CHARACTERISTICS OF TICK-BORNE INFECTIONS IN THE UNDEREXPLORED AREAS OF THE TRANS-BAIKAL TERRITORY

Lagunova E.K.,
Khasnatinov M.A.,
Danchinova G.A.

Scientific Centre for Family Health and Human Reproduction Problems (Timiryazeva str. 16, Irkutsk 664003, Russian Federation)

Corresponding author:
Maxim A. Khasnatinov,
e-mail: khasnatinov@gmail.com

ABSTRACT

Background. *Infections that are transmitted to humans through the bites of ixodid ticks remain a significant problem for public healthcare. However, in many regions endemic for tick-borne diseases, the diversity and prevalence of infections transmitted by ticks are still underexplored.*

The aim of the study. *To characterize the modern diversity and prevalence of tick-borne pathogens in the ecosystems of the valley of the Chikoy River (Trans-Baikal Territory, Russian Federation).*

Materials and methods. *Real-time polymerase chain reaction was used to examine 48 imagoes of Ixodes persulcatus ticks, one Haemaphysalis concinna female tick and 38 small mammals specimens for infection with seven tick-borne pathogens.*

Results. *The H. concinna tick tested negative for all studied pathogens. In I. persulcatus ticks, the prevalence of infection with Borrelia burgdorferi s.l. comprised 39.5 %, Anaplasma phagocytophilum – 16.7 %, B. miyamotoi – 8.3 % and Ehrlichia sp. – 2.1 %. Tick-borne encephalitis virus (TBEV), Rickettsia sibirica and R. heilongjiangensis were not detected in I. persulcatus. Four species of vertebrate hosts of ticks and tick-borne infections were found: Myodes rufocanus (44.7 %) Apodemus peninsulae (39 %), Microtus oeconomus (13.2 %) and M. rutilus (2.6 %). In rodent populations, the prevalence of TBEV infection comprised 5.3 %, B. burgdorferi s. l. – 39.5 %, B. miyamotoi – 28.9 %, Ehrlichia sp. – 21.1 %, A. phagocytophilum – 18.4 %.*

Conclusion. *The widespread distribution of taiga ticks, the presence of numerous populations of competent vertebrate hosts of infections, and the high prevalence of infections among vertebrate and invertebrate hosts indicate that active natural foci of tick-borne encephalitis, Lyme disease, tick-borne relapsing fever caused by B. miyamotoi, human granulocytic anaplasmosis, and human monocytic ehrlichiosis (HME) are present in the ecosystems of Chikoy River valley.*

Key words: *the Trans-Baikal Territory, Borrelia, tick-borne encephalitis virus, Anaplasma phagocytophilum, Riskettsia, Ixodes persulcatus*

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ХАРАКТЕРИСТИКА КЛЕЩЕВЫХ ИНФЕКЦИЙ В МАЛОИЗУЧЕННЫХ РАЙОНАХ ЗАБАЙКАЛЬСКОГО КРАЯ

Лагунова Е.К.,
Хаснатинов М.А.,
Данчинова Г.А.

ФГБНУ «Научный центр проблем здоровья семьи и репродукции человека» (664003, г. Иркутск, ул. Тимирязева, 16, Россия)

Автор, ответственный за переписку:
Хаснатинов Максим Анатольевич,
e-mail: khasnatinov@gmail.com

РЕЗЮМЕ

Обоснование. *Инфекции, передающиеся человеку при укусах иксодовых клещей, остаются актуальной проблемой здравоохранения. Однако во многих частях нозоареала разнообразие и распространённость клещевых инфекций остаются недостаточно исследованными.*

Цель исследования. *Охарактеризовать современное разнообразие и распространённость возбудителей клещевых инфекций в долине р. Чикой (Забайкальский край, Россия), входящей в буферную зону Байкальской природной территории.*

Материалы и методы. *С помощью полимеразной цепной реакции в реальном времени на заражённость семью возбудителями клещевых инфекций исследованы 48 имаго клещей Ixodes persulcatus, 1 особь Haemaphysalis concinna и 38 особей мелких млекопитающих.*

Результаты. *Клещ H. concinna не был заражён ни одним из исследуемых патогенов. Зараженность I. persulcatus Borrelia burgdorferi s. l. составила 39,5 %, Anaplasma phagocytophilum – 16,7 %, B. miyamotoi – 8,3 % и Ehrlichia sp. – 2,1 %. Вируса клещевого энцефалита (ВКЭ), Rickettsia sibirica и R. heilongjiangensis в таёжных клещах не обнаружено. Выявлены 4 вида позвоночных хозяев клещей и инфекций: Myodes rufocanus (44,7 %) Apodemus peninsulae (39 %), Microtus oeconomus (13,2 %) и M. rutilus (2,6 %). В популяциях мелких млекопитающих зараженность ВКЭ составила 5,3 %, B. burgdorferi s. l. – 39,5 %, B. miyamotoi – 28,9 %, Ehrlichia sp. – 21,1 %, A. phagocytophilum – 18,4 %.*

