



<https://doi.org/10.29326/2304-196X-2024-13-1-78-86>



Role of ixodid ticks in tick-borne pathogen spread and circulation in the Belarusian Lakeland

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ABSTRACT

Results of ixodid tick analysis for their ecological, epizootological and epidemiological significance for tick-borne pathogen spread across the Belarusian Lakeland are presented. The ticks were collected in publicly accessible areas of the Vitebsk Raion in April – November 2022: 8 routes were tracked, 18 flag-km were passed, 529 tick specimens were collected, including 350 imago ticks and 179 nymph ticks. The ixodid tick genus and species were determined using N. A. Filippova's ixodid tick determinator. All caught ticks were tested for *Borrelia* spp., *Anaplasma* spp. (*Ehrlichia* spp.), *Babesia* spp. and *Tick-borne encephalitis virus* genetic materials with real-time polymerase chain reaction using the reagent kit for nucleic acid extraction from environmental samples in accordance with the manufacturer's instructions. The specimens were grouped in accordance with the MG 3.1.1027-01 "Collection, recording and preparation for laboratory tests of blood-sucking arthropods being vectors of natural focal infections"; therewith, one specimen includes only one tick. Differences in the numbers of ixodid ticks and the occurrence of genetic markers of tick-borne pathogens in them were found to be associated with ecological characteristics of the examined territories. The following epidemically and epizootically significant ticks contributing to transmissible infection and invasion spread were found in the Belarusian Lakeland: ticks of *Ixodes* and *Dermacentor* genera; their frequency index was 70.1 and 29.9%, respectively. Tick-transmitted pathogen prevalence rate in the examined territories of the Vitebsk Raion was as follows: 61.7% for *Borrelia* spp., 25.8% for *Anaplasma* spp. (*Ehrlichia* spp.) and 25% for *Babesia* spp., mixed infections were found in 10.8% of the ticks. No *Tick-borne encephalitis virus* genetic materials were found in the specimens. Total infection rate for ixodid ticks was 22.7%.

Keywords: Belarusian Lakeland, ixodid ticks, transmission infections, borreliosis, anaplasmosis (ehrlichiosis), babesiosis

For citation: Osmolovsky A. A., Subotsina I. A. Role of ixodid ticks in tick-borne pathogen spread and circulation in the Belarusian Lakeland. *Veterinary Science Today*. 2024; 13 (1): 78–86. <https://doi.org/10.29326/2304-196X-2024-13-1-78-86>

Conflict of interests: The authors declare that there is no conflict of financial/non-financial interests associated with the paper.

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УДК 576.895.42-047.37(476)

Роль иксодовых клещей в распространении и циркуляции возбудителей клещевых инфекций и инвазий на территории Белорусского Поозерья

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РЕЗЮМЕ

Приведены результаты анализа эколого-эпизоотологической и эпидемиологической значимости иксодовых клещей в распространении и циркуляции возбудителей клещевых инфекций на территории Белорусского Поозерья. Сбор образцов проводили с апреля по ноябрь 2022 г. на объектах открытой природы Витебского района, при этом пройдено 8 маршрутов, отработано 18 флаго-километров, собрано 529 экземпляров клещей, в том числе 350 взрослых имаго и 179 нимф. Родовую и видовую принадлежность иксодовых клещей устанавливали с помощью определителя Н. А. Филипповой. Все отловленные особи были исследованы на наличие генетического материала *Borrelia* spp., *Anaplasma* spp. (*Ehrlichia* spp.), *Babesia* spp. и *Tick-borne encephalitis virus* методом полимеразной цепной реакции в режиме реального времени с использованием набора реагентов для экстракции нуклеиновых кислот из объектов окружающей среды в соответствии с инструкцией производителя. Группировку проб осуществляли в соответствии с МУ 3.1.1027-01 «Сбор, учет и подготовка к лабораторному исследованию кровососущих членистоногих – переносчиков возбудителей природно-очаговых инфекций», при этом в одну пробу включали только одного клеща. Определено, что различия в показателях численности иксодовых клещей и встречаемости в них генетических маркеров возбудителей клещевых инфекций имеют определенную связь с экологическими особенностями изучаемых территорий. Установлено, что в Белорусском Поозерье фауна эпидемически и эпизоотически значимых видов клещей, способствующих распространению трансмиссивных инфекций

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и инвазий, представлена клещами родов *Ixodes* и *Dermacentor*, индекс встречаемости составил 70,1 и 29,9% соответственно. Показано, что спектр возбудителей инфекционных заболеваний, передаваемых иксодовыми клещами, на исследуемых территориях Витебского района в 61,7% случаев представлен *Borrelia* spp., в 25,8% – *Anaplasma* spp. (*Ehrlichia* spp.) и в 25% – *Babesia* spp., при этом микст-инфицированность переносчиков составила 10,8%. Генетический материал вируса клещевого энцефалита в пробах обнаружен не был. Общая инфицированность иксодовых клещей составляла 22,7%.

