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BLOOD BIOCHEMICAL PARAMETERS IN RESERVOIR HOSTS UNDER IXODID TICK-BORNE BORRELIOSIS

M.A. PEREVOZCHIKOVA✉, I.A. DOMSKY, YU.A. BEREZINA, A.A. SERGEEV

Zhitkov Russian Game Management and Fur Farming Research Institute, 79, ul. Preobrazhenskaia, Kirov, 610000, Russia, e-mail mperevozchikova@mail.ru (✉ corresponding author), vniioz43@mail.ru, uliya180775@bk.ru, metalbird@mail.ru
ORCID:
Perevozchikova M.A. orcid.org/0000-0003-3638-3712
Berezina Yu.A. orcid.org/0000-0001-5082-716X
Domsky I.A. orcid.org/0000-0003-1633-1341
Sergeev A.A. orcid.org/0000-0002-9461-5131
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Abstract

Currently, there is a wide distribution of natural foci of ixodid tick-borne borreliosis (ITB, Lyme disease) in Europe, Asia, Australia and America, as well as high infection rate of people and animals. Studies have shown that this new natural focal infection occupies a leading position in terms of morbidity and socio-economic damage. Many pathogenic microorganisms, including *Borrelia burgdorferi*, which cause disease in humans and animals, persist in certain natural foci. Ticks feed on different animals at different stages of development. The size of the tick population mainly depends on the number of adult reservoir hosts. The research, for the first time, revealed in ITB infection reservoir hosts with diagnostically significant titers of antibodies to *B. burgdorferi* the blood serum biochemical parameters which indicate the pathological effect of the pathogen on the body of wild animals, the development of multiple organ failure and harm to their health. The aim was to study the natural foci of ixodid tick-borne borreliosis and to obtain new data on the epizootology of the disease, including an assessment of the effect of parasitism of ixodid ticks infected with *B. burgdorferi* on the blood chemical composition of reservoir hosts, the mountain hare and moose. Blood serum from adult males of mountain hare (n = 11) was used, including 5 samples that had diagnostically significant titers of antibodies to *B. burgdorferi* in the indirect immunofluorescence assay (IDIF) (1:40 and 1:80), and 6 samples that did not have diagnostically significant titers of antibodies to *B. burgdorferi* (control). We also studied blood serum from moose (n = 114) of different sex and age groups (animals aged 6-7 months and adults), including 24 samples with diagnostically significant titers of antibodies to *B. burgdorferi* and 90 samples from clinically healthy animals (Kirov region), including individuals whose sera in the IDIF did not have diagnostically significant titers of antibodies to *B. burgdorferi*. Animals were hunted during scientific shooting during the autumn hunting seasons of 2005-2020. Blood samples for laboratory studies were taken from the jugular vein immediately after the animal was shot. Anti-borreliosis antibodies in blood serum was detected in IDIF test using *B. afzelii* corpuscular antigen (strain Ip-21) and fluorescein isothiocyanate (FITC) labeled luminescent immune serum against globulins of various animal species (rabbit, dog, bull, pig, chicken). Biochemical studies of blood serum were performed using a semi-automatic analyzer Biochem SA (High Technology Inc., USA) with a set of reagents (Eco-Service, Russia) to measure the concentration of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase, lactate dehydrogenase (LDH), alpha-amylase, total protein, albumin, total bilirubin, direct bilirubin, creatinine and cholesterol. It was found that the animals with diagnostically significant titers of antibodies to *B. burgdorferi* had statistically significant ($p < 0.05$) differences in the activity of AST, ALT, alkaline phosphatase, the content of total protein, total and direct bilirubin, creatinine. Thus, in a hare with diagnostically significant titers of antibodies to *B. burgdorferi*, AST activity was 342.2 % higher compared to animals without titers. An increase in AST activity in moose was noted, by 35.0 % in young females, by 35.3 % in young males, by 31.2 % in adult females, and by 24.0 % in adult males. ALT activity in hare with diagnostic titers to *B. burgdorferi* was 32.8 % higher compared to the control. An increase in ALT activity was also found in moose,

by 53.8% in young females, by 90.4 % in young males, by 188.6 % in adult females, and by 173.9 % in adult males. In hare, the value of the de Ritis coefficient testified to the predominance of the heart pathology, and in moose, on the contrary, to liver damage. An increase ($p < 0.05$) in the activity of alkaline phosphatase during borreliosis in adult moose was noted, by 132.5 % in females and by 206.3 % in males, and a decrease in the enzyme activity in young females. In hare, an increase in the content of total bilirubin by 42.4 % was revealed, in young female moose by 86.1 %, in young males by 121.9 %, in adult females by 118.8 %, in adult males by 70.4 %. In addition, the content of direct bilirubin increased in male moose, by 59.1 % in young and by 102.8 % in adults. The amount of total protein in all groups of animals with diagnostically significant antibody titers to *B. burgdorferi* increased: in hare by 123.6 %, in young female moose by 24.3 %, in young males by 53.5 %, in adult females by 76.7 %, in adult males by 19.9%. In hares with diagnostic titers of antibodies to *B. burgdorferi*, the renal failure led to a 40.7 % increase for creatinine, the end product of metabolism. Univariate analysis (ANOVA) allowed us to establish a significant effect of *B. burgdorferi* on the increase in serum concentrations of aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, total protein, total and direct bilirubin, and creatinine. The data obtained indicate multiple organ failure in reservoir hosts under borreliosis and pathological effects of *B. burgdorferi* on animals.