Заключение. *Повсеместное распространение таёжных клещей, наличие многочисленных популяций компетентных позвоночных хозяев инфекций, а также высокие показатели заражённости позвоночных и беспозвоночных хозяев свидетельствуют о широком распространении в долине р. Чикой активных природных очагов клещевого энцефалита, болезни Лайма, клещевой возвратной лихорадки, вызываемой B. miyamotoi, гранулоцитарного анаплазмоза и моноцитарного эрлихиоза человека.*

Ключевые слова: *Забайкальский край, Borrelia, вирус клещевого энцефалита, Anaplasma phagocytophilum, Riskettsia, Ixodes persulcatus*

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INTRODUCTION

Zoonotic infections are still recognized as one of most significant threats to the human health in Russian Federation. This is particularly true for infections transmitted by ixodid ticks due to the wideness of spread and the severity of the illnesses. In the Asian part of the Russian Federation, the main vector of many tick-borne infections are *Ixodes persulcatus* ticks (Schulze, 1930) [1].

The most epidemically significant causative agents of tick-borne diseases (TBD) are tick-borne encephalitis virus (TBEV), Lyme disease agent *Borrelia burgdorferi* sensu lato, the agent of the tick-borne relapsing fever *B. miyamotoi* a few species of rickettsiae, anaplasmas, ehrlichiae and other pathogens [2]. Ticks can be infected either by a single human pathogen (mono-infection) or simultaneously by two or more species of pathogens (co-infection). Mono- and co-infections are clinically distinctive depending on the combination of pathogens. This requires the development of new approaches to the diagnosis, prevention and treatment of known tick-borne infections, including co-infections [3, 4].

The main source of information for assessing the epidemiological situation is active monitoring of natural foci of tick-borne infections, based on direct evaluation of the number of tick vectors and mammals – reservoir hosts of infections and tick feeders in the ecosystem, as well as evaluation of the prevalence of infection with the studied pathogens. Active monitoring requires highly qualified personnel and is labor-intensive and time-consuming. As a result, in many territories that are endemic for TBD, there is a lack of information about the prevalence and diversity of tick-borne infections, as well as the structure and risk assessment of their natural foci. Despite the obvious advantages of this approach, it has a number of significant drawbacks. This method, for example, does not consider a number of important aspects of the infection process, such as multiplexing of several pathogens in one parasitic system, the intensity of contact between people and tick vectors, the socio-demographic state of the population, and migration processes in human and wildlife populations [5]. Besides, in order to obtain reliable data during active monitoring, it is necessary to study large numbers of vectors and feeders (ticks and vertebrates) at the same time at sampling key sites located in different landscapes The study should be performed using unified methods of detection and identification of pathogens for the entire sample of materials collected during field expeditions [6, 7]. As a result, in many areas endemic for tick-borne infections, the peculiarities of the spread and circulation of pathogens in nature remain poorly studied.

One of such areas is the Krasnochikoysky district of the Trans-Baikal Territory, which has the highest incidence rates of tick-borne encephalitis and Lyme disease [8]. Despite this, research on tick-borne infections in these areas has previously been sporadic and was mainly focused on tick-borne encephalitis virus and Lyme disease agents, while no information is available concerning the circulation of other pathogens transmitted by ixodid ticks. The Chikoy is one of the largest rivers in the Lake Baikal catchment area, and the Chikoy valley along with the adjacent ranges of the Khentii Mountains is part of the Baikal Natural Area. Large areas of indigenous cedar forest unaffected by human activity, peculiar flora and fauna, unique natural objects, and deposits of natural resources (uranium ores) predetermine the potential for further recreational, economic and industrial development of the Chikoy valley [9]. Given the foregoing, characterization of the current situation in natural foci of TBD seems to be a significant task both scientifically and practically.

THE AIM

To characterize the current diversity and prevalence of causative agents of tick-borne infections in the Chikoy River valley (Krasnochikoysky District, Trans-Baikal Territory, Russian Federation).

MATERIALS AND METHODS

Materials

Ixodid ticks were collected from vegetation in the most common biotopes of the study area using flannel flag. The abundance of the ticks was evaluated using the standard methods [10] and expressed as the number of ticks per flag per kilometer of the route. The captured ticks were delivered to the laboratory alive. Information about each tick was registered in the information-analytical system "Field ticks" [11]. Small mammals were captured using Sherman live traps on survey lines. The abundance of animals was expressed as a number of specimens per trap per day. Animals were euthanized at the place of capture in compliance with the "Ethical Principles for Medical Research Involving Human Subjects" [12]. For each captured animal, species, sex, age group (juveniles or adults), and infestation with ectoparasites were individually determined. Afterwards the tissue sampling of brain, spleen, kidney and lung was performed. Tissue samples were stored in liquid nitrogen until delivery to the laboratory and then at –80 °C until study.

Preparation of tick and mammalian organ suspensions

Each tick was individually washed in 70 % ethanol and dried on filter paper, and then the species, sex and condition of the tick were determined. The tick species was identified morphologically using the key guide of the USSR ixodid tick fauna [13]. Prepared ticks were homogenized using a TissueLyzer II automatic homogenizer (QIAgen, Germany) and sterile 3 mm diameter tungsten carbide beads. Homogenates were resuspended in 300 µl of sterile phosphate buffered saline PBS; (pH = 7.4) and examined immediately after preparation.