Ключевые слова: Белорусское Поозерье, иксодовые клещи, трансмиссивные инфекции, боррелиоз, анаплазмоз (эрлихиоз), babesиоз

Для цитирования: Осмоловский А. А., Субботина И. А. Роль иксодовых клещей в распространении и циркуляции возбудителей клещевых инфекций и инвазий на территории Белорусского Поозерья. *Ветеринария сегодня*. 2024; 13 (1): 78–86. <https://doi.org/10.29326/2304-196X-2024-13-1-78-86>

Конфликт интересов: Авторы заявляют об отсутствии конфликта финансовых/нефинансовых интересов, связанных с написанием статьи.

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INTRODUCTION

In the last decade, there has been a steady trend towards increasing the incidence of tick-borne infections in animals and humans, expanding their nosoareals, detecting mixed infections, as well as registering previously unknown pathogens and new nosological disease forms in Europe, including the Republic of Belarus, and in Asia [1, 2, 3, 4, 5].

So far, in the Republic of Belarus, almost all attention has been focused on only two tick-borne infections – viral tick-borne encephalitis and Lyme borreliosis. In fact, the reports on vector-borne diseases non-typical for Belarus – anaplasmosis, tularemia, tick-borne rickettsiosis, have become more often and Crimean hemorrhagic fever, monocytic ehrlichiosis, etc. have become spread intensively in neighboring countries [6]. Detection of human granulocytic anaplasmosis in some countries bordering the Republic is of the greatest interest for practical health-care. Antibodies to anaplasmas were most often detected in sera from chronic patients with neurological disorders who had a history of tick bite (18.2%), as well as from patients with Lyme borreliosis (13.5%) [1]. Therefore, the attention of epidemiologists and infectious disease specialists has been recently focused on tick-borne mixed infections.

To date, it has been reliably proven that infection of the tick with 2–3 viral as well as bacterial and/or protozoal pathogens is not an exception, but a pattern [7]. According to the literature, the mixed infection can reach 36% in the structure of tick-borne infections in endemic territories [8]. Moreover, up to 5% of ixodid ticks harbor Lyme borreliosis and tick-borne encephalitis agents [9]. Clinical and serological tests have shown that tick-borne encephalitis can develop both in the form of mono- and more severe mixed tick-borne viral and bacterial diseases in individuals who have reported tick bites [10, 11]. Therefore, any disease caused by tick bite should be considered as a potential mixed infection [6].

Analysis of the available literature showed that the largest number of ecological and epizootiological studies of ixodid ticks were carried out in the central and southern regions of Belarus (Gomel, Brest, Minsk Oblasts and city of Minsk); there are also some data for the Grodno and

Mogilev Oblasts but sporadic study data – for the Vitebsk region (Belarusian Lakeland) [12].

Geographically, the Belarusian Lakeland is a lake plateau in the north of the country, forming the southeastern part of the Baltic Uplands between the middle part of the Western Dvina River and the middle part of the Neman River basin, and occupies about 19% of the territory of Belarus. The major (larger) part of the Lakeland is located in the Vitebsk Oblast territory, and its small parts are located in the Minsk and Grodno Oblasts [13]. The Lakeland was named after large number of lakes (over 3,000) located in this area. The lakes form the following very picturesque landscape groups: Braslavskaya, Narochanskaya, Lepelskaya, Glubokskaya.

The Lakeland climate is the coldest and wettest in Belarus. The area is a part of the northern moderately warm humid agro-climatic zone.

Forests are the basic natural vegetation in the Lakeland. They occupy about 40% of the territory, which corresponds to the national average. The most wooded (up to 50%) areas are sandy lowlands, covered by coniferous forests that are often marshy. Coniferous species (mainly pine) predominate in the forests, their proportion reaches 60%. The highest proportion of spruce forests (17%) in the Republic is a peculiarity of its vegetation. There are very few oak forests (less than 2%), and other broad-leaved species are almost not found. Birch, alder and aspen forests predominate among small-leaved forests [14].

Meadows occupy a very small area. Drylands predominate among them, and there are significantly fewer lowland and floodplain meadows. The meadows are heavily overgrown.

There are few marshes in the Lakeland, but in some places they form large massifs (Yelnya, Obolsky marshes, Belmont). The widespread upper sphagnum and transitional bogs are a distinctive features of the land.

Thus, all above-described natural and geographical features of the Belarusian Lakeland are similar to subtaiga landscapes that creates unique conditions for ixodid tick habitats.

The study was aimed at analysis of the ecological, epizootiological and epidemiological role of ixodid ticks in tick-borne pathogen occurrence and circulation in the Belarusian Lakeland.

MATERIALS AND METHODS

The numbers and species of sexually mature ixodid ticks were recorded from April to November 2022. Due to the peculiarities of daily activity of ixodid ticks, the recording was performed during the period of maximum ixodid tick activity: on clear days – in the morning, from the moment the dew dries up to the onset of daytime heat, and in the evening after the heat decreases until dusk or evening temperature drop; on cloudy days without midday heat – during the day. At the same time, the night air temperature should have been at least 8 °C [15].

