

## Experience of PCR research on *Lyme borreliosis* in children from the Ternopil Region

SVITLANA NYKYTYUK<sup>1, A, E, F</sup>, SERHIY KLYMNYUK<sup>2, C</sup>, VOLODYMYR PANICHEV<sup>3, A</sup>,  
 ORCID ID: 0000-0003-3146-9664 ORCID ID: 0000-0002-1308-3250 ORCID ID: 0000-0003-3473-8399

OLHA MARCHUK<sup>4, A, B</sup>, IVAN KLISHCH<sup>5, D</sup>  
 ORCID ID: 0000-0002-9089-0873 ORCID ID: 0000-0001-6226-4296

<sup>1</sup> Department of Children's Diseases and Pediatric Surgery, I. Horbachevsky Ternopil National Medical University, Ternopil, Ukraine

<sup>2</sup> Department of Microbiology, Virology and Immunology, I. Horbachevsky Ternopil National Medical University, Ternopil, Ukraine

<sup>3</sup> State Institution "Ternopil Region Centre for Diseases Control and Prevention of the Ministry of Health of Ukraine", Ternopil, Ukraine

<sup>4</sup> Interdepartmental Training and Research Laboratory Center I. Horbachevsky Ternopil National Medical University, Ternopil, Ukraine

<sup>5</sup> Department and Laboratory Diagnostics, I. Horbachevsky Ternopil National Medical University, Ternopil, Ukraine

**A** – Study Design, **B** – Data Collection, **C** – Statistical Analysis, **D** – Data Interpretation, **E** – Manuscript Preparation, **F** – Literature Search, **G** – Funds Collection

**Summary Background.** *Lyme borreliosis* (LB) is a multisystem infectious disease caused by *Borrelia burgdorferi sensu lato* and is transmitted to humans through tick bites. *Lyme borreliosis* has become more common among children. Improving the methods of etiological diagnosis of Lyme disease is especially important.

**Objectives.** The survey covered 30 parents of children who were bitten by ticks. *Borrelia burgdorferi sensu lato* (*B. afzelii*, *B. burgdorferi sensu stricto* and *B. garinii*), *B. miyamotoi* and *A. phagocytophilum* were identified.

**Material and methods.** For the identification of pathogens of blood-borne infections among children, we observed a group of children (30) aged 1 to 14 years. Ticks were identified using a stereomicroscopic SEO system. The DNA of *B. burgdorferi sensu lato* (*s.l.*) (*B. burgdorferi sensu stricto*, *B. afzelii* and *B. garinii*), *B. miyamotoi* and *A. phagocytophilum* were determined by real-time PCR and dark field microscopy.

**Results.** In the study of blood of children *B. burgdorferi* was found in 2 people (6.6%), *A. phagocytophilum* – in 2 (6.6%), *B. miyamotoi* – in 3 (10.0%), *B. burgdorferi s.l.* compatible with *A. phagocytophilum* and *B. miyamotoi* – detected in 1 child (3.3%).

**Conclusions.** Early detection and identification of *Lyme borreliosis* in particular and preventive treatment can prevent an increase in the incidence of this infection in children. It is necessary to take into account the epidemiological risks not only for borreliosis but also granulocytic anaplasmosis.

**Key words:** Lyme disease, child, research.

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### Background

Today, the incidence of Lyme disease remains high. The number of attacks on people is increasing [1], and the risks of co-infection for the urban population, which leads to the severity of the clinical picture of the disease, the peculiarities of its polymorphism and chronicity. Improving the methods of etiological diagnosis of Lyme disease in children is especially important. Lyme disease has various clinical manifestations which should be excluded in all patients who have been bitten by ticks.

The study was conducted as a part of the study "Mono- and mixed tick-borne infections. Improvement of medical and diagnostic technologies and biosafety measures". The authors state that all procedures and experiments of this study were conducted according to the ethical standards of the 1975 Declaration of Helsinki, revised in 2008 [2], as well as to national legislation. Informed consent was obtained from the patients involved in the study. This work obtained a positive opinion from the Bioethics Committee (#69 from 12.04.2022).

The purpose of the work is to identify the causative agents of tick-borne infections in children who have been bitten by ticks, as well as to generalise the accuracy of diagnostic tests and testing modes.

