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Analysis of the species composition and infection of ticks with pathogens of Lyme disease in the territory of the Republic of Tatarstan for the period 2012–2022

Irina A. Karpova[1, 2], Gulshat R. Khasanova[1], Luciya F. Sadreeva[2], Yurij A. Tyurin[1, 3], Nail N. Shaykhullin[2]

[1] Kazan State Medical University, Kazan, Russia;
[2] Hygienic and Epidemiological Center in Republic of Tatarstan (Tatarstan), Kazan, Russia;
[3] Kazan Research Institute of Epidemiology and Microbiology, Kazan, Russia

ABSTRACT

BACKGROUND: The role of ixodid ticks in the transmission of pathogens of a number of natural focal infections can hardly be overestimated. Analysis of the manifestations of the epizootic process is important for planning preventive and anti-epidemic measures and is an integral component of epidemiological surveillance of natural focal infections.

AIM: To analyze the species composition of ticks and the infection of ticks with borreliosis pathogens in the Republic of Tatarstan for the period 2012–2022.

MATERIALS AND METHODS: An analysis of the species composition of ticks collected by the zoological group of the Center for Hygiene and Epidemiology in the Republic of Tatarstan from environmental objects during the period 2012–2022 was carried out. Testing of the ticks for *Borrelia burgdorferi* and *Borrelia miyamotoi* was carried out using the polymerase chain reaction method with hybridization-fluorescent detection. An analysis of statistical observation form No. 2 "Information on infectious and parasitic diseases" (the section "Tick bites") for the Republic of Tatarstan from 2012 to 2022 was carried out. Statistical processing was performed using descriptive statistics methods in Microsoft Excel.

RESULTS: The total number of people who applied for tick bites in the Republic of Tatarstan in 2012–2022 was 77,703, of which 59,168 (76.2%) were people over 18 years of age. During the study period, 5536 ticks were collected for research on the territory of the Republic of Tatarstan from environmental objects. The species composition of ticks was distributed as follows: *Dermacentor reticulatus* — 75.4%, *Ixodes ricinus* — 14.1%, *Ixodes persulcatus* — 10.5%. Infection of different types of ticks with Borrelia was not the same and was 20.4% for *I. persulcatus*, 14% for *I. ricinus,* 4.4% for *D. reticulatus*. In 4 regions of the republic, from ticks of the species *I. ricinus* pathogenic genomospecies *B. miyamotoi* were found (the infestation of this type of tick with *B. miyamotoi* was 0.4%). Indicators of morbidity of the population with ixodid tick-borne borreliosis and indicators of infection rates for ticks vary in different regions of the republic.

CONCLUSIONS: The dominant species of ixodid ticks in natural epitopes of the Republic of Tatarstan in 2012–2022 were *D. reticulatus* (75.4% of the total number of collected ticks). For ticks of this species, the lowest level of infection with Borrelia was demonstrated (4.4% of ticks). This may be one of the factors responsible for the low, in comparison with other regions, incidence rates of tick-borreliosis in the Republic of Tatarstan. The identification of ticks of the species *I. ricinus* infected with *B. miyamotoi* confirms the need for targeted molecular genetic and serological examination for recurrent fever in patients with non-erythematous forms of febrile illness after an episode of tick bite.

Keywords: Lyme disease; Borrelia infections; tick bites; epidemiology; ticks.

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Анализ видового состава и заражённости клещей возбудителями иксодовых клещевых боррелиозов на территории Республики Татарстан за период 2012–2022 годов

И.А. Карпова[1, 2], Г.Р. Хасанова[1], Л.Ф. Садреева[2], Ю.А. Тюрин[1, 3], Н.Н. Шайхуллин[2]

[1] Казанский государственный медицинский университет, Казань, Россия;
[2] Центр гигиены и эпидемиологии в Республике Татарстан (Татарстан), Казань, Россия;
[3] Казанский научно-исследовательский институт эпидемиологии и микробиологии, Казань, Россия

АННОТАЦИЯ

Обоснование. Роль иксодовых клещей в передаче возбудителей целого ряда природно-очаговых инфекций трудно переоценить. Анализ проявлений эпизоотического процесса важен для планирования профилактических и противоэпидемических мероприятий и выступает неотъемлемым компонентом эпидемиологического надзора за природно-очаговыми инфекциями.