Adult (imago) and nymph ticks were collected in publicly accessible natural environment in the following Vitebsk region territories: Tulovsky botanical reserve, Tulovo agri-settlement (a/s); park named after Soviet Army; beach of “Burevestnik” children’s health camp and territories adjacent to it, s. Zui; Pridvinye biological reserve, s. Shevino; Luzhesnyansky dendrological park, s. Luzhesno; “Chertova Boroda” botanical reserve; Ruba Ski Centre territory; forest area near s. Sokolniki. All territories had subtaiga landscapes.

The coordinates of the examined territories were determined using satellite navigators (GLONASS/GPS receivers) in the global positioning system.

In open (clearings, lawns, glades) and forest areas with tall grass and shrubs, flag made of plain light fleecy fabric (waffle, flannel) was used for tick collection. A piece of fabric (60 × 100 cm) is attached with its narrow side to a stick and dragged through vegetation in an unfolded form; the person dragged the flag in front of or at the side of himself/herself and periodically inspected it.

The route length was calculated based on tracked 10-meter distance intervals, the 10-meter distance interval was predetermined by counting double steps. Stops was made to take notes and to inspect own clothes after completion of each 10-meter distance interval.

The length of the observation route (1 flag-km of natural biotope) was the main unit for the tick population estimation.

A total of 8 routes were tracked, 18 flag-km were passed, 529 tick specimens were collected, including 350 imago ticks and 179 nymph ticks.

The ixodid tick genus and species were determined using N. A. Filippova’s ixodid tick determinator¹. Tick species were identified *in vivo* with binocular microscope (16×).

Collected ticks were placed in glass tubes with cotton-gauze plug or in plastic tubes with screw caps. Each tube was labeled. The following information was indicated in the label: collection place and time, tick species, sex, life stage and feeding level^{2,3}.

Caught ticks were tested for animal and human disease agents by detection of disease agent genetic materials with real-time polymerase chain reaction (PCR) using thermocycler. The specimens were grouped in accordance

with MG 3.1.1027-01 “Collection, recording and preparation for laboratory tests of blood-sucking arthropods being vectors of natural focal infections”⁴, therewith, one tick was considered as one specimen. Genetic materials were extracted from the specimens with the reagent kit for nucleic acid extraction from environmental objects in accordance with the manufacturer’s instructions.

Differences in mean values were assessed for their statistical significance with Student’s t-test (t).

RESULTS AND DISCUSSION

A total of 529 tick specimens, including 350 imago ticks (66.2%) and 179 (33.8%) nymph ticks were collected in publicly accessible natural environment along the designated routes (Table 1). Therewith, nymph ticks were caught along three out of eight routes.

Species and sex composition of collected ixodid ticks is given in Table 2. Tick frequency index was calculated based on the data given in Table 2: number of specimens in which ticks of particular species were detected expressed as percentage of total number of tested specimens.

It was found that epidemiologically and epizootologically significant tick species in the Belarusian Lakeland (Vitebsk Raion), responsible for the spread of tick-borne infections and invasions, were represented by ticks of *Ixodes* and *Dermacentor* genera, that was generally consistent with other studies. Thus, the frequency index for *Ixodes* ticks was 70.1%, and for *Dermacentor* ticks was only 29.9%.

Ixodes ricinus (European forest, castor bean, sheep tick) is a temporary ectoparasite with long feeding period and “ambush” technique for attack.

Adult ticks in fasting period are absolutely flat, have a small size, 3–4 mm only, and fasting nymph ticks are about 2 mm in size. During feeding the ticks enlarge significantly in size, especially females (hundreds of times), and their bodies become of ovoid shape. The body of the adult tick is a leathery sac, harder on the back (scutum); ticks lack eyes and wings, but have 4 pairs of legs. The scutum completely covers the body of male ticks, while in female ticks it covers only the anterior third of the body. Females are larger than males and of reddish color; they change their color to gray in engorged state. Nymphs are of light-brown color and change their color to grayish-brown in engorged state. Non-continuous poor-developed marginal groove is a distinctive phenotypic feature of *Ixodes ricinus*.

All active phases of the tick life cycle (egg development and molting) take place in the forest litter.

Ixodes ricinus ticks live in sparse splashing sites and young small-leaved forests (birch, aspen, alder), where there are clearings with high herbage, and the soil is covered with thick leaf layer. Ticks also like to settle in willow thickets forming along melioration ditches running between fields.

Ixodes ricinus is a carrier of *Tick-borne encephalitis virus* of the European genotype, less pathogenic to humans, *Borrelia burgdorferi sensu lato* complex (ixodid tick-borne borreliosis, or Lyme disease), *Anaplasma phagocytophilum* (granulocytic anaplasmosis), *Babesia* spp. (canine piroplasmosis, babesiosis of cattle) [16].