The species of small mammals was identified morphologically in accordance with the key guide of the USSR rodent fauna [14]. The infestation of animals by ixodid ticks was assessed based on the index of abundance (IA; average number of ticks per specimen) and the index of occurrence (IO; proportion of animals in the sample on which ixodid ticks were found).

Animal tissue samples (30–50 mg) were homogenized using a TissueLyzer II and chilled sterile 5 mm steel beads. Homogenates were resuspended in 300 µl of chilled sterile PBS (pH = 7.4) and examined immediately after preparation.

Nucleic acids extraction

Total RNA/DNA was extracted from individual ticks or tissue samples. Briefly, 100µl of suspension was purified using RealBest Extraction 100 or RealBest UniMag kits (VectorBest, Novosibirsk) and KingFisher Flex (Thermo Fisher Scientific, Finland) magnetic particle processor according to the manufacturer's instructions. The resulting nucleic acids were dissolved in 300 µl of elution buffer.

Real-time PCR

Polymerase chain reaction (PCR) with real-time hybridisation-fluorescence detection was used to reveal causative agents of tick-borne infections. Reagent kits "RealBest DNA *Borrelia burgdorferi s. l.*/RNA VKE", "RealBest DNA *Anaplasma phagocytophilum/Ehrlichiamuris, Ehrlichia chaffeensis*", "RealBest DNA *Borrelia miyamotoi*" and "RealBest DNA *Rickettsia sibirica/Rickettsia heilongjiangensis*" (VectorBest, Novosibirsk). Since *E. muris* and *E. chaffeensis* were detected in a single PCR reaction without species identification, the identified Ehrlichiae were hereafter designated as *Ehrlichia*sp.

Fifty microliters of DNA/RNA preparation was used as a matrix for reverse transcription PCR. Reaction and real-time results were recorded using a CFX C1000 Touch amplifier (BioRad, USA) according to the kit manufacturer's instructions. BioRad CFX Manager 3.1 software (BioRad, USA) was used to process and analyze the results.

Quantitative PCR

Quantification of *Borrelia* sp. was performed using multiplex quantitative PCR (qPCR) targeting the *16S* rRNA gene [15]. Briefly, the *16S rRNA* gene fragments of *B. burgdorferi* sensu lato (strain B31) and *B. miyamotoi* (strain HT31) were PCR amplified using BspF-16s [5`-GCTGTAAACGATGCACACTTGGT-3`] and BspR-16s [5`- GGCGGCACACTTAACACGTTAG-3`] primers. The obtained PCR fragments (70 nucleotide bases in length) were independently cloned in the plasmid vector pCR4-TOPO (Thermo Fisher Scientific, USA) and grown in competent DH5α strain of *Escherichia coli* (Nippon Gene, Japan) as described previously [15]. The number of spirochetes in the samples was estimated using calibration curve produced by serial tenfold dilution of standard DNA samples of the corresponding Borrelia spp. and expressed as log10 genome copies per tick. PCR was performed in a reaction volume of 25 µL. The reaction mixture contained 1U Taq polymerase HSTaq (Eurogen), 2.5 µl of DNA template, primers BspF-16s and BspR-16s at a concentration of 900 nM each and probes FAM-LD [5`-FAM-TTCGGTACTAACTTTTAGTTAA-BHQ1-3`] and VIC-RF [5`-R6G-CGGTACTAACCTTTCGATTA-BHQ1-3`] at a concentration of 200 nM each. PCR conditions were set up as follows: an initial cycle at 50 °C for 2 min, followed by a cycle at 95 °C for 2 min, then 45 cycles of 95 °C for 15 s and 63 °C for 60 °C. The fluorescence was measured at the 63 °C stage of every cycle and compared to the calibration curve readings, with the FAM channel determining the presence and concentration of B. burgdorferi s. l. DNA, and the VIC channel – the presence and concentration of B. miyamotoi DNA..

Statistical methods of data processing

Sex ratio and infection prevalence were assessed as the proportion of infected specimens (in percent) and 95% confidence intervals (95% CI) were calculated.

To assess associations between TBEV infections with *B. burgdorferi* s. l. l., *B. miyamotoi*, *A. phagocytophilum* and *Ehrlichia* sp. in ticks, 2×2 contingency tables were constructed for each pair of microorganisms [16]. In this case, infected and uninfected tick conditions were taken as binary determinants. Analysis of variance based on Fisher's *F*-criterion [17] was used to assess the statistical significance of differences between group mean values.

Statistical analyses were performed using MS Excel software (Microsoft Corp., USA), MaxStat Light and R version 4.0.2 (R Foundation, Austria). Statistical significance was set at 0.95. All tests of statistical significance were two-tailed. Differences were considered statistically significant at $p < 0.05$.

RESULTS AND DISCUSSION

The tick and animal surveys and sample collection were conducted between the 3rd and 10th of July, 2021 during a field survey along the Chikoy River valley in the Republic of Buryatia and Krasnochikoysky District of the Trans-Baikal Territory. A total of 13 sampling sites were studied (Fig. 1). The survey routes for tick abundance assessment and sample collection comprised 15.7 km. The survey of small mammal's abundance and specimen collection resulted in 455 traps-nights. In total, 49 specimens of ixodid ticks and 38 specimens of rodents were collected. Besides this, 116 larvae and nymphs of taiga ticks were found on small mammals, but were excluded from PCR tests.