Цель исследования — провести анализ видового состава и заражённости клещей возбудителями иксодовых клещевых боррелиозов на территории Республики Татарстан за период 2012–2022 годов.

Материалы и методы. Проведён анализ видового состава клещей, собранных зоологической группой ФБУЗ «Центр гигиены и эпидемиологии в Республике Татарстан» с объектов окружающей среды в течение 2012–2022 годов. Тестирование исследуемого материала на *Borrelia burgdorferi* и *Borrelia miyamotoi* осуществлено методом полимеразной цепной реакции с гибридизационно-флуоресцентной детекцией. Проанализирована форма статистического наблюдения № 2 «Сведения об инфекционных и паразитарных заболеваниях» по Республике Татарстан с 2012 по 2022 год в части «Укусы клещами». Статистическая обработка выполнена с использованием методов описательной статистики в программе Microsoft Excel.

Результаты. Общее число людей, обратившихся по поводу укусов клещей в Республике Татарстан в 2012–2022 годах, составило 77 703, из них 59 168 (76,2%) — лица старше 18 лет, остальные — дети. За исследуемый период на территории Республики Татарстан с объектов окружающей среды было собрано для исследования 5536 особей клещей. Видовой состав клещей распределился следующим образом: *Dermacentor reticulatus* — 75,4%, *Ixodes ricinus* — 14,1%, *Ixodes persulcatus* — 10,5%. Боррелиофорность различных видов клещей не была одинаковой и составила 20,4% для *I. persulcatus*, 14% — для *I. ricinus*, 4,4% — для *D. reticulatus*. В 4 районах республики в клещах вида *I. ricinus* были обнаружены патогенные геномовиды *B. miyamotoi* (заражённость этого вида клещей *B. miyamotoi* составила 0,4%). Показатели заболеваемости населения иксодовым клещевым боррелиозом и показатели боррелиофорности клещей варьируют в разных районах республики.

Заключение. Доминирующим в природных эпитопах Республики Татарстан видом иксодовых клещей в 2012–2022 годах явился *D. reticulatus*, на долю которого пришлось 75,4% собранных особей. Для клещей этого вида продемонстрирован наименьший уровень боррелиофорности (4,4% клещей), что может быть одним из факторов, ответственных за невысокие, в сравнении с другими регионами, показатели заболеваемости иксодовым клещевым боррелиозом в Республике Татарстан. Выявление клещей вида *I. ricinus*, поражённых *B. miyamotoi*, подтверждает необходимость прицельного молекулярно-генетического и серологического обследования на предмет возвратной лихорадки пациентов с безэритемной формой лихорадки после эпизода укуса клеща.

Ключевые слова: болезнь Лайма; боррелиозы; укусы клещей; эпидемиология; клещи.

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ЭКО•ВЕКТОР

BACKGROUND

Ixodic tick-borne borreliosis (ITB, Lyme disease) is the leading natural focal bacterial zoonosis registered in Russia. Russia is the habitat of most of the global ITB range, which is confirmed by the annual registration of disease cases in more than 70 entities of the country. High ITB incidence is consistently registered in the Siberian, Ural, Volga, and Northwestern Federal Districts [1]. Additionally, the Republic of Tatarstan is endemic for ITB. Monitoring of the immunity stress of the population of cities and districts of the Republic of Tatarstan to pathogens transmitted by ticks and mosquitoes showed that the proportion of people seropositive to ITB was 3.8% [1].