¹ Filippova N. A. Fauna of the USSR. New edition, No. 114. Arachnids. Vol. 4. Issue 4. Ixodid ticks of *Ixodinae* subfamily. Leningrad: Nauka; 1977. 396 p.

² MG 3.1.3012-12 Collection, recording and preparation for laboratory tests of blood-sucking arthropods in natural foci of dangerous infectious diseases: Methodical Guidelines. Moscow: Federal Centre of Hygiene and Epidemiology of the Federal Service for the Oversight of Consumer Protection and Welfare of the Russian Federation; 2012. 55 p.

³ Domonova E. A., Tvorogova M. G., Podkolzin A. T., Shipulina O. Yu., Karan L. S., Yatsyshina S. B. et al. Collection, transportation and storage of biological material for PCR diagnostics: Methodical Guidelines. Moscow: Central Research Institute for Epidemiology; 2021. 112 p.

⁴ Collection, recording and preparation for laboratory tests of blood-sucking arthropods being vectors of natural focal infections: Methodical Guidelines. Moscow: Federal Centre for State Epidemiological Surveillance of the Ministry of Health of the Russian Federation; 2002. 55 p.

Table 1
Absolute numbers of ixodid ticks in examined natural biocenoses

| Examined territories | Number of imago ticks, absolute units | Number of nymph ticks, absolute units |
|--|---------------------------------------|---------------------------------------|
| Tulovsky botanical reserve, Tulovo agrisettlement (a/s) | 31 | – |
| Park named after Soviet Army | 32 | – |
| Beach of “Burevestnik” children’s health camp and territories adjacent to it, s. Zui | 11 | – |
| Pridvinye biological reserve, s. Shevino | 47 | – |
| Luzhesnyansky dendrological park, s. Luzhesno | 78 | – |
| “Chertova Boroda” botanical reserve | 26 | 39 |
| Ruba Ski Centre territory | 48 | 8 |
| Forest area near s. Sokolniki | 77 | 132 |

Table 2
Species and sex composition of ixodid ticks in the examined natural biocenoses, absolute units

| Examined territory | <i>Dermacentor</i> | | | <i>Ixodes</i> | | |
|--|--------------------|----|--------|---------------|----|--------|
| | ♀ | ♂ | nymphs | ♀ | ♂ | nymphs |
| Tulovsky botanical reserve, Tulovo agrisettlement (a/s) | 18 | 12 | – | 1 | – | – |
| Park named after Soviet Army | 22 | 9 | – | 1 | – | – |
| Beach of “Burevestnik” children’s health camp and territories adjacent to it, s. Zui | 2 | 1 | – | 3 | 5 | – |
| Pridvinye biological reserve, s. Shevino | 23 | 20 | – | 2 | 2 | – |
| Luzhesnyansky dendrological park, s. Luzhesno | 4 | 1 | – | 34 | 39 | – |
| “Chertova Boroda” botanical reserve | 4 | 2 | 8 | 18 | 2 | 31 |
| Ruba Ski Centre territory | 5 | 3 | 2 | 32 | 8 | 6 |
| Forest area near s. Sokolniki | 4 | 8 | 10 | 56 | 9 | 122 |
| Total, <i>n</i> = 529 | 82 | 56 | 20 | 147 | 65 | 159 |

Dermacentor reticulatus (meadow tick) can live in open spaces in leafy and mixed forests – forest clearings, forest edges, but prefer meadows and pastures with dense tall grass, can survive under water during flooding, live in flood meadows, can be found on lawns, prefer wet places – ravines, road ditches, springs. So, the tick mass reproduction foci can form in livestock grazing areas [17]. The ticks are highly cold-resistant. The activity of meadow ticks starts almost a month earlier than that one of forest ticks – in April – May.

Ticks of *Dermacentor* genus have special features: all phases of the tick life cycle take place within one year, and imago ticks can survive two to three winter periods without feeding [18].

The characteristic morphological features of *Dermacentor* genus are light enamel pigments in the form of spots of various shapes and sizes, most apparent on the dorsal scutum and less apparent on the legs and proboscis. The shape of enamel spots and their number vary greatly. The male tick is of light color with dark markings. In female tick, only scutum is of light color, but female tick body is dark-brown [19].

Meadow tick is a vector of thick-borne encephalitis, borreliosis, babesiosis (piroplasmosis), anaplasmosis, ehrlichiosis, rickettsiosis, tularemia, listeriosis, erysipeloid, Q fever, tick typhus, Omsk hemorrhagic fever [18, 19].

All collected ticks were tested for genetic materials of *Borrelia* spp., *Anaplasma* spp. (*Ehrlichia* spp.), *Babesia* spp. and *Tick-borne encephalitis virus* with real-time PCR.