Keywords: *Borrelia burgdorferi,* ixodid ticks, reservoir hosts, *Alces alces*, *Lepus timidus*, blood biochemistry, blood serum

Ixodid tick-borne borreliosis (ITB, Lyme disease) is a relatively new group of naturally focal vector-borne infectious diseases. The pathogen was discovered in 1982 [1]. ITB is a typical spirochetosis with the clinical and pathogenetic features of this group of infectious diseases. The ability of spirochetes to persist for a long time in the body of humans and animals leads to the formation of a chronic process that occurs with systemic damage to organs [2, 3].

The pathogen enters the body with the saliva of the tick. Its primary accumulation occurs in the basal and papillary layers of the epidermis, which is accompanied by vascular changes and manifests itself as erythema migrans, which sometimes serves as the only marker of the acute period of the disease [3]. Skin lesions (pathognomonic signs of the disease in humans) in sick animals are observed only in isolated cases due to the presence of fur and skin pigmentation.

As Borrelia accumulate, they spread hematogenously and lymphogenously from the primary focus throughout the body, entering internal organs and other areas of the skin (secondary erythema). Generalization of the infection is clinically accompanied by symptoms of general intoxication and damage to various organs (brain and spinal cord with involvement of the meninges, liver, kidneys, heart, spleen, muscles, joints, lymph nodes in the inflammatory process) [3].

Currently, for the countries of Europe, Asia, Australia and America, the relevance of ITB is determined by the wide distribution of natural foci, as well as the high infection rate of people and animals. Studies have shown that this new natural focal infection occupies a leading place in terms of morbidity and socio-economic damage [2-4].

To date, no specific prevention measures have been developed for the disease, and in nature, ixodid ticks have practically no natural enemies. As a result, only the human factor remains the main regulator of the numbers of these arthropods [5)]. On the territory of the Russian Federation, a decrease in the area of acaricidal treatments is the main reason for the epidemiological problems with tick-borne infections [6]. In addition, the expansion of borreliosis foci is caused by the reduction of arable land, the abandonment of intensive agriculture, and the increase in suburban construction and landscaping of urban areas. There is a mosaic growth of forests and the formation of a favorable environment for ixodid ticks and their hosts [7-10].

Many pathogenic microorganisms, including *Borrelia burgdorferi*, which cause diseases in humans and animals, persist in the body of reservoir hosts. This reservoir function is closely related to the association between the animal species and the pathogen, which must remain viable without interfering with the survival

of the host. The specificity of arthropod vectors for different animal species is also important in the transmission of the pathogen and is of particular importance for the development of a predictive model of disease risk.

At different stages of development, ticks feed on different types of animals. Tick-borne pathogens are transmitted to susceptible organisms from small to medium-sized mammals and birds (mainly by nymphs and larvae), while tick population size is primarily dependent on the number of adult reservoir hosts. Medium-sized mammals such as the mountain hare (*Lepus timidus*, Linnaeus 1758), and large ungulates such as moose (*Alces alces*, Linnaeus 1758), white-tailed deer (*Docoileus virginianus* Zimmermann, 1780), cattle (*Bos taurus taurus* Linnaeus, 1758) and horses (*Equus caballus* Linnaeus, 1758), serve as feeders for all stages of tick development. Large ungulates are the primary food source for adults and are not capable of pathogen accumulation but are nonetheless important for pathogen transmission because they provide food for large numbers of adult females, contributing to increased tick numbers [11]. Large wild and domestic animals are considered incompetent reservoirs, meaning ticks that feed on them can infect each other when feeding together [12]. In addition, incompetent reservoirs serve as a supporting reservoir for all stages of mite development. Reducing the population of incompetent reservoirs can reduce potential transmission, the prevalence of Borrelia, and the risk of disease in humans.

Incompetent reservoirs determine the increase in tick numbers in the area where they live, and if competent hosts also have high numbers, the risk of disease in humans increases significantly. Studies have shown that in areas inhabited by European roe deer (*Capreolus capreolus* Linnaeus, 1758) and cattle, Ixodes ticks are found in greater numbers [11, 13-15] and the number of reported cases of borreliosis is higher [16]. In general, for a correct and complete interpretation of the epizootology of any anthropozoonotic disease, the causative agent, vector and reservoir hosts should be considered as environmental system. Humans are always incidental hosts, and their risk of infection is based on the presence of competent and incompetent reservoir hosts [17, 18].

Biochemical blood parameters are widely used to assess the condition of the body of mammals, determine the presence of parasites [19], and can also indicate the state of the feed supply [20]. L.B. Keith et al. [21] and I.M. Keith et al. [22] studied parasites of the American hare (*Lepus americanus* Erxleben, 1777) in North America. V. Haukisalmi et al. [23, 24] studied parasites of voles in northern Finland at different stages of the population cycle. Their results suggest that parasites do not have any obvious effect on population cycles.

Borrelia persistence has been confirmed in some vertebrate species. Bacteremia is subject to changes that depend on the health of the host and the viability of the pathogen [18]. According to D.C. Duffy et al. [25] and T. Boulinier et al. [26], if a large number of infected ticks parasitize reservoir hosts, the reproductive dynamics of host populations may be disrupted.