Most often, ITB occurs through ixodid tick bites. Additionally, Borrelia can be transmitted through tick feces after they get on the skin and are then rubbed in when scratching. Over 200 species of wild mammals are tick feeders in natural foci. Infection of people and animals commonly occurs from April to October, during the period of seasonal tick activity. *Ixodes ricinus* and *Ixodes persulcatus* are of the most epidemiological significance in the spread of ITB. These ticks are believed to be the most aggressive toward humans [2].

The epidemic of borreliosis depends on the activity of natural foci of infection. The accumulation of the Lyme disease pathogen in foci is influenced by various factors, including landscape transformation with subsequent changes in the population size of the main carriers and feeders and the state of the biocenosis of animals sensitive to the pathogen.

The susceptibility of individuals to infection is extremely high.

Analysis of the epidemic situation for ixodid tick-borne borreliosis in 2022 and the prognosis for 2023 in Russia, prepared by the Reference Center for Monitoring Borreliosis of the Omsk Research Institute of Natural Focal Infections of Rospotrebnadzor, revealed a significant association between the infection rates of ticks in natural stations and ticks removed from humans and the infection rate of the population; however, the correlations were detected only for *I. persulcatus*, but not for other types of ticks [3].

Moreover, analysis of the manifestations of the epizootic process is crucial for planning preventive and anti-epidemic measures and for the epidemiological surveillance of natural focal infections [3].

This study aimed to analyze the species composition and infection rate of ticks with pathogens of Lyme disease in the Republic of Tatarstan in 2012–2022.

MATERIALS AND METHODS

Research design

An analysis of biological material, namely, ticks collected by the zoological group of the Center for Hygiene and Epidemiology in the Republic of Tatarstan (Tatarstan) from environmental objects using a flannel flag using the standard method [4], was performed according to the schedule approved by the Office of Rospotrebnadzor in the Republic of Tatarstan (Tatarstan). Collection was performed in 2012–2022 (from April to October of each year).

Data on laboratory testing of ticks were evaluated in the testing laboratory center of the Center for Hygiene and Epidemiology in the Republic of Tatarstan (Tatarstan) and the Kazan Research Institute of Epidemiology and Microbiology of Rospotrebnadzor.

Ticks (*I. persulcatus*, *D. reticulatus*, and *I. ricinus*) were tested by polymerase chain reaction with hybridization-fluorescence detection using the AmpliSens TBEV, *B. burgdorferi* s.l., *A. phagocytophilum*, and E. chaffeensis/E. muris-Fl reagent kit (Russia) for *B. burgdorferi* and the RealBest DNA Borrelia miyamotoi reagent kit (Russia) for the pathogen *B. miyamotoi*. Sample preparation included the preparation of a tick suspension. In studying tick pools, 10 individuals or less were included in one sample. The ticks to be tested were placed in Eppendorf tubes, 500 µl of 96% ethanol was added, and the resulting suspension was mixed using Vortex technology. Then, 500 µl of 0.15 M sodium chloride solution was added to the test tube with ticks and vortexed again, and the remaining liquid was carefully removed using a vacuum aspirator. Tick suspensions were mechanically prepared from the resulting material. Then, 100 µl of supernatant was collected for RNA/DNA extraction from *Ixodes* ticks and 50 µl for RNA/DNA extraction from *Dermacentor* ticks. RNA/DNA was extracted using the RIBO-prep reagent kit (Russia).

The reporting forms for 2012–2022 "Review and prognosis of the state of populations and numbers of small mammals and arthropods — carriers and transmitters of pathogens of natural focal diseases, epizootological and epidemiological situation in the constituent entity of the Russian Federation" were analyzed and approved by the Order of the Federal Service for Surveillance on Consumer Rights Protection and Human Wellbeing (Rospotrebnadzor) dated January 14, 2013, no. 6, and prepared by the Center for Hygiene and Epidemiology in the Republic of Tatarstan (Tatarstan). Statistical processing was performed using Microsoft Excel.