It was found that 120 (22.7%) out of 529 ticks were carriers of infectious animal and human disease agents (carrier ticks) and 409 (77.3%) ticks were provisionally “clean” ticks since the range of detectable DNA markers was limited. Therewith, *Borrelia* spp. DNA was detected in the largest number of the carrier ticks: 74 (61.7%) out of 120 ticks; *Anaplasma* spp. (*Ehrlichia* spp.) DNA was detected in 31 (25.8%) out of 120 carrier ticks and *Babesia* spp. DNA was detected in 30 (25.0%) out of 120 carrier ticks (Fig. 1).

Ixodid tick-borne borreliosis is one of the critical health concerns associated with transmissible natural focal infections in many countries, including the Republic of Belarus, in the view of its ever-increasing social, medical and economic importance [20, 21, 22]. In general, borreliosis is a pronounced transmissible tick-borne human and animal disease. For example, according to literature data [23], in Russia the proportion of ixodid tick-borne borreliosis amounts to about 30% out of the total number of natural focal infections and amounts to more than 55% out of infections caused by the ixodid tick-transmitted agents. Other studies results have shown that spontaneous bacteriophoricity (infection of ticks with *Borrelia*) of ixodid ticks in natural foci can reach 70% and even 90% [24].

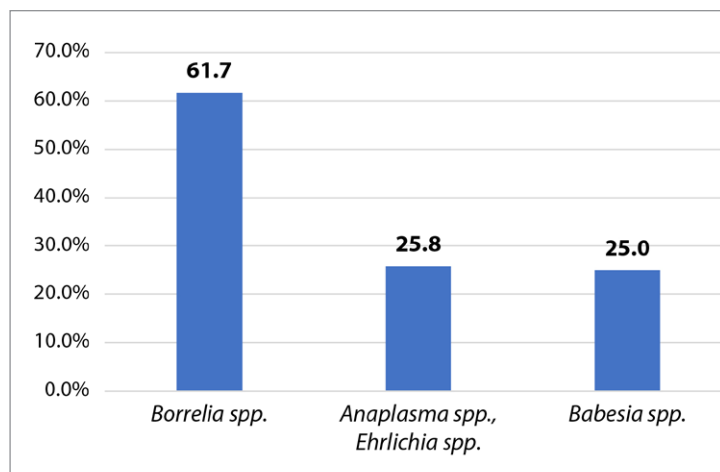


Fig. 1. Detection of tick-borne pathogen DNAs in ixodid ticks-carriers

The natural foci of ixodid tick-borne borreliosis directly depend on natural landscapes: there are especially many of them in mixed forests located in the moderately humid climatic zone, that is highly consistent with that one in the Vitebsk region. Many infected wild and domestic vertebrate species (rodents, deer, moose, goats, cows, dogs, horses, etc.) as well as birds transmitting infected ixodid ticks over long distances during migratory flights serve as *Borrelia* reservoirs and carriers.

According to the data obtained by staff-members of the Group for Arbovirus Infections of the Republican Research and Practical Center for Epidemiology and Microbiology monitoring the transmissible infection pathogen circulation dynamics in the Republic of Belarus, tick bacteriophoricity in natural environment has increased in the Republic on average from 12.4 to 19.1% over the last decade.

Anaplasmas are agents of transmissible seasonal diseases of cattle and humans.

So far, anaplasmas have been known only as animal disease agents, and anaplasmoses have been of veterinary interest, but currently humans are proven to be also susceptible to the infection [25]. PCR amplification method has made it possible to make significant amendments to anaplasmas taxonomy that greatly facilitates the disease diagnosis.

Sick animals and anaplasma carriers are the source of the invasion. The pathogen is transmitted by 11 ixodid tick species, one Argas tick species and insects. Besides cattle, reindeer, zebu, buffaloes, moose, roe deer and antelopes are susceptible to *Anaplasma marginale* [26].

Anaplasmosis is widespread almost everywhere. The disease is usually reported in spring, summer and autumn. However, the disease cases can be reported in winter in case of poor animal management and feeding, as well as violations of aseptic and antiseptic rules during veterinary and zootechnical manipulations (blood collection with one needle, tagging, etc.). Anaplasmosis is often reported in the form of mixed invasion with piroplasmidoses [27].

According to veterinary reports, the Bryansk, Pskov Oblasts bordering to the Republic of Belarus, as well as Rязan, Kaliningrad, Vladimir Oblast and other regions of the Russian Federation are anaplasmosis-affected [5, 11]. Mass infection and disease in livestock animals result in deaths, reduced growth in young animals and reduced milk yields

in cows, inefficient feed conversion, increased costs due to expensive treatment, extensive testings and measures to be taken to combat the disease. All this causes huge economic losses.