At present, the pathological effect of Borrelia on the human body and some species of domestic animals has been well studied, but it has not been established whether *B. burgdorferi* has any effect on the body and on the population of hosts and reservoir hosts of ixodid ticks as a whole. In the literature available to us, we have not found data regarding the effect of *B. burgdorferi* on competent and incompetent reservoir hosts, including on biochemical blood parameters.

In this work, for the first time, biochemical parameters of blood serum were established in reservoir hosts with ITB, which have diagnostically significant titers of antibodies to *B. burgdorferi* that indicate the pathological influence of the pathogen on the body of wild animals, the development of multiple organ failure

and harm to health.

Our goal was to study the natural foci of ixodid tick-borne borreliosis and obtain new data on the epizootology of the disease, including assessing the effect of parasitism of ixodid ticks infected with *Borrelia burgdorferi* on the blood chemical composition of the reservoir hosts, the mountain hare and moose.

Materials and methods. Blood serum from adult male white hare (n = 11) was used, including 5 samples that in the indirect immunofluorescence assay (IDIF) had diagnostically significant titers of antibodies to *B. burgdorferi* (1:40 and 1:80), and 6 samples that did not have diagnostically significant antibody titers to *B. burgdorferi* (control). We also examined blood serum from moose (n = 114) of various sex and age groups (young animals aged 6-7 months and adults), including 24 samples with diagnostically significant antibody titers to *B. burgdorferi*, and 90 samples from clinically healthy animals in the Kirov Province, including individuals whose blood serum did not have diagnostically significant antibody titers to *B. burgdorferi* in the IDIF test.

The animals were caught by shooting during the autumn hunting seasons of 2005-2020, the white hare in the Kotelnichsky District of the Kirov Province with the center in the village of Sloboda (58°39′58″N, 50°43′42″E), the moose on the territory of the scientific and experimental hunting farm (Zhitkov All-Russian Institute of Hunting and Fur Farming), located in the Slobodsky District of the Kirov Province with its center in the village Rogovoe (58°33′04″N, 50°43′42″E). All animals were wild and moved freely, feeding on local vegetation.

Immediately after shooting the animal, blood for laboratory studies was sampled from the jugular vein (*venae jugularis*) into UNIVAC vacuum tubes (Eiliton LLC, Russia) with a coagulation activator (4 ml each) and centrifuged (a Liston C 2204 centrifuge, Liston LLC, Russia) for 20 min at 1500 rpm.

Anti-borreliosis antibodies in blood serum were detected in the indirect immunofluorescence assay (IDIF) using the corpuscular antigen of *B. afzelii* (strain Ip-21) and luminescent immune serum labeled with fluorescein isothiocyanate (FITC) against globulins of various animal species (rabbit, dog, bovine, pig, chicken). IDIF test was performed according to the recommendations of E.I. Korenberg et al. [27]. The results were recorded using a luminescent microscope LUMAM (JSC LOMO, Russia; an immersion lens). Titers of specific antibodies at a dilution of 1:40 or higher were considered diagnostically significant [28, 29].

Biochemical studies of blood serum (a semi-automatic Biochem SA analyzer, High Technology, Inc., USA) were carried out with a set of reagents (Eco-Service, Russia) for quatification of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase, lactate dehydrogenase (LDH), alpha-amylase, total protein, albumin, total bilirubin, direct bilirubin, creatinine and cholesterol.

Statistical analysis was performed using Microsoft Excel Office 2019 and Statgraphics 19-X64 software by generally accepted methods [30]. Mean values (M), standard deviations (±SD), medians (Me), percentiles (25% and 75%) were calculated. To compare parameters between groups, the nonparametric Wilcoxon-Mann-Whitney test (U) was used. Relationships between traits were assessed using Spearman rank correlation. To assess the effect of *B. burgdorferi* on blood biochemical parameters, one-way analysis of variance (ANOVA) was used. The influence of the factor was considered statistically significant at $p < 0.05$.

Results. Biochemical parameters of blood serum of clinically healthy wild animals and individuals with diagnostically significant titers of antibodies to *B. burgdorferi* (1:40 and 1:80) are given in Tables 1-3.

1. Blood biochemical parameters of adult male mountain hare (*Lepus timidus* Linnaeus, 1758) with diagnostic values of antibodies to *Borrelia burgdorferi* and animals with the antibody titers below the cutoff titer values (Kirov Province, Kotelnichsky District, 2005-2020)