Analysis of statistical observation form no. 2 "Information on infectious and parasitic diseases" in the Republic of Tatarstan from 2012 to 2022 in the part "Tick bites" was performed.

Study conditions

Ticks were collected from environmental objects by the zoological group of the Center for Hygiene and Epidemiology in the Republic of Tatarstan (Tatarstan) in the different municipalities of the republic. Laboratory testing of ticks was performed in the testing laboratory center of the Center for Hygiene and Epidemiology in the Republic of Tatarstan (Tatarstan) and Kazan Research Institute of Epidemiology and Microbiology of Rospotrebnadzor (Kazan).

Study duration

In 2012–2022, ticks were collected and subsequently subjected to laboratory testing. In 2023, laboratory and statistical data were analyzed.

Methods of statistical data analysis

Intensive and extensive incidence rates were calculated. Categorical data are presented as a share. Correlation analysis was used to assess the relationship between the indicators. Statistical processing was performed using Microsoft Excel.

RESULTS

Research objects

The materials of the study included the following:

- 5,536 ticks collected from environmental sites
- Reporting forms "Review and prognosis of the state of populations and numbers of small mammals and arthropods as carriers and transmitters of pathogens of natural focal diseases, epizootological and epidemiological situation in the constituent entity of the Russian Federation" for 2012–2022 approved by the Order of the Federal Service for Surveillance on Consumer Rights Protection and Human Wellbeing (Rospotrebnadzor) dated January 14, 2013, no. 6, and prepared by the Center for Hygiene and Epidemiology in the Republic of Tatarstan (Tatarstan)
- Statistical observation forms no. 2 "Information on infectious and parasitic diseases" for the Republic of Tatarstan from 2012 to 2022.

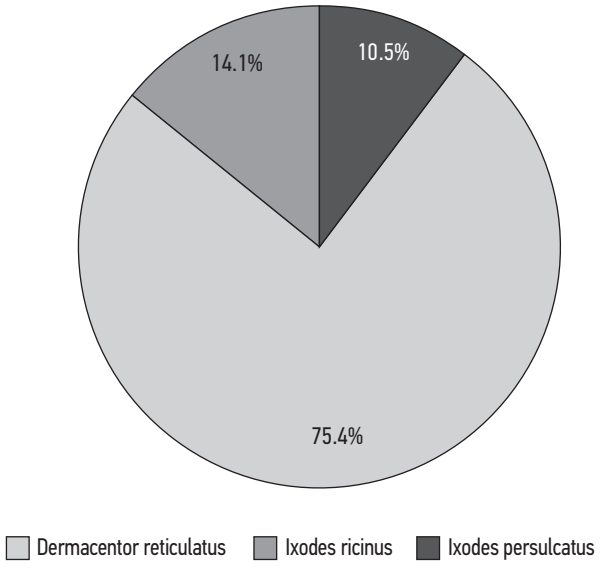

Fig. 1. Index of dominance of ticks of different types in natural biotopes of the Republic of Tatarstan in 2012–2022.

Main results of the study

The species composition of ticks was distributed as follows: *D. reticulatus*, 4173 individuals; *I. ricinus*, 780 individuals; and *I. persulcatus*, 583 individuals (Fig. 1).

During subsequent laboratory testing, pathogenic genomospecies *B. burgdorferi* s.l. were detected in biomaterials of 410 of 5,536 ticks examined; the proportion of affected ticks was 7.4% of the individuals collected. Moreover, the borreliosis carriage of ticks of different species was not the same and was 20.4% for *I. persulcatus*, 14% for *I. ricinus*, and 4.4% for *D. reticulatus*.

Additionally, pathogenic genomospecies *B. miyamotoi* were detected in ticks collected in four eastern and central regions of the republic (districts Novosheshminsky, Yelabuzhsky, Almetyevsky, and Sarmanovsky), that is, 0.68% of the collected individuals (in 14 of 2,288 samples).