Ehrlichiosis as well as anaplasmosis is caused by rickettsia-like bacteria and transmitted by ticks. Both humans and animals (dogs, cats, wild canine and feline animals, etc.) are affected by ehrlichiosis. Different tropism to blood cells is a distinctive feature of these tick-borne rickettsioses. Thus, *Anaplasma* affect leukocyte granulocytes (mainly neutrophils) and *Ehrlichia* have a tropism for monocytes, that was the reason to name the diseases transmitted by them as human granulocytic anaplasmosis and human monocytic ehrlichiosis, respectively. Both anaplasmosis and ehrlichiosis are new infections for the Republic of Belarus and require more detailed study. According to the scientific data published by the Republican Research and Practical Center for Epidemiology and Microbiology, the proportion of human granulocytic anaplasmosis cases among 64 patients with tick-borne infections was 23.4%, and monocytic ehrlichiosis cases in humans were sporadic [1].

Babesiosis (piroplasmosis) is a natural focal ixodid tick-borne disease caused by hemoprotozoan parasites of *Babesia* genus [28]. Ixodid ticks of *Rhipicephalus*, *Dermacentor*, *Hyalomma*, *Ixodes ricinus*, *Haemaphysalis leachi* genera, as well as *Argas* ticks are main vectors of the infection and main hosts of the parasite [29]. Cattle and canine animals are mainly affected. In humans, the disease is caused by three *Babesia* species: *Babesia microti* – in America, *Babesia divergens* and *Babesia rodhaini* – in Europe. More than 100 cases of human babesiosis have been described in the literature, the most of them were fatal [30].

Western tick-borne encephalitis is a disease caused by arbovirus, the life cycle of which requires *Ixodes ricinus* ticks as a participant of the virus circulation and as a reservoir of the virus in nature. Tick-borne encephalitis has been studied in Belarus since the early 40s of the last century, when its natural foci were identified in the Belovezhskaya Pushcha territory, and the virus was isolated from *Ixodes ricinus* ticks [12].

According to the Republican Research and Practical Center for Epidemiology and Microbiology, the number of *Ixodes ricinus* ticks infected with *Tick-borne encephalitis virus* has been slowly but steadily increased with periods of stabilization in the Republic of Belarus over the past decade [20].

It should be noted that none of the parasites collected during this study had the *Tick-borne encephalitis virus* genetic markers. At the same time, it is believed that tick-borne encephalitis remains the priority tick infection for the Polesie region, where as in the Republic as a whole, number of ixodid ticks in natural biotopes steadily increases, number of the ticks naturally infected with various pathogens also increases, and infected vector habitats expand [12].

Many cases of mixed infections (from 18 to 32%) in ixodid ticks have been described in the literature [1, 2, 4, 10, 15]; but in our study, only 13 out of 120 ticks were found to be infected with several pathogens (Fig. 2). While, more than two pathogens were detected in two specimens only.

Detection of several pathogens in one tick makes the tick-borne mixed infections an important and priority problem for the Republic of Belarus and requires comprehensive study.

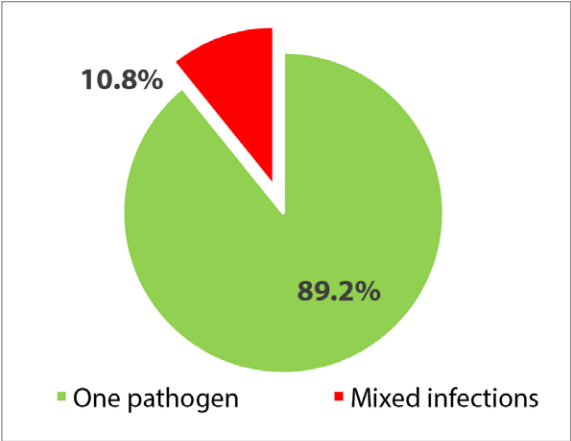


Fig. 2. Mixed infections in examined ticks

The study showed that the largest number of infected ticks (38.5%) was collected on the territory of the “Chertova Boroda” botanic reserve, the smallest number of infected ticks (9.1% at $p = 0.00015$) was collected on the beach of “Burevestnik” children’s health camp and in the territories adjacent to it (s. Zui). The proportion of infected

parasites was approximately the same in other examined publicly accessible natural environment areas and ranged from 15.6 to 21.5% (Fig. 3).

The results of tests of ixodid ticks-carriers collected in the territory of “Chertova Boroda” botanic reserve and on the beach of “Burevestnik” children’s health camp and in the territories adjacent to it (s. Zui) for DNAs of transmissible tick infection pathogens are shown in Figure 4.

Detected differences in ixodid tick numbers and tick-borne pathogen genetic marker occurrence correlate with the ecological characteristics of the examined territories.

The “Chertova Boroda” botanical reserve is a locally important nature reserve located in the Chretova Boroda stow to the south-west of Vitebsk, to west of the Orekhovo microraion, on the right bank of the Western Dvina River. Its terrain is hilly with wet ravines and characteristic mixed-forest landscape with many shrubby thickets and grass deadwood. The territory is ideal for a permanent habitat for ixodid ticks.