Ixodid tick abundance ranged from low to moderate and ranged from 0.4 to 13 individuals per flag/km. Considering the suboptimal survey season (early July), such tick abundance suggest that there are natural foci of tick-borne infections with medium and high tick activity in the surveyed area. The highest tick numbers were associated with mixed grass pine-birch forests with spiraea and rosehip as underbrush. One tick captured at survey point No. 7 (Fig. 1) was identified as a female *Haemaphysalis concina*

(Koch, 1844). The remaining 48 ticks were identified as *Ixodes persulcatus* (Shulze, 1930). All ticks were imagoes, with a sex ratio of 0.6 males per female.

Relative abundance of small mammals ranged from 10 to 21.5 exv./100 trap-nights, with no animals captured at three locations. Species diversity was represented by four predominant species: Korean field mouse *Apodemus peninsulae* (Thomas, 1907), grey red-backed vole *Myodes rufocanus* (Sundevall, 1846), tundra vole *Microtus oeconomus* (Pallas, 1776), and grey red-backed vole *Myodes rutilus* (Pallas, 1779). *M. rufocanus* (44.7 %) and *A. peninsulae* (39.5 %) were the most common species captured, whereas *M. oeconomus* and *M. rutilus* were scarce (13.2 % and 2.6 % of the sample, respectively).

I. persulcatus larvae were found only on rodents of two species: *A. peninsulae* and *M. rufocanus*, with *A. peninsulae* playing a slightly more significant role in the feeding of taiga tick larvae (IA = 5.3; IO = 60 %) compared to *M. rufocanus* (IA = 2.1; IO = 35.3 %), despite the greater abundance of the latter. Only grey-sided vole served as feeding hosts for taiga tick nymphs, with IA = 0.12 ticks per specimen and IO = 11.8 %. Ixodid ticks of any other species have not been found on rodents.

Diversity and prevalence of tick-borne infections among ixodid ticks

The prevalence of tick-borne infections in ticks

The only female of *H. concinna* was not infected by any of the pathogens studied. The agents of the North Asian tick typhus *R. sibirica* and the Far-Eastern spotted fever *R. heilongjiangensis* have not been observed in ticks or in tissue samples from rodents, and therefore these pathogens are not discussed further.

In taiga ticks collected per flag from vegetation, the most common pathogen was *B. burgdorferi* s. l., which was detected in 39.6 % *of* samples. Second abundant pathogen appeared to be *A. phagocytophilum* (16.6 %), followed by *B. miyamotoi* (8.3 %). The proportion of *Ehrlichia* sp. was 2 %. No tick-borne encephalitis virus was detected in ticks (Table 1).

The quantitative borrelia load was estimated for eight specimens of *I. persulcatus* infected with *B. burgdorferi* s. l. and for two specimens infected with *B. miyamotoi.* The mean concentration of *B. miyamotoi* was 2.5 ± 5.1 with a maximum of 2.9 log10 genome copies per tick. The concentration of *B. burgdorferi* s. l. varied from 1.1 to 3.8 and averaged 2.7 ± 0.7 log10 genome copies per tick. It can be assumed that the load of *B. burgdorferi* s. l. per tick is slightly higher than *B. miyamotoi*, although the differences are not statistically significant.

Co-infection of taiga ticks with two or more pathogens

In more than 90 % of cases, ticks were infected with only one of the tested microorganisms, but 3 (6.2 %) ticks were simultaneously infected with *B. burgdorferi* s. l. and *A. phagocytophilum*. No statistically significant associations between these infections was revealed. No other combinations of pathogens were revealed in the studied sample of taiga ticks.