The borreliosis carriage of ticks varies in different regions of the republic. In 15 districts, the indicators exceeding the republic average indices (7.4%) were noted, where the proportion of affected ticks ranged from 8.0% to 20.7% (Fig. 2). Exceeding the republic average indicators by two times or more was revealed in three districts of the Republic of Tatarstan, namely, Alekseevsky (20.7%), Vysokogorsky (19.3%), and Nurlatsky (18.8%). Moreover, the ticks collected in these municipal districts are largely represented by the genus *Ixodes*, including 33.3% of ticks collected in Alekseevsky, 42.5% in Vysokogorsky, and 33.0% in Nurlatsky. It was for this type of tick that the highest indicator of borreliosis carriage was revealed.

Unfortunately, laboratory tests of ticks for the presence of Borrelia were not conducted in ten districts of the Republic of Tatarstan during the study period, although in some of these districts, cases of ITB among the population are registered annually. Thus, the average long-term incidence rate for the study period was 0.93 per 100 thousand people in Arsky, 0.61 in Kaybitsky, 0.49 each in Apastovsky and Mamadyshsky, 0.36 each in Kukmorsky and Drozhanovsky, and 0.25 in Bavlinsky. It was established that in the districts where the incidence rates of ITB exceed the average long-term annual values (i.e., Agryzsky, Verkhneuslonsky, Zainsky, Laishevsky, Leninogorsky, and Tetyushsky) (Fig. 3), laboratory tests of ticks were not conducted at all or only ticks of the genus *Dermacentor* were tested, which are not main carriers of ITB. Thus, a connection between the incidence rates of the population and borreliosis carriage of ticks was not established. Notably, in some municipalities, cases of ITB were not registered during the study period (these areas are not presented in the diagram), which may be due to a number of reasons requiring detailed analysis.

The total number of people who sought medical attention for tick bites in 2012–2022 was 77,703, including 59,168 (76.2%) aged >18 years, and the rest were children. The highest number of bite patients was recorded in 2015, 2019, and 2022, which coincides with the peaks in ITB incidence in the population [5].