The route ran through the territory belonging to “Burevestnik” children’s health camp, mixed forest, settlement of Zui (Vitebsk Raion), 6 km from Vitebsk. The landscape is predominantly mixed forest with meadows located closer to the northeastern part of the camp and to the Laucesa

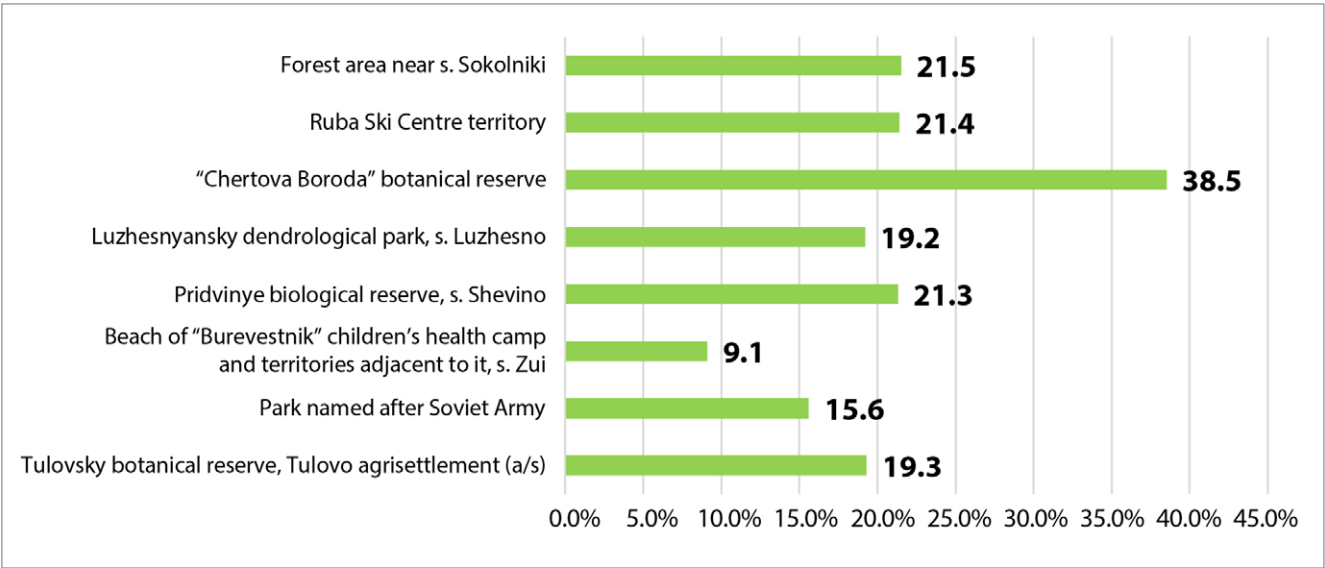


Fig. 3. Proportion of infected ticks in the examined territories

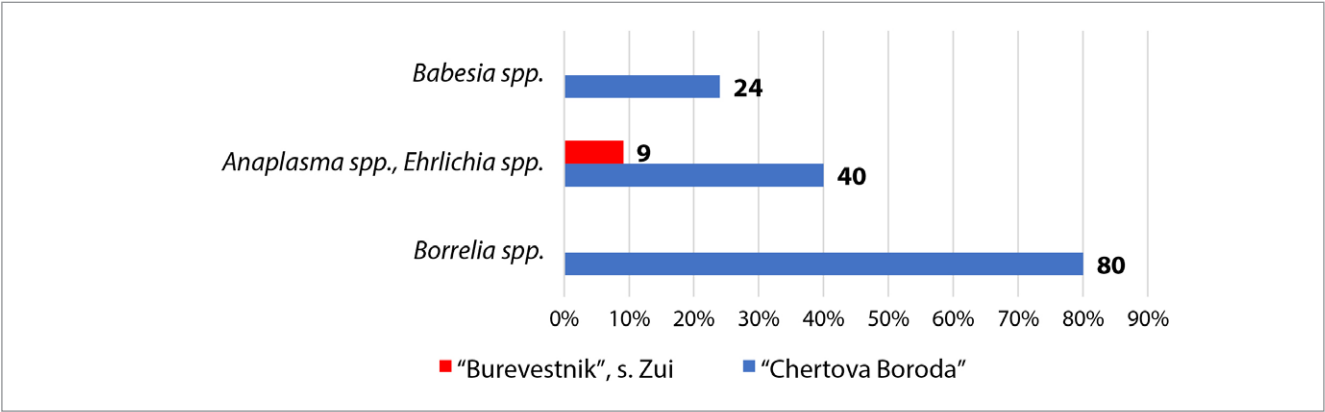


Fig. 4. Results of tests for detection of tick-borne pathogen DNAs in ixodid ticks-carriers in the “Chertova Boroda” botanic reserve as well as on the beach of “Burevestnik” children’s health camp and in the territories adjacent to it (s. Zui)