Parameter	Animals with diagnostic antibody titers (n = 5)	Animals without diagnostic antibody titers (n = 6)
Aspartate aminotransferase, U/l:		
min-max	44.2-115.9	15.6-25.8
M±SD	85.5±31.09	21.8±4.20
Me	99.3*	22.4
25 %-75 %	61.2-106.8	19.3-25.2
Alanine aminotransferase, U/l:		
min-max	61.2-91.2	39.4-69.8
M±SD	77.5±12.96	56.9±11.02
Me	78.1*	58.7
25 %-75 %	68.0-88.9	51.5-63.7
Lactate dehydrogenase, U/l:		
min-max	697.0-1242.5	620.1-902.1
M±SD	889.9±210.40	753.5±112.1
Me	821.6	740.5
25 %-75 %	786.3-902.1	670.3-838.2
α-Amylase, U/l:		
min-max	138.5-294.1	163.9-261.5
M±SD	212.7±68.21	228.4±35.69
Me	237.5	236.6
25 %-75 %	145.0-248.5	219.6-252.4
Total protein, g/l:		
min-max	96.1-162.7	58.3-76.7
M±SD	131.5±28.66	64.1±7.17
Me	136.9*	61.2
25 %-75 %	108.3-153.8	59.3-66.7
Albumen, g/l:		
min-max	63.2-88.5	56.2-86.9
M±SD	77.6±9.70	70.7±11.27
Me	79.3	69.7
25 %-75 %	73.6-83.4	63.6-77.8
Total bilirubin, mmol/l:		
min-max	3.3-7.8	1.6-3.6
M±SD	5.0±1.85	2.8±0.66
Me	4.2*	3.0
25 %-75 %	3.7-6.0	2.7-3.2
Direct bilirubin, mmol/l:		
min-max	0.1-2.5	0.2-1.0
M±SD	1.1±0.87	0.7±0.34
Me	1.0	0.8
25 %-75 %	0.8-1.1	0.5-1.0
Creatinine, mmol/l:		
min-max	100.2-144.6	72.2-124.3
M±SD	124.8±16.5	96.0±18.70
Me	129.1*	91.8
25 %-75 %	119.2-131.1	86.3-106.7
Cholesterol, mmol/l:		
min-max	0.1-0.3	0.1-0.4
M±SD	0.3±0.09	0.2±0.09
Me	0.3	0.2
25 %-75 %	0.2-0.3	0.2-0.3

* Differences between the groups are statistically significant at $p < 0.05$.

We identified a strong correlation between some biochemical parameters in animals with diagnostically significant titers of antibodies to *B. burgdorferi*. In mountain hare, there were correlations between AST activity and the total bilirubin content ($r = -1$, $p = 0.00$), total protein and direct bilirubin ($r = -1$, $p = 0.00$). In young female moose correlations were between AST and ALT ($r = -1$, $p = 0.00$), AST and total protein ($r = 1$, $p = 0.00$), total and direct bilirubin ($r = 1$, $p = 0.00$); in young male moose between total protein and creatinine ($r = 0.89$; $p = 0.02$). Unlike control animals, in young female moose with diagnostically significant titers of antibodies to *B. burgdorferi*, a strong positive correlation ($r = 1$; $p = 0.00$) ocurred between the activity of alkaline phosphatase and the total protein content,

while a strong negative correlation ($r = -1$; $p = 0.00$) occurred between AST activity and total bilirubin, AST and direct bilirubin, AST and creatinine.

2. Blood biochemical parameters of young moose (*Alces alces* Linnaeus, 1758) with diagnostic values of antibodies to *Borrelia burgdorferi* and animals with the antibody titers below the cutoff titer values (Kirov Province, Kotelnichsky District, 2005-2020)

Parameter	Femails		Mails	
	with diagnostic antibody titers ($n = 3$)	clinically healthy ($n = 20$)	with diagnostic antibody titers ($n = 7$)	clinically healthy ($n = 20$)
Aspartate aminotransferase, U/l:				
min-max	197.5-240.4	117.5-219.1	166.7-349.4	120.2-207.5
M±SD	216.0±22.07	161.2±28.30	238.7±76.33	160.0±30.92
Me	210.1*	155.6	209.3*	154.7
25 %-75 %	203.8-225.2	146.9-178.9	189.5-283.1	131.6-193.5
Alanine aminotransferase, U/l:				
min-max	78.0-112.4	47.2-72.0	70.8-173.1	48.0-71.2
M±SD	95.2±17.17	60.8±6.42	116.7±37.08	58.7±6.74
Me	95.1*	61.9	108.8*	57.2
25 %-75 %	86.6-103.8	56.8-63.9	90.3-141.7	54.8-64.7
Alkaline phosphatase, U/l:				
min-max	176.2-221.6	165.2-296.0	153.8-470.8	169.2-270.8
M±SD	199.1±22.70	230.4±40.8	289.2±111.48	222.2±31.14
Me	199.4	232.0	261.8	219.1
25 %-75 %	187.8-210.5	197.7-269.2	210.0-359.1	200.9-253.8
Total protein, g/l:				
min-max	68.1-84.3	46.5-83.0	72.0-178.6	45.5-86.1
M±SD	76.5±8.13	61.6±9.91	121.4±41.03	66.4±13.53
Me	77.1*	62.0	108.3*	70.5
25 %-75 %	72.6-80.7	53.7-67.0	90.6-154.7	53.2-77.8
Albumen, g/l:				
min-max	34.6-53.1	33.0-49.1	34.6-54.6	32.2-53.3
M±SD	44.7±9.37	40.9±4.48	46.5±8.22	42.9±6.94
Me	46.5	41.8	49.2	42.9
25 %-75 %	40.5-49.8	38.2-43.7	40.8-52.7	36.0-50.0
Total bilirubin, mmol/l:				
min-max	13.1-25.6	5.6-10.9	9.9-25.5	5,6-10,1
M±SD	17.9±6.77	7.9±1.54	18.1±5.29	8,4±1,11
Me	14.9*	8.0	18.7*	8,4
25 %-75 %	14.0-20.3	6.3-9.1	15.1-21.2	7,6-9,3
Direct bilirubin, mmol/l:				
min-max	2.1-3.5	1.4-3.7	2.1-5.7	0.9-3.8
M±SD	2.9±0.70	2.6±0.60	3.7±1.33	2.4±0.9
Me	3.2	2.6	3.8*	2.4
25 %-75 %	2.6-3.3	2.3-3.1	2.5-4.5	1.8-2.8
Creatinine, mmol/l:				
min-max	149.7-209.6	117.7-180.7	110.5-215.6	109.3-180.4
M±SD	185.2±31.47	152.8±20.3	156.0±43.51	149.1±23.78
Me	196.4	155.1	132.4	142.9
25 %-75 %	173.0-203.0	139.8-169.5	124.8-192.0	133.3-173.0

* Differences between the groups are statistically significant at $p < 0.05$.