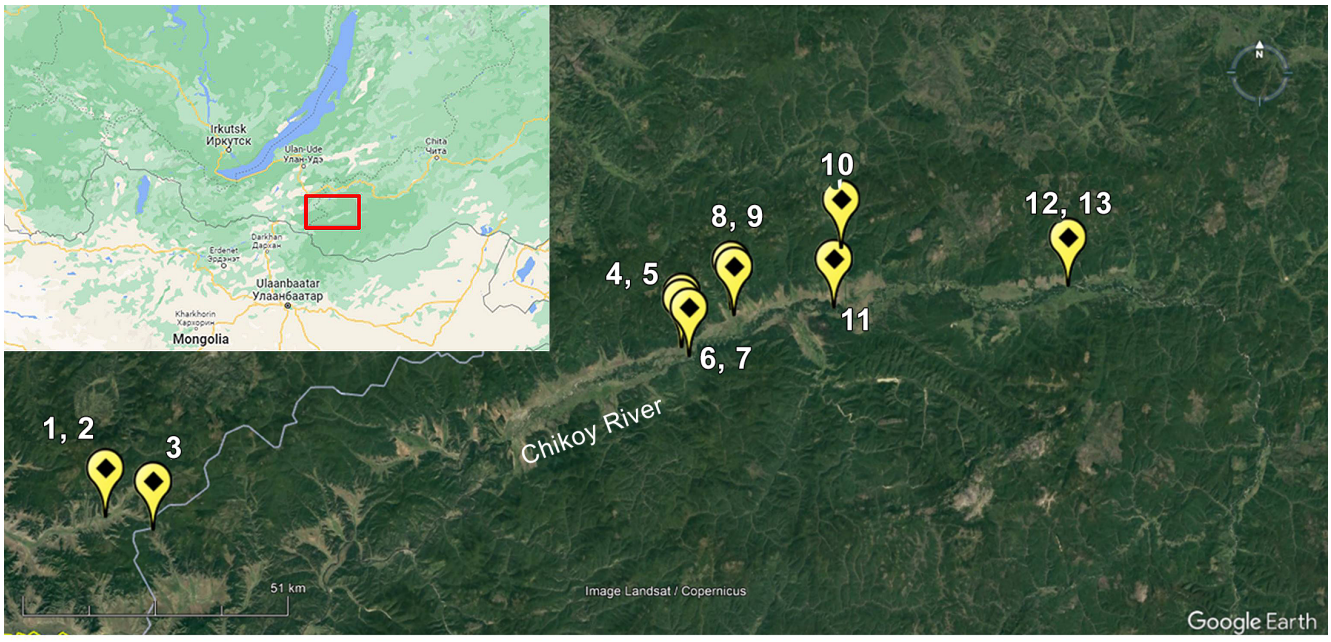

FIG. 1.

Survey area and localization of sampling sites. Mapping of tick and rodent capture sites and image creation were performed using GoogleEarthPro 7.3 software. Survey points: **1, 2** *– Republic of Buryatia, Kyakhtinsky District, vicinity of Ulady village;* **3** *– border of Buryatia and Trans-Baikal Territory;* **4–7** *– Trans-Baikal Territory, Krasnochikoysky District, Fomichevo village;* **8** *– Trans-Baikal Territory, Krasnochikoyskiy District, Zakharovo village;* **9–11** *– Trans-Baikal Territory, Krasnochikoyskiy District, Shimbylik village;* **12, 13** *– Trans-Baikal Territory, Krasnochikoyskiy District, Steklozavod village*

Diversity and prevalence of tick-borne infections among small mammalian ixodid tick feeders

All tick-borne pathogens found in taiga ticks were detected in small mammals, and two rodents were infected with TBEV – an adult female grey-sided vole and a juvenile male East Asian wood mouse – were also revealed. A. peninsulae was the most infected – about 53 % of animals were infected with at least one of the studied microorganisms. Among five specimens of *M. oeconomus*, two were infected with tick-borne pathogens. The infestation of rodents of different species is summarized in Table 2.

Mammalian infestation with tick-borne encephalitis virus

In contrast to taiga ticks, TBEV was observed in the tissues of 5 % of vertebrate hosts. In one *A. peninsulae* specimen, viral RNA was revealed in brain, and in one *M. rufocanus* specimen, systemic infection was observed with the presence of viral RNA in the brain, spleen, kidney and lungs. The prevalence of infections in of TBEV infection was comparable in both species comprising 7 % and 6 %, respectively.

Mammalian infestation and patterns of infection with B. burgdorferi s. l.

The agents of Lyme disease were the most common pathogens among vertebrate hosts and, in average, were observed in 39 % of animals, which is almost identical to the infection rate among taiga ticks. *B. burgdorferi* s. l. DNA was found in all examined mammalian species with nearly the same prevalence of 39–40 %. The exception was *M. rufocanus*, which had 35 % infection rate, although the difference was statistically insignificant.

In small mammals, *B. burgdorferi* s. l. DNA was detected in all organs examined (Table 3), although with different frequencies. In kidney tissues borreliae were found rare and only among numerous species of *A. peninsulae* and *M. rufocanus*. Most often (in almost all infected animals) the *B. burgdorferi* s.l. infection was observed in lung tissues. Among the low abundant species of *M. oeconomus* and *M. rutilus*, all infected animals

TABLE 1

THE PREVALENCE OF TICK-BORNE PATHOGENS AMONG *I. PERSULCATUS* TICKS IN THE ECOSYSTEMS OF THE CHIKOY RIVER VALLEY

	Specimens studied	Of those infected, *n*, % (95% CI)					Not infected, % (95% CI)
		TBEV	B. b. s. l.	B. m.	A. ph.	E. sp.	
I. persulcatus, females	30	0	12 40 (22; 58)	2 7 (0; 16)	4 13 (1; 25)	1 3 (0; 10)	12 40 (22; 58)
I. persulcatus, males	18	0	7 39 (16; 61)	2 11 (0; 26)	4 22 (3; 41)	0	7 39 (16; 61)
Total	48	0	19 40 (26; 53)	4 8 (1 ;16)	8 17 (6; 27)	1 2 (0; 6)	19 40 (26; 53)

Note. TBEV – tick-borne encephalitis virus; B. b. s. l. – *B. burgdorferi* sensu lato; B. m. – *B. miyamotoi*; A. ph. – *Anaplasma phagocytophilum*; E. sp. – *Ehrlichia* sp.