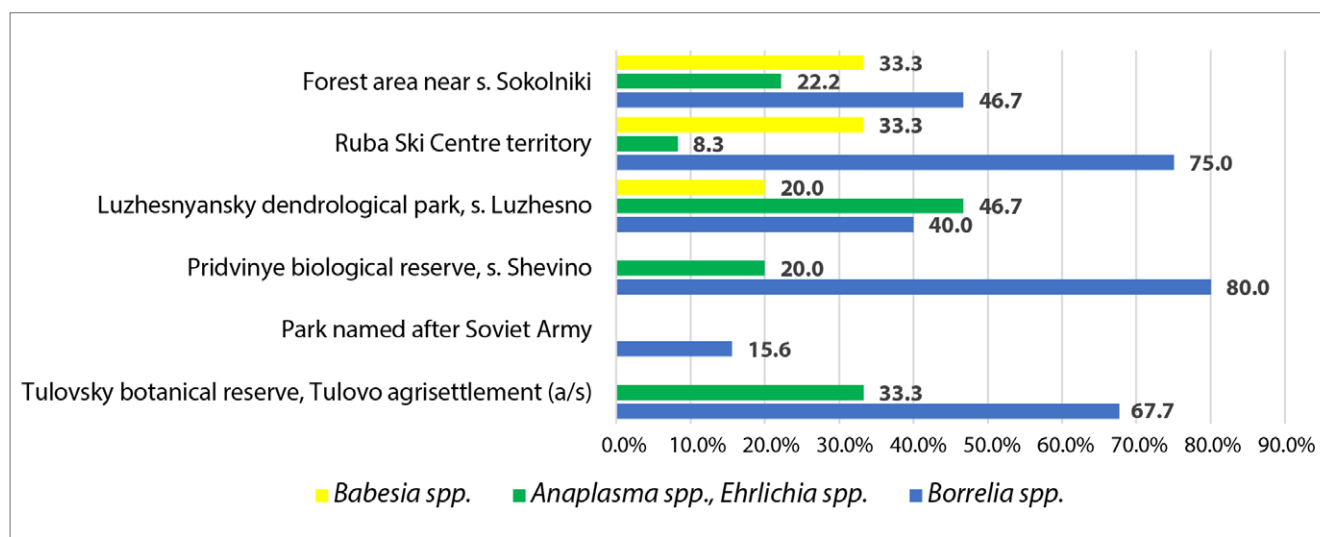


Fig. 5. Detection of tick-borne pathogen DNAs in ixodid ticks-carriers in other six examined territories

River shore. The small number of collected ticks can be accounted for regular measures for destruction of ectoparasites and larvae thereof taken in the territories adjacent to the camp.

The results of tests of ixodid ticks-carriers collected in six other examined territories for DNAs of transmissible tick infection pathogens are shown in Figure 5.

The range of transmissible infection pathogens carried by ixodid ticks was found to be different in examined territories.

Thus, only *Borrelia* spp. DNA was detected in carrier ticks collected in the territory of the Park named after Soviet Army; only *Borrelia* spp. and *Anaplasma* spp. (*Ehrlichia* spp.) DNAs were detected in the ticks collected from the vegetation in the Pridvinye biological reserve (settlement of Shevino) and the Tulovsky botanical reserve (Tulovo agrisettlement). Whereas, *Borrelia* spp. and *Anaplasma* spp. (*Ehrlichia* spp.) as well as *Babesia* spp. genetic materials were detected in the carrier ticks collected along the routes running through the Luzhesnyansky dendrological park (s. Luzhesno), Ruba Ski Centre territory and the forest area near s. Sokolniki.

It is important to note that *Borrelia* spp. was the pathogen most often detected in carrier ticks collected along all routes (15.6 to 80% bacteriophoricity). Thus, tick-borne borreliosis is the predominant transmissible infection in the territory of the Belarusian Lakeland.

CONCLUSION

It was found that epidemiologically and epizootologically significant tick species in the Belarusian Lakeland responsible for spread of transmissible infections and invasions were represented by ticks of *Ixodes* and *Dermacentor* genera, their frequency index was 70.1 and 29.9%, respectively.

It was shown that 22.7% of ixodid ticks in the Belarusian Lakeland were potential carriers of the following human and animal infectious diseases: tick-borne borreliosis, anaplasmosis (ehrlichiosis), babesiosis.

No Tick-borne encephalitis virus genetic materials were found in the specimens tested during the study.

The range of infectious disease agents transmitted by ixodid ticks in the Belarusian Lakeland (Vitebsk region)

was found to be as follows: *Borrelia* spp. were detected in 61.7% of tested ticks, *Anaplasma* spp. (*Ehrlichia* spp.) – in 25.8% of tested ticks and *Babesia* spp. – in 25% of tested ticks. Therewith, the proportion of mixed infected vectors was 10.8%.

Numbers of ixodid ticks and presence of tick-borne pathogen genetic markers in them were found to correlate with the ecological features of the examined territories.

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Received 11.12.2023

Revised 15.01.2024

Accepted 31.01.2024

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