In moose with diagnostically significant antibody titers, a strong correlation ($r = 1$; $p = 0.00$) was idetnitied between adult and young females, young females and adult males, adult females and males for alkaline phosphatase; between young and adult females, young females and adult males for total protein; and between young and adult females, young females and adult males for total bilirubin.

The one-way analysis (ANOVA) estimates the effect of *B. burgdorferi* on blood biochemical parameters of reservoir hosts. In the mountain hare, there was a statistically significant effect of *B. burgdorferi* on increasing the activity of AST ($p = 0.00$; 73.67% influence rate), ALT ($p = 0.01$; 47.44% influence rate), total protein content ($p = 0.00$; 77.77% influence rate), total bilirubin ($p = 0.02$; 44.1% influence rate), and creatinine ($p = 0.02$; 44.44% influence rate). In young female moose, *B. burgdorferi* had a significant effect on increasing the activity of AST ($p = 0.00$; 32.59% influence rate), ALT ($p = 0.00$; 69.17% influence rate), on total protein ($p = 0.02$; 22.48% influence rate), total bilirubin ($p = 0.00$; 65.57%

influence rate), creatinine (p = 0.02; 21.85% influence rate); in young male moose on an increase in the activity of AST (p = 0.00; 37.65% influence rate), ALT (p = 0.00, 65.64% influence rate), on total protein (p = 0.00; 53.57% influence rate), total bilirubin (p = 0.00; 71.7% influence rate), direct bilirubin (p = 0.00; 24.88% influence rate). In adult female moose, *B. burgdorferi* infection caused an increase in activity of AST (p = 0.02, 20.34% influence rate), ALT (p = 0.00, 62.49% influence rate), alkaline phosphatase (p = 0.00, 77.89% influence rate), in total protein (p = 0.00, 64.26% influence rate), total bilirubin (p = 0.00, 67.61% influence rate); in adult male moose an increase in activity of AST (p = 0.01, 14.19% influence rate), ALT (p = 0.00, influence rate 52.72%), alkaline phosphatase (p = 0.00, 67.83% influence rate), in total protein content (p = 0.00, 30.77% influence rate), total bilirubin (p = 0.00, 55.65% influence rate), direct bilirubin (p = 0.00, 46.12% influence rate).

3. Blood biochemical parameters of adult moose (*Alces alces* Linnaeus, 1758) with diagnostic values of antibodies to *Borrelia burgdorferi* and animals with the antibody titers below the cutoff titer values (Kirov Province, Kotelnichsky District, 2005-2020)

Parameter	Femails		Mails	
	with diagnostic antibody titers (n = 4)	clinically healthy (n = 20)	with diagnostic antibody titers (n = 10)	clinically healthy (n = 30)
Aspartate aminotransferase, U/l:				
min-max	301.6-326.9	157.1-341.6	198.6-452.3	162.3-342.5
M±SD	317.3±11.43	253.8±52.38	301.2±74.05	250.9±47.52
Me	320.2*	244.1	301.6*	243.3
25 %-75 %	312.3-325.2	219.6-296.0	247.4-338.2	208.4-299.4
Alanine aminotransferase, U/l:				
min-max	56.9-223.4	29.4-55.4	58.8-299.8	30.4-53.2
M±SD	133.9±71.36	43.6±7.35	141.5±80.23	41.9±6.33
Me	127.5*	44.2	111.2*	40.6
25 %-75 %	91.0-170.4	39.1-49.7	89.2-153.5	38.3-46.9
Alkaline phosphatase, U/l:				
min-max	109.1-195.2	49.0-89.1	124.5-438.1	46.6-88.3
M±SD	156.3±37.1	69.3±12.62	226.2±96.12	69.9±11.31
Me	160.5*	69.0	215.6*	70.4
25 %-75 %	137.7-179.2	57.5-79.3	152.7-251.3	61.4-79.2
Total protein, g/l:				
min-max	62.2-104.5	45.4-70.1	63.2-94.1	59.4-79.4
M±SD	92.7±20.36	57.4±7.48	79.8±13.34	68.1±4.93
Me	102.0*	57.8	82.4*	68.7
25 %-75 %	91.5-103.2	52.4-62.0	66.7-92.1	63.4-71.4
Albumen, g/l:				
min-max	39.8-69.9	30.9-53.8	34.9-59.6	33.4-53.2
M±SD	49.8±13.61	43.7±5.62	44.5±7.58	42.3±5.65
Me	44.8	44.9	44.3	41.5
25 %-75 %	42.8-51.8	40.2-46.5	39.9-46.8	38.8-46.6
Total bilirubin, mmol/l:				
min-max	9.8-24.7	6.7-10.7	10.1-26.5	6.5-11.5
M±SD	17.9±6.30	8.6±1.02	16.3±5.2	9.1±1.43
Me	18.6*	8.5	15.6*	9.2
25 %-75 %	15.1-21.5	8.1-9.3	12.3-18.8	8.3-10.1
Direct bilirubin, mmol/l:				
min-max	2.1-3.9	1.6-3.7	2.3-10.7	1.3-4.0
M±SD	3.1±0.77	2.6±0.55	6.0±2.90	2.7±0.79
Me	3.2	2.7	5.7*	2.8
25 %-75 %	2.7-3.6	2.2-2.9	3.5-8.2	2.1-3.3
Creatinine, mmol/l:				
min-max	144.12-202.3	156.4-225.7	135.4-229.6	126.3-223.2
M±SD	174.96±25.94	183.93±18.59	183.98±33.98	182.1±23.66
Me	176.71	185.7	169.5	181.2
25 %-75 %	159.16-192.51	169.6-195.35	162.75-219.27	167.5-200.1