TABLE 2

THE PREVALENCE OF TICK-BORNE PATHOGENS AMONG SMALL MAMMALS IN THE ECOSYSTEMS OF THE CHIKOY RIVER VALLEY

Type	Specimens studied	Of those infected, *n*, % (95% CI)					Not infected, % (95% CI)
		TBEV	B. b. s. l.	B. m.	A. ph.	E. sp.	
Apodemus peninsulae	15	1 7 (0; 19)	6 40 (15; 65)	4 27 (4; 49)	1 7 (0; 19)	5 33 (9; 57)	7 47 (21; 72)
Microtus oeconomus	5	0	2 40 (0; 83)	0	1 20 (0; 55)	1 20 (0; 55)	3 60 (17; 103)
Myodes rufocanus	17	1 6 (0; 17)	6 35 (13; 58)	2 12 (0; 27)	3 18 (0; 36)	1 6 (0; 17)	9 53 (29; 77)
Myodes rutilus	1	0	1	0	1	0	0
Total	38	2 5 (0; 12)	15 39 (24; 55)	6 16 (4; 27)	6 16 (4; 27)	7 18 (6; 31)	19 50 (34; 66)

Note. TBEV – tick-borne encephalitis virus; B. b. s. l. – *B. burgdorferi* sensu lato; B. m. – *B. miyamotoi*; A. ph. – *Anaplasma phagocytophilum*; E. sp. – *Ehrlichia* sp.

had signs of systemic *B. burgdorferi* s. l. infection involving at least three organs, i.e., brain, spleen and lungs. Among *A. peninsulae*, the systemic infection was observed in three specimens with involvement of two or more organs, and in three specimens DNA of *B. burgdorferi* s. l. was detected only in lungs. In *M. rufocanus* a similar pattern was observed: three specimens exhibited systemic infection involving 3 or 4 organs, and in three cases borreliae were detected only in lungs (two specimens) or brain (one specimen).

The quantitative load of *B. burgdorferi* s. l. was possible to be determined for three specimens of *M. rufocanus* and one each of *A. peninsulae*, *M. oeconomus* and *M. rutilus* (Table 4). The attempts to quantify the spirochete concentrations in brain tissues were not successful.

Trace amounts of *B. burgdorferi* s. l. DNA were detected in the kidneys of only one of *M. rufocanus*. In spleen and lung tissues, *B. burgdorferi* s. l. DNA concentrations were comparable for most samples and were on the order of 10 genomic copies per 10 mg of tissue. In tissues of a single specimen of the *M. oeconomus* the concentration of *B. burgdorferi* s. l. DNA was 3.5 log10 genomic copies, which is about 1000 times higher than similar parameters of *M. rufocanus* and *A. peninsulae* (Table 4).

Infection and features of mammalian infection with B. miyamotoi

The agent of tick-borne relapsing fever *B. miyamotoi*, has only been found among abundant rodent species. The prevalence of infection in *A. peninsulae* was more than twice higher than in *M. rufocanus* (27 % and 12 %, respectively). In rodent organism, *B. miyamotoi* infection was more disseminated than *B. burgdorferi* s. l. (Table 5) and affected 2 or more organs in all cases, with the exception of one specimen of *A. peninsulae*, in which these spirochetes were found only in spleen.

The quantitative load of *B. miyamotoi* was determined for three infected specimens of *A. peninsulae* and two *M. rufocanus* (Table 6). Overall, the concentration of *B. miyamotoi* in mammalian tissues was about 10-fold higher than that of *B. burgdorferi* s. l. and ranged from 50 to about 10,000 genome copies per 10 mg

TABLE 3

OCCURRENCE OF *B. BURGDORFERI* S. L. IN ORGANS OF SMALL MAMMALS

Mammalian species	*B. burgdorferi* s. l. infected, specimens	Organs in which *B. burgdorferi* s. l. DNA was revealed, *n* (%)			
		brain	spleen	kidneys	lungs
A. peninsulae	6	1 (16.7)	2 (33,3)	1 (16.7)	6 (100)
M. oeconomus	2	2 (100)	2 (100)	0 (0)	2 (100)
M. rufocanus	6	3 (50)	3 (50)	2 (33.3)	5 (83.3)
M. rutilus	1	1 (100)	1 (100)	0 (0)	1 (100)

TABLE 4

MEAN BACTERIAL LOAD OF *B. BURGDORFERI* S. L. IN ORGANS OF SMALL MAMMALS

Mammalian species	*B. burgdorferi* s. l. concentration in tissues (lg genome copies/10 mg)		
	spleen	kidneys	lungs
A. peninsulae	1	0	0.8
M. oeconomus	3.5	0	3.3
M. rufocanus	0	0.1	0.9
M. rutilus	0.6	0	1.4

of tissue. In *A. peninsulae*, the quantitative load of spirochetes in different organs was approximately equal, whereas in *M. rufocanus*, spleen tissues were prominent, in which the DNA concentration of *B. miyamotoi* was 2 orders of magnitude higher than in other organs (Table 6).