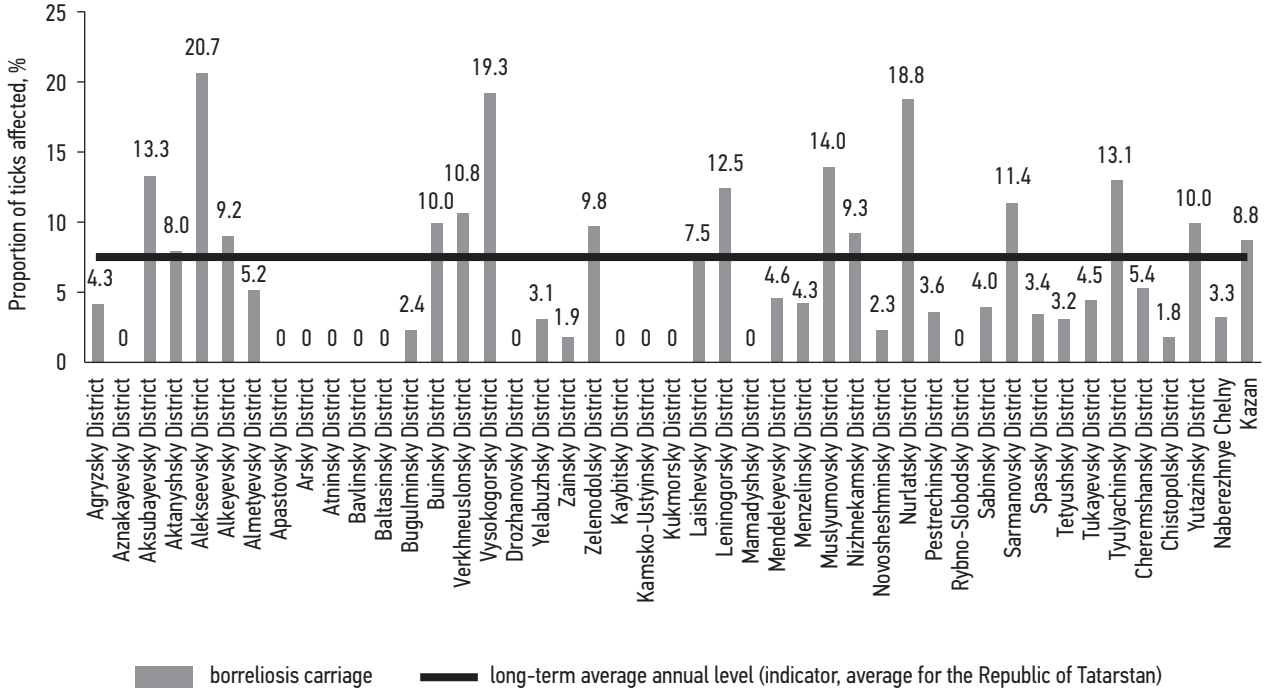

Fig. 2. Infection of ticks with *Borrelia* in municipal districts of the Republic of Tatarstan (2012–2022).

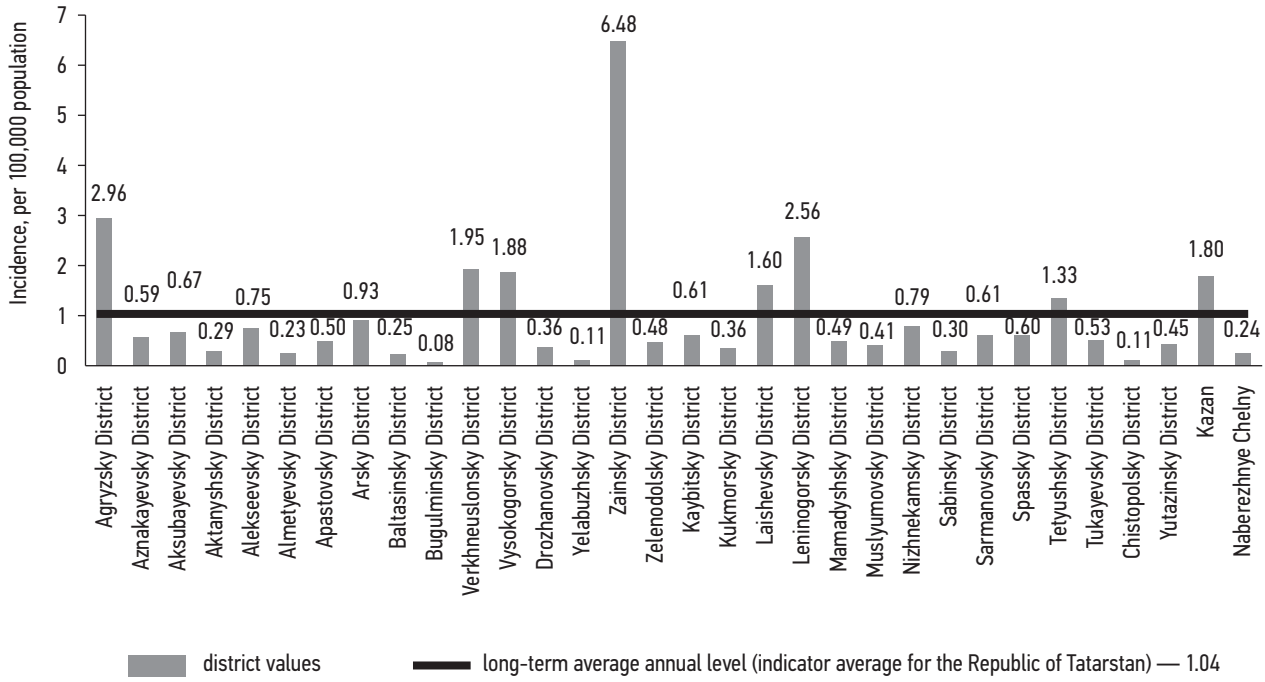

Fig. 3. Territorial distribution of borreliosis incidence rates for the period 2012–2022 in the Republic of Tatarstan (in terms of per 100 thousand population).