* Differences between the groups are statistically significant at $p < 0.05$.

It should be noted that the entire territory of the Kirov Province in landscape and climate terms is favorable for the reproduction and maintenance of the population of ixodid ticks and their reservoir hosts [31].

Currently, the epidemiological situation regarding ITB in the Kirov Province remains unfavorable and the incidence exceeds the average for the Russian Federation. Thus, in 2020, this figure was 2.2 times higher than the Russian average (2.85 cases of the disease per 100 thousand population) and amounted to 6.29 cases per 100 thousand population. The highest incidence was registered in the Uninsky District (53.79 cases per 100 thousand population). The childhood morbidity rate was 2.7 times higher than the average for Russia (1.75 cases per 100 thousand population) and amounted to 4.71 cases per 100 thousand child population. Borreliosis has been registered in Kirov and 19 districts. In 2020, the virology laboratory of the Center for Hygiene and Epidemiology in the Kirov Region examined 1,815 ticks, 1,673 from people and 142 from environmental objects. Among ticks from people, 42.4% were positive for borreliosis (46.8% in 2018). Of ticks from environmental objects, 43.7% were positive for borreliosis (71.8%i in 2018, 49.2% in 2019) [32].

Our earlier serological and bacteriological studies [33-36] found out that the borrelia IDIF positive animals (mountain hare, fox *Vulpes vulpes* Linnaeus, 1758, elk, raccoon dog *Nyctereutes procyonoides* Gray, 1834, black grouse *Lyrurus tetrix* Linnaeus, 1758, capercaillie (*Tetrao urogallus* Linnaeus, 1758) and animals from internal organs of which borrelia were isolated on the BSK-H medium (badger *Meles meles* Linnaeus, 1758, wild boar *Sus scrofa* Linnaeus, 1758) serve as feeders for ixodid ticks and the reservoir hosts, distributors and accumulators of borreliosis pathogens (Fig. 1).

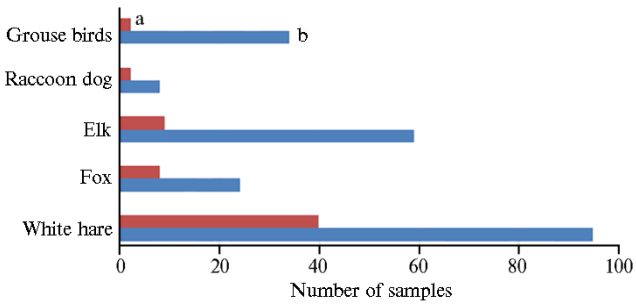

Fig. 1. Results of a study of blood serum of some animals for borreliosis in the reaction of indirect immunofluorescence: a — positive samples, b — studied samples [34, 36].

The high infestation of the mountain hare (Fig. 2) is explained by the greater likelihood of its contact with ticks due to high activity during the spring rutting period, abundant multi-layered fur, and sizes corresponding to the layer of high concentration of ticks on vegetation.

We believe that the number of white hare will make it possible to predict the epizootic situation regarding ITB, since even its slight fluctuations can affect the number and infection of tick vectors by increasing the likelihood of *Borrelia* horizontal transmission. A decrease in the number of reservoir hosts, on the contrary, will lead to a reduction in the population of the vector and, as a consequence, the pathogen. Similar data on the dynamics of parasitic systems in natural foci of ITB are given by Yu.V. Kovalevsky et al. [37].

H.E. Lyubeznova et al. [31] analyzed the dependence of tick infection with Borrelia on the number of rodents, white hare and fox. When comparing the number of mouse-like rodents and the infection of ticks with Borrelia, an average correlation was revealed ($r = 0.4$, $p < 0.05$) with a shift after 1 year. With an increase in the population of the white hare ($r = 0.8$, $p < 0.01$) and fox ($r = 0.72$, $p < 0.01$) in the second and third years, an increase in tick infection occurred.

Fig. 2. Hungry (1) **and feeding** (2) **ixodid ticks on a mountain hare (*Lepus timidus*, Linnaeus 1758).** Photo by I.A. Domsky.

Human borreliosis is characterized by polymorphism of clinical signs and the predominance of chronic forms [38-40]; for animals, it is a latent form. This makes it difficult to make a diagnosis.