Prevalence and patterns of infection with *A. phagocytophilum* in rodents

Overall, the causative agent of human granulocytic anaplasmosis (HGA), *A. phagocytophilum*, was found in 16 % of mammals but the incidence of infection with this pathogen varied between species. Thus, *M. rufocanus* and *M. oeconomus* were characterized by infection rates close to the mean value, but among *A. peninsulae* the prevalence of infection was three times lower (18–20 % vs. 7 %, respectively; Table 2).

In most cases, *A. phagocytophilum* infection resulted in a systemic spread of the pathogen involving all organs examined, except the kidneys (Table 7). The only exception was an infected specimen of *A. peninsulae*, in which HGA agent DNA of the human granulocytic anaplasmosis pathogen was detected only in the spleen (Table 7).

Prevalence and patterns of infection with Ehrlichia sp. in rodents

Ehrlichia infection was most common of *A. peninsulae*, in which these microorganisms were observed in 33 % of cases. Among other mammalian species, these bacteria were found in single specimens (Table 2).

TABLE 5

OCCURRENCE OF *B. MIYAMOTOI* IN ORGANS OF SMALL MAMMALS

Mammalian species	*B. miyamotoi* infected, specimens	Organs in which *B. miyamotoi* DNA was revealed, *n* (%)			
		brain	spleen	kidneys	lungs
A. peninsulae	4	2 (50)	4 (100)	0 (0)	3 (75)
M. rufocanus	2	0 (0)	1 (50)	1 (50)	2 (100)

TABLE 6

MEAN BACTERIAL LOAD OF *B. MIYAMOTOI* IN ORGANS OF SMALL MAMMALS

Mammalian species	*B. miyamotoi* concentration in tissues, lg genome copies/10 mg			
	brain	spleen	kidneys	lungs
A. peninsulae	2	2.7	0	2.3
M. rufocanus	0	4.1	1.4	2.2

TABLE 7

OCCURRENCE OF *A. PHAGOCYTOPHILUM* IN ORGANS OF SMALL MAMMALS

Mammalian species	Infected with *A. phagocytophilum*, specimens	Organs in which *A. phagocytophilum* DNA was revealed, *n* (%)		
		brain	spleen	lungs
A. peninsulae	1	0	1 (100)	0
M. oeconomus	1	1 (100)	1 (100)	1 (100)
M. rufocanus	3	2 (66.7)	3 (100)	3 (100)
M. rutilus	1	1 (100)	1 (100)	1 (100)

In contrast to all other pathogens studied, *Ehrlichia* sp. infection was always systemic, and in *A. peninsulae*, ehrlichiae DNA was detected in all organs examined (Table 8).

Co-infection of small mammals with two or more pathogens

Co-infection with two or more pathogens appeared to be widespread among vertebrate hosts of tick-borne infections. The only *M. rufocanus* specimen studied was simultaneously infected with two pathogens, i. e. *B. burgdorferi* s. l. and *A. phagocytophilum*. Of the two infected *M. oeconomus*, one was infected with three bacteria species, i. e. *B. burgdorferi* s. l., *A. phagocytophilum* and *Ehrlichia* sp. The pattern of infection with tick-borne pathogens among multiple mammalian species is summarized in Table 9.

Korean field mice *A. peninsulae* were co-infected more often – about 40 % of animals were simultaneously infected with two or more pathogens, with mono-infections only be-

TABLE 8

DISTRIBUTION OF *EHRLICHIA* SP. IN VARIOUS ORGANS OF SMALL MAMMALS

Mammalian species	*Ehrlichia* sp. infected, specimens	Organs in which *Ehrlichia* sp. DNA was revealed, n (%)			
		brain	spleen	kidneys	lungs
A. peninsulae	5	4 (80)	5 (100)	1 (20)	5 (100)
M. oeconomus	1	1 (100)	1 (100)	0	1 (100)
M. rufocanus	1	1 (100)	1 (100)	0	1 (100)

TABLE 9

CO-INFECTION PATTERNS OF TICK-BORNE PATHOGENS IN SMALL MAMMALS

Pathogens	Mono-infection, *n* (%)	Co-infection, *n* (%)	Co-infection with (%)				
			B. b. s. l.*	B. m.	A. ph.	E. sp.	more than 2 pathogens
			A. peninsulae (*n* = 15)				
TBEV	0	1 (6.7)	0	0	0	0	1 (6.7)
B. b. s. l.	0	6 (40)	–	1 (6.7)	1 (6.7)	2 (13.3)	2 (13.3)
B. m.	1 (6.7)	3 (20)	1 (6.7)	–	0	0	2 (13.3)
A. ph.	0	1 (6.7)	1 (6.7)	0	–	0	0
E. sp.	1 (6.7)	4 (26.7)	2 (13.3)	0	0	–	2 (13.3)
Total	2 (13.3)	6 (40)	4 (26.7)	1 (6.7)	1 (6.7)	2 (13.3)	–
			M. rufocanus (*n* = 17)				
TBEV	1 (5.9)	0	0	0	0	0	0
B. b. s. l.	2 (11.8)	4 (23.5)	–	1 (5.9)	2 (11.8)	0	1 (5.9)
B. m.	1 (5.9)	1 (5.9)	1 (5.9)	–	0	0	0
A. ph.	0	3 (17.6)	2 (11.8)	0	–	0	1 (5.9)
E. sp.	0	1 (5.9)	0	0	0	–	1 (5.9)
Total	4 (23.5)	4 (23.5)	3 (17.6)	1 (5.9)	2 (11.8)	0	–