DISCUSSION

In contrast to tick-borne viral encephalitis, ITB incidence in Russia showed an upward trend, and the disease ranks first (38.3% in 2022) among the natural focal infections registered in the country [1]. The incidence rate in 2022 was 4.98 per 100 thousand population, exceeding the long-term average annual threshold of 2010–2019 by 3.4%. The highest rates were recorded in the Republic of Tyva and Tomsk and Sverdlovsk regions [1]. Despite the introduction of advanced laboratory diagnostic technologies and increasing information on transmissible infections, the

incidence of ITB and prevalence of chronic forms of the disease in many regions of Russia, including the Republic of Tatarstan, remain unclear. Only some infections are verified. According to expert estimates, the actual incidence exceeds the number of registered cases by five or more times [6, 7]. The epidemiological situation of ITB in the Republic of Tatarstan can be considered favorable compared to that in other regions of Russia. Nevertheless, the incidence is wave-like, with a significant increase in incidence rates over the past 3 years [5]. High-quality epidemiological surveillance of transmissible zoonoses is impossible without considering the human–environment relationship and studying the dynamics of the population of carriers and their infestation. The species composition of ticks should be considered when planning measures for acaricidal treatment of the territory, because different tick species have different activity peaks.

The results of the study of borreliosis carriers (ticks of the ixodid family) demonstrate the dominance of ticks of the genus *Dermacentor* in the carrier structure in the republic during the study period (2012–2022). *I. ricinus* and *I. persulcatus* ticks are common but few species. Most probably, this distribution is due to the presence of biotopes in the Republic of Tatarstan that are more suitable for arthropods of this genus. The Republic of Tatarstan is located in the East European Plain. The total area of the Republic of Tatarstan is 68,000 km^2, and its climate is moderately continental. The average temperatures are from −13°C to −18°C in January, and +19°C to 20°C in July. The amount of precipitation is up to 500 mm per year. About 16% of the territory is covered by forest. The Republic of Tatarstan is located on the border of two large zoogeographic zones, forest and steppe, which determines the species composition of the fauna. These natural conditions are most suitable habitats for *D. reticulates* [8].

The highest borreliosis carriage (20.4%) was noted in *I. persulcatus*. For comparison, in different regions of Russia, the infection rate of *I. persulcatus* ticks collected from vegetation in 2022 with Borrelia varied from 17% to 48.03% [3]. The infection rate of *D. reticulatus* ticks was minimal, indicating the low efficiency of reproduction of Borrelia in ticks of this genus. According to studies conducted in the neighboring Kirov region from 2010 to 2015, the average proportion of *D. reticulatus* individuals infected with Borrelia was 36.93% [9]. The high infection rate was attributed to the recent spread of this species of ticks in the Kirov region.

B. miyamotoi was detected in *I. ricinus* ticks (infection rate: 0.4%). In the Tver oblast, the infection rate of *I. ricinus* ticks with this species of Borrelia was 2.9%, and that of *I. persulcatus* ticks was 1.8%. In the Tomsk, Novosibirsk, and Kemerovo regions, the genetic material of *B. miyamotoi* was revealed in 2.2% of *I. persulcatus* and *I. pavlovskyi* ticks [10]. Thus, according to data from the Republic of Tatarstan and other regions of Russia, the infection rate of ixodid ticks with *B. miyamotoi* is significantly lower than that with Borrelia of the *B. burgdorferi* s.l. complex.

Study limitations

The relationship between the incidence rates of the population and borreliosis carriage of ticks was not assessed owing to lacking data on tick examination in individual municipalities.

