Spread of arthropod-borne infections in Kyrgyzstan

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Abstract. The article presents the results of studying the spread of ticks carrying blood parasitic diseases, as well as species of arthropod-borne infections, which were registered among animals in Kyrgyzstan. For research, the material was ticks that parasitize animals, and the blood of sick animals. In total, 6 types of ticks were identified that were carriers of such diseases as: anaplasmosis, babesiosis, theileriosis, nuttaliosis, hemobartonellosis and ehrlichiosis. The work showed a fairly high incidence of blood parasitic diseases among pets. At the same time, it was determined which diseases on which types of ticks circulate on the territory of Kyrgyzstan, and they turned out to be: *Dermacentor marginatus, Rhipicephalus turanicus, Rhipicephalus sanguineus, Hemophysalis punctate, Hyalomma scupense, Hyalomma marginatum.*

1 Introduction

At present, due to the changed conditions of agriculture and climatic changes in the environment, there are also changes in the habitats of ticks, which in turn manifests itself in the emergence of new natural foci of blood parasitic diseases. This phenomenon has been confirmed by many researchers [1-3]. According to A.Kh. Gubeidullina et al., in the forests of the Ulyanovsk region when comparing two observation periods (1966–1969 and 2007–2008) in the forests of the Volga right bank, the habitat of *Ixodes ricinus* decreased significantly, while the number of *Dermacentor reticulatus* increased [1]. According to Roberta Marques et al., climate change is among the most important problems worldwide affecting the geographical distribution of vectors and pathogens, as a result of which there is a threat of the spread of blood parasitic diseases [2]. Blood parasitic diseases of animals are a group of widespread diseases of agricultural, domestic and wild mammals, birds, fish and amphibians (cases of human infection are known), causing pathological changes in the blood and internal organs of animals, the etiological role in which unicellular microorganisms play. Economic damage from these diseases is caused by the death of animals (mortality 30-60%), reduced productivity and reproductive properties of animals, significant costs of preventive and therapeutic measures [4]. These pathogens cause annual losses of US $13–18 billion worldwide [5, 6].

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According to Radiuk E.V. (2018) *Anaplasma phagocytophilum* is a causative agent of granulocytic anaplasmosis not only in dogs, but also in other animal species, including humans. Human granulocytic anaplasmosis (HGA) is recorded worldwide; it can be mild or fatal [7]. Radiuk E.V. claims that the infection of ticks with this pathogen varies depending on the region and ranges from 0% to 46.7% in individual natural foci.

According to Jane E. Sykes [8] presumably *Ixodes ricinus persulcatus* transmit *A. phagocytophilum,* and *Rhipicephalus sanguineus* is the main transmitter of *A. Platys.* The author points out that ticks *Ixodes persulcatus and Dermacentor silvarum* are carriers in Asia and Russia. Other species of *Ixodes spp.* ticks can also be implicated in transmission.

The main transmitter of E. canis is the tick Rhipicephalus sanguineus, known as the brown dog tick [9, 10], however, there is an opinion about the possibility of transmitting the disease to other types of ticks [9].

All Babesia species are transmitted by ticks with a limited number of hosts. The main arthropod carriers are Ixodes ricinus. In some areas, the species Rhipicephalus are the main vectors of Babesia, especially for the species bigemina and bovis.

Babesia canis has intermediate pathogenicity and is widespread in Europe and Asia. *B. gibsoni* is found mainly in the Middle East, South Asia, Japan, North Africa, and South America and is a novel infectious disease in the United States, as well as recently discovered in Italy, Hungary, and Australia. Species such as *Rhipicephalus sanguineus, Dermacentor spp.* and *Haemaphysalis ellipticum can* transmit *Babesia canis*, while *Babesia* gibsoni is transmitted *through* Haemaphysalis *bispinosa and* Haemaphysalis longicornis.

According to scientists in Kyrgyzstan (N.A. Duisheev 1984) [13], the following types of blood-parasitic diseases among cattle and small ruminants have been registered in the republic: piroplasmosis, babesiosis, anaplasmosis and tehleriosis. Currently, there are frequent cases of infection of horses with piroplasmosis and nuttaliosis, outbreaks of hemosporidiosis among domestic animals (dogs and cats) and birds in the republic.

According to A. Aitmyraz kyzy the prevalence of cattle anaplasmosis is 1.7%. The results of PCR and partial DNA sequencing of the 16S ribosomal RNA (rRNA) gene showed that Anaplasma Centrale, A. phagocytophilum like-1 and the human pathogenic novel genotype A. capra circulate in cattle herds in Kyrgyzstan [14].

The distribution and abundance of parasitiform ticks and their carriers of vector-borne infections have not been studied in Kyrgyzstan for the past 30 years. During this period, the living conditions and distribution of ixodic ticks have changed, which in turn can lead to a change in the species composition of the causative agent of vector-borne diseases.

In this regard, this paper presents data on studies of the distribution of the species composition of pathogens of blood parasitic diseases among animals and the species composition of disease vectors.

2 Materials and methods

Objective: to study the spread of blood parasitic disease vector ticks and the types of vector-borne diseases recorded among animals in Kyrgyzstan