 to 11 years. This is due to their activity and the lack of due attention from the parents.

3. Analysis of the distribution of pathogens in different life stages of ticks showed that carriers of pathogens of infections may be practically all stages of the tick of *Ixodes ricinus*. Nymphs are carriers of *A. rhagocytophylum* more than females (ratio approximately 2:1). Spirochet *B. burgdorferi* s.l. both stages carry in the same way. And females and nymphs are often carriers of the *B. burgdorferi* complex with *A. rhagocytophylum* and *B. miyamotoi* with *A. phagocytophylum*.

4. The presence of history of contact children with the ticks affected by *B. burgdorferi* and the occurrence of a patient with erythema migrans is sufficient to diagnose Lyme's disease and start treatment.

5. Laboratory diagnostics of Lyme-borreliosis is based on the identification of the pathogen itself (bacterioscopy and bacteriological methods of diagnosis) or its DNA, and determination of specific blood antibodies (2 stage diagnostic by serological method).

6. The necessity to prescribe the preventive treatment of Lyme borreliosis in children who have been exposed to ticks has been proved.

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Abbreviations

Neuroborreliosis (NB)
Lyme borreliosis (LB)
History of present illness (HPI)
Past medical history (PMH)
Pertinent physical examination (PE)
Complete blood count (CBC)
Erythema migrans (EM)
Lyme arthritis (LA)

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