### Material and methods

Pathogens of transmissible infections in the blood of children bitten by ticks were identified. 30 children aged 1 to 14 years were under observation. The average age was 7.7 years. There were 20 boys (66.6%) and 10 girls (33.3%).

Real-time PCR determined DNA fragments of *B. burgdorferi sensu lato* (*sl*) (*B. burgdorferi sensu stricto*, *B. afzelii* and *B. garinii*), *Borrelia miyamotoi*, *Anaplasma phagocytophilum*, *Ehrlichia muris*, *Ehrlichia chafeensis* and *Babesia speciaes*.

Ticks were identified using a stereomicroscopic SEO system. Infection of ticks with *Borrelia* was also determined by dark field microscopy.



## Results of the research and discussion

The survey covered 30 parents of children who were bitten by ticks.

In the study of blood in children, *B. burgdorferi* was found in 2 people (6.6%), *A. phagocytophilum* – in 2 (6.6%), *B. miyamotoi* – in 3 (10.0%), *B. burgdorferi s.l.* with *A. phagocytophilum* and *B. miyamotoi* – in 1 child (3.3%).

According to their parents, 48 hours had passed since the notice of the tick bite in 5 (16.6%) children, up to 24 hours – in 16 (53.3%) and up to 12 hours – in 9 children (30.1%).

The locations of attachment of ticks are mainly head, earlobes, upper and lower extremities and perineum. It was established that the probability of infection with *LB* pathogens in the case of mites correlates with the duration of their suction [3].

The children complained of itching and redness at the site of the bite (2 patients), lymphadenitis was found in 1 child, and fever and arthralgia was noticed in 1 child. Another child was bitten by a mite infected with *A. phagocytophilum*. A week later, she was hospitalised in a neuropsychiatric hospital with acute mental disorders.

All children were preventively prescribed with antibacterial therapy of the penicillin group (amoxicillin 50 mg/kg for 7 days).

In the scientific world, there is a discussion about the need to identify the pathogen. According to some recommendations [2], the removed mite should be examined for species identification, in particular for *B. burgdorferi*. At present, 22 species of *B. burgdorferi s.l.* have been identified, up to 10 of which have confirmed the presence of pathogenic potential in humans: *B. afzelii*, *B. bavariensis*, *B. bissettii*, *B. burgdorferi sensu stricto*, *B. garinii*, *B. kurtenbachii*, *B. lusitaniae*, *B. mayonii*, *B. spielmanii*

and *B. valaisiana* [4]. A huge contribution to human *LB* world-wide still belongs to *B. afzelii*, *B. burgdorferi sensu stricto* and *B. garinii*. Different types of *Borrelia* have different organ tropism and mainly cause clear clinical manifestations of the disease [5].

According to some authors, only 2% of tick bites lead to infection [6]. Monoinfections (79.1%) are dominant over coinfections (20.9%). *A. phagocytophilum/Rickettsia* spp. (70%) was the most common among coinfection; however, coinfections also included *B. afzelii/A. phagocytophilum*, *B. afzelii/Rickettsia* spp., *B. miyamotoi/A. phagocytophilum* and *B. afzelii/B. garinii/B. lusitaniae* [7].

The species *B. miyamotoi* genetically belongs to the group of *Borrelia* – the causative agent of recurrent fevers [8, 9].

Thus, ticks are important vectors of various pathogenic microorganisms that lead to clinical diseases in humans. Information on the infection of ticks and pathogenic microorganisms is important for the detection and prevention of the disease.

## Conclusions

Early detection and identification of Lyme disease pathogens and preventive treatment of children prevent an increase in the incidence of this infection.

Information on tick infestation is important for assessing epidemic risks in endemic areas.

Differential diagnosis of tick infections in children should be carried out taking into account the combined infection of ticks with two or more pathogens.

When assessing the condition of children after a tick bite, it is necessary to take into account the epidemic risks not only for borreliosis but also granulocytic anaplasmosis.

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Address for correspondence:

Svitlana Nykytyuk, MD, PhD, Assoc. Prof.

Department of Children's Diseases and Pediatric Surgery

I. Horbachevsky Ternopil National Medical University

Ternopil, Ukraine

E-mail: androx@tdmu.edu.ua