CONCLUSION

The Republic of Tatarstan is endemic for Lyme disease. The incidence of ITB, as well as the borreliosis carriage of ticks, has territorial differences. The dominant species of ixodid ticks in the natural epitopes of the Republic of Tatarstan in 2012–2022 was *D. reticulatus*, which accounted for 75.4% of the collected species. The lowest level of borreliosis carriage was established for ticks of this species, as only 4.4% of ticks were affected (i.e., the borreliosis carriage of *I. persulcatus* was 20.4%, and that for *I. ricinus* was 14%). The relatively low incidence rates of ITB in the Republic of Tatarstan in comparison with other regions may be due to this fact. The detection in small quantities of *I. ricinus* ticks infected with spirochete *B. miyamotoi* discovered relatively recently in Russia [11] confirms the need for a specific examination of patients with a non-erythematous form of fever that developed after a tick bite to diagnose relapsing fever.

It is crucial to conduct a study of ticks, especially in areas with incidence rates exceeding the average long-term annual level in the Republic of Tatarstan, with the determination of the species composition and infection of tick carriers, to monitor the epidemic situation of ITB in the region and develop proposals to reduce the risk of infection of the population.

ADDITIONAL INFORMATION

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Competing interests. The authors declare that they have no competing interests.

Authors' contribution. All authors made a substantial contribution to the conception of the work, acquisition, analysis, interpretation of data for the work, drafting and revising the work, final approval of the version to be published and agree to be accountable for all aspects of the work. I.A. Karpova — data collection and analysis, article writing; G.R. Khasanova — data analysis, writing and editing the article; L.F. Sadreeva — data analysis, editing a section of the article; Yu.A. Tyurin — conducting laboratory research on ticks, editing a section of the article; N.N. Shaykhullin — collection of biological material, writing the article.

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AUTHORS' INFO

*** Irina A. Karpova**;
address: 13a Sechenova street, 420061 Kazan, Russia;
ORCID: 0009-0005-9244-1384;
eLibrary SPIN: 7610-5819;
e-mail: fbuzkarpovairina@mail.ru

Gulshat R. Khasanova, MD, Dr. Sci. (Medicine), Professor;
ORCID: 0000-0002-1733-2576;
eLibrary SPIN: 6704-2840;
e-mail: gulshatra@mail.ru

Luciya F. Sadreeva;
ORCID: 0009-0007-1161-8577;
eLibrary SPIN: 1624-6805;
e-mail: sadreeva_65@mail.ru

Yurij A. Tyurin, MD, Dr. Sci. (Medicine), Assistant Professor;
ORCID: 0000-0002-2536-3604;
eLibrary SPIN: 5089-5565;
e-mail: tyurin.yurii@yandex.ru

Nail N. Shaykhullin;
ORCID: 0009-0008-0294-0430;
eLibrary SPIN: 2446-0820;
e-mail: nailfbuz@yandex.ru

ОБ АВТОРАХ

*** Карпова Ирина Александровна**;
адрес: Россия, 420061, Казань, ул. Сеченова, д. 13а;
ORCID: 0009-0005-9244-1384;
eLibrary SPIN: 7610-5819;
e-mail: fbuzkarpovairina@mail.ru

Хасанова Гульшат Рашатовна, д-р мед. наук, профессор;
ORCID: 0000-0002-1733-2576;
eLibrary SPIN: 6704-2840;
e-mail: gulshatra@mail.ru

Садреева Люция Фаатовна;
ORCID: 0009-0007-1161-8577;
eLibrary SPIN: 1624-6805;
e-mail: sadreeva_65@mail.ru

Тюрин Юрий Александрович, д-р мед. наук, доцент;
ORCID: 0000-0002-2536-3604;
eLibrary SPIN: 5089-5565;
e-mail: tyurin.yurii@yandex.ru

Шайхуллин Наиль Нурсаетович;
ORCID: 0009-0008-0294-0430;
eLibrary SPIN: 2446-0820;
e-mail: nailfbuz@yandex.ru

* Corresponding author / Автор, ответственный за переписку