Currently, borreliosis in dogs is the most studied. Clinical signs are reported in 5-20% of cases [41]. An acute disease begins after several months, and sometimes years, of a prosperous period. Borreliosis sugns are fever, lameness, sore muscles and joints, migratory arthritis, enlargement and swelling of the lymph nodes. Most often, one joint is affected, usually from the side of the tick bite [42]. Neurological disorders, diseases of the heart, liver, kidneys, bladder and eyes are recorded somewhat less frequently in the acute period [43]. However, nephritis is a lethal form of borreliosis in dogs and develops in them at the age of 3 years and older [44]. According to N.S. Pustovit [45], symptoms of renal failure and pathology of the urinary tract were detected in 21% of seropositive dogs.

In cattle, borreliosis is also asymptomatic. The dominant clinical signs are lameness and joint swelling, and less commonly, erythematous skin rash, fever, laminitis, abortion, weight loss, and decreased productivity [46-48]. The birth of calves with severe internal organ pathologies in infected cows has been reported [48]. In cows, the presence of *B. burgdorferi* has been proven in joint fluid, blood, urine, feces and milk [47, 49].

Biochemical research methods provide significant assistance in assessing the effect of a pathogen on the body [50]. Thus, in everyday practice, when diagnosing liver diseases, generally accepted biochemical tests for bilirubin content, aminotransferase activity, and alkaline phosphatase are used. Traditionally, these tests are combined to diagnose clinical and biochemical syndromes (cytolysis, cholestasis, etc.) [50].

We found that animals in whose blood sera diagnostically significant titers of antibodies to *B. burgdorferi* (1:40 and 1:80) were detected by IDIF had statistically significant differences ($p < 0.05$) in the activity of AST, ALT, alkaline phosphatase, the content of total protein, total bilirubin, direct bilirubin and creatinine from the control group.

Cytolysis syndrome (violation of the integrity of hepatocytes) is caused by impaired permeability of cell membranes, disintegration of membrane structures, necrosis of hepatocytes with the release of enzymes into the plasma, which entails an increase in the activity of AST and ALT. In this case, increased amounts of both fractions of bilirubin are determined in the blood serum [50]. In the mountain hare with diagnostically significant antibody titers to *B. burgdorferi*, AST activity was 342.2% higher compared to animals without antibody titers. An increase in enzyme concentration was also noted in moose, by 35.0% in young females, by 35.3% in young males, by 31.2% in adult females, and by 24.0% in adult males. ALT activity in the mountain hare with diagnostic titers to B. burgdorferi was higher by 32.8% compared to animals without titers. An increase in ALT activity was also found in moose, by 53.8% in young females, by 90.4% in young males, by 188.6% in adult females, and by 173.9% in adult males.

ALT is a predominant marker of liver disease. Since ALT is localized in the cytoplasm, and AST is localized in the mitochondria, the AST index increases to a lesser extent in liver diseases. In addition, a significant increase in AST activity indicates severe damage to hepatocytes and serves as one of the early markers of disorders in the heart muscle [50].

In clinical practice, the de Ritis coefficient (ratio of AST to ALT) is used for the differential diagnosis of liver and myocardial diseases. Since ALT activity predominantly increases in liver diseases, this coefficient decreases. In cardiac pathology, on the contrary, an increase in AST activity predominates and the de Ritis coefficient increases [50]. In a mountain hare with diagnostically significant titers of antibodies to *B. burgdorferi*, the de Ritis coefficient was 1.25, in animals without them it was 0.38, which indicated heart pathology. In moose, the opposite results were obtained. In young females, the de Ritis coefficient was 2.20, in young males 1.92, in adult females 2.50, in adult males 2.71. In animals from the control group, these values were respectively 2.51, 2.70, 5.52, and 5.99. Thereof, our data indicated liver damage in this reservoir host species.

Cholestasis syndrome is caused by both a violation of the biliary function of hepatocytes and damage to the bile canaliculi (intrahepatic cholestasis), and a disorder of the outflow of bile through the hepatic and common bile ducts due to their obstruction (extrahepatic cholestasis). Both forms are characterized by increased activity of alkaline phosphatase and some other excretory enzymes, hypercholesterolemia, and hyperbilirubinemia [50]. We revealed a statistically significant ($p < 0.05$) increase in alkaline phosphatase activity in adult moose, by 132.51% in females and by 206.32% in males. The increase may be due to cholestasis of any etiology and localization (hepatitis, cirrhosis when intrahepatic, obstructive jaundice when extrahepatic). Cholestasis is doubtful under normal alkaline phosphatase level [50]. A decrease in the activity of this enzyme was also revealed in young females that might be associated with some slowdown in growth rate and a decrease in osteoblastic activity under infection and possible lack of energy. T. Soveri et al. [51] note that parasites influence blood parameters of the mountain hare. Thus, *Trichostrongylus retortaeformis* causes a decrease in alkaline phosphatase activity.

The nature of pigment metabolism disorders is assessed based on the results of a study of serum bilirubin. With parenchymal (hepatic) jaundice in patients with hepatitis, cirrhosis and other liver diseases, hepatocytes are damaged and bilirubin conversion is impaired. We detected an increase in the content of total bilirubin in all groups of animals with diagnostically significant titers of antibodies to *B. burgdorferi*. In the white hare, the indicator increased by 42.42%, in young female moose by 86.12%, in young males by 121.91%, in adult females by 118.77%, in adult males by 70, 44%. In addition, in young male moose the amount of direct bilirubin was increased by 59.09%, in adults by 102.85%.