Note. TBEV – tick-borne encephalitis virus; B. b. s. l. – *B. burgdorferi* sensu lato; B. m. – *B. miyamotoi*; A. ph. – *Anaplasma phagocytophilum*; E. sp. – *Ehrlichia* sp.

ing observed for *B. miyamotoi* and *Ehrlichia* sp. The only case of TBEV infection in this rodent species was observed only in co-infection with *B. burgdorferi* s. l., *B. miyamotoi* and *Ehrlichia* sp. Among the bacteria, *B. burgdorferi* s. l. were observed with approximately equal frequency in all co-infections; *B. miyamotoi* was observed either in multiple co-infections or in combination with *B. burgdorferi* s. l., the only case of *A. peninsulae* infection with anaplasmas was in combination with *B. burgdorferi* s. l., whereas the more common ehrlichiae occurred in about equal numbers, both as mono-infections and in various combinations with other microorganisms.

Among *M. rufocanus*, approximately 25 % of animals were infected with one pathogen and the same number – with two or more pathogens. Mono-infections have been reported for TBEV, *B. burgdorferi* s. l. and *B. miyamotoi*. Among the co-infections, the most notable were *B. burgdorferi* s. l., which occurred in all available combinations except TBEV. For the other bacteria, co-infections in this rodent species were not common and were limited to single cases of multiple infections or co-infections with *B. burgdorferi* s. l.

CONCLUSION

In the Krasnochikoysky district of the Trans-Baikal Territory within the Chikoy River valley, a stable circulation of at least five causative agents of tick-borne infections has been revealed for the first time: tick-borne encephalitis virus, *B. burgdorferi* s. l., *B. miyamotoi, A. phagocytophilum* and *Ehrlichia* sp. It has been shown that these pathogens not only infect tick vectors, but are also spread among natural vertebrate hosts – small mammals. *I. persulcatus* ticks are the most relevant vectors of tick-borne infections in the surveyed area, while the most important vertebrate hosts and tick feeders are *M. rufocanus* and *A. peninsulae*.

No cases of infection of taiga ticks and rodents with causative agents of tick-borne rickettsiosis of northern Asia *R. sibirica* and Far Eastern tick-borne rickettsiosis *R. heilongjiangensis* were revealed. However, the existence of natural foci of these diseases in the ecosystems of the Chikoy River catchment cannot be excluded. A limited sample size of ticks and vertebrate hosts, on the one hand, does not allow us to assert the completeness of the description of the ixodid tick fauna of the Chikoy River valley, as well as the biodiversity of pathogens infecting them. In contrast, the discovery of the *H. concinna* tick (which is the competent invertebrate host of *R. heilongjiangensis*), also suggests that the species diversity of both tick vectors and tick-borne pathogens in the Krasnochikoisky district may be even wider than established in the present study.

Summarising the results obtained, we can note a high risk of infection with tick-borne encephalitis, Lyme disease, tick-borne relapsing fever caused by *B. miyamotoi*, HGA and HME in the Krasnochikoisky district of the Trans-Baikal Territory, which is part of the Baikal Natural Area, including the most attractive areas for residence and recreation of the local population, Russian and foreign tourists. This evaluation is supported by the widespread distribution of epidemically significant vectors of tick-borne infections – *I. persulcatus* ticks, the presence of numerous populations of competent vertebrates – hosts of infections and tick feeders, high prevalence of infections in of ticks and small mammals, as well as a significant proportion of animals simultaneously infected with two or more pathogens. All this indicates the need to further improve the surveillance of tick-borne infections with the involvement of all the possibilities of modern science for both short-term and medium- and long-term forecasting of the epidemiological situation.

Conflict of interest

The authors of this article declare no conflicts of interest.

Compliance with the principles of biomedical ethics

The study was designed and performed in compliance with the ethical principles required by the World Medical Association Declaration of Helsinki. The study was performed with the approval of the Biomedical Ethics Committee of the Scientific Centre for Family Health and Human Reproduction Problems (Protocol No. 2 from February 18, 2020).

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Information about the authors

Ekaterina K. Lagunova – Laboratory Assistant at the Laboratory of Vector-Borne Infections, Scientific Centre for Family Health and Human Reproduction Problems, e-mail: lagunovakatya1994@yandex.ru, https://orcid.org/0009-0007-3688-023X

Maxim A. Khasnatinov – Dr. Sc. (Biol.), Leading Research Officer at the Laboratory of Vector-Borne Infections, Scientific Centre for Family Health and Human Reproduction Problems, e-mail: khasnatinov@yandex.ru, https://orcid.org/0000-0002-8441-3640

Galina A. Danchinova – Dr. Sc. (Biol.), Head of the Laboratory of Vector-Borne Infections, Scientific Centre for Family Health and Human Reproduction Problems, e-mail: dan-chin@yandex.ru, https://orcid.org/0000-0002-6705-3970

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