Note that disruption of the uptake of free bilirubin by the liver cell and its binding to glucuronic acid causes an increase in the amount of free (indirect) bilirubin in the blood. The release of bilirubin glucuronide (direct bilirubin) from the liver cell into the bile capillaries, caused by inflammation, destruction, necrosis and a decrease in the permeability of hepatocyte membranes, leads to regurgitation of bile back into the sinusoids and into the general bloodstream and, accordingly, to an increase in the content of bound (direct) bilirubin in the blood. Disorder of hepatocyte function is accompanied by a loss of the ability of the liver cell to capture and metabolize urobilinogen absorbed in the intestine, which enters the general bloodstream in large quantities and is excreted in the urine. As a consequence, with parenchymal jaundice, the blood content of both free (indirect) and bound (direct) bilirubin increase [50].

Determination of total protein in blood serum is used to diagnose liver, kidney, and cancer diseases. Increased protein content is recorded during the development of acute and chronic infectious diseases and autoimmune pathologies.

In our study, an increase in the total protein content was recorded in all groups of studied animals that had diagnostically significant titers of antibodies to *B. burgdorferi*, in the mountain hare by 123.6%, in young female moose by 24.3%, in young males by 53.5%, in adult females by 76.7%, in adult males by 19.9%. Literature data on the content of total protein in the blood serum of animals infected with *B. burgdorferi* are contradictory. N.S. Pustovit [45] notes that total blood protein increases in infected animals. O.A. Laktyushina [52] found its decrease in dogs with borreliosis.

The renal failure leads to an increase in the content of the final product of metabolism — creatinine. We observed an increase in this indicator by 40.7% in hares with diagnostically significant antibody titers to *B. burgdorferi*. According to T. Soveri et al. [51] who studied the blood biochemical parameters of the mountain hare, in clinically healthy animals the total protein was 54.0±5.40 mmol g/l, creatinine 92.0±12.90 mmol/l. These figures are consistent with our data. T. Soveri et al. [51] also found that high host animal densities facilitate parasite transmission, and poor feed supply may reduce host resistance to various diseases.

According to V.I. Starostina et al. [53], in people with borreliosis, the content of total protein and AST activity in the blood increase. N.N. Vorobyova et al. [54, 55] found that 31.1% of people infected with Borrelia have a moderate enlargement of the liver, which is accompanied by an increase in the activity of ALT, AST, alkaline phosphatase, and hyperbilirubinemia in the blood serum. M.V. Savelyeva et al. (56) also report an increase in the activity of ALT and AST in 52.9% of people with ITB. According to D.V. Dmitrenko (57), liver pathology in borreliosis manifests itself as anicteric hepatitis which is clinically inconspicuous (minor dyspeptic disorders, moderate increase in liver size). It is detected in patients most often during a biochemical blood test as a moderate increase in the number of transaminases (usually ALT) and/or hyperbilirubinemia which indicates cytolysis syndrome. The results of biochemical studies showed in 50% of sick dogs an increased blood AST content which ranged from 44 up to 87.0 IU/l. ALT was elevated in 30% of cases and ranged from 75.0 to 112.0 IU/l; 30% of dogs showed an increase in creatinine levels from 153.2 to 212.0 mmol/l [49].

P.V. Aksenova [58], studying the blood of free-living bison, found antibodies to *B. burgdorferi* in 16.4% of samples. In Belovezhskaya Pushcha, from 20.0 to 30.0% of bison were seropositive for *B. burgdorferi*. However, the clinical signs of the disease have not been established. The results of our research are also confirmed by the data of N.S. Pustovit [45] who notes that in infected animals the levels of AST, ALT, alkaline phosphatase, creatinine, total protein, and bilirubin increase.

Thus, indirect immunofluorescence assay identified diagnostically significant titers of antibodies to *Borrelia burgdorferi* (1:40 and 1:80) in blood serum of white hare and moose, considered as reservoir hosts of *B. burgdorferi*. Infected amimals statistically significantly differ ($p < 0.05$) from control animals in blood aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase activity and in total protein, total and direct bilirubin, and creatinine levels. In animals with diagnostically significant titers of antibodies to *B. burgdorferi*, some blood parameters correlate, that is, in mountain hare, AST activity with total bilirubin, total protein with direct bilirubin; in young female moose, AST activity with ALT activity, AST activity with total protein, total bilirubin with direct bilirubin; in young male moose, total protein with creatinine. Unlike control animals, in young female moose with diagnostically significant antibody titers to *B. burgdorferi*,

blood alkaline phosphatase activity and total protein correlate positively while correlations between AST activity and total bilirubin, direct bilirubin, and creatinine are negative. In moose with diagnostically significant antibody titers, there are strong correlations for alkaline phosphatase activity between adult and young female, young females and adult males, adult females and males, for total protein level — between young and adult females, young females and adult males, for total bilirubin — between young and adult females, young females and adult males. One-way ANOVA analysis revealed a significant effect of *B. burgdorferi* on increasing biochemical blood parameters, including the AST, ALT, alkaline phosphatase, total protein, total and direct bilirubin, and creatinine concentrations Our data indicate pathology of the cardiovascular system, kidneys and liver in the mountain hare and, mainly, liver pathology in moose under borreliosis. An increase in the concentration of total protein with normal albumin levels in reservoir hosts occurs due to an increase in globulin fractions which indicates acute and/or chronic infection. A significant change in the blood biochemical parameters in reservoir hosts with diagnostically significant titers of antibodies to *B. burgdorferi* indicates its pathological effect on animals, multiple organ failure and irreparable harm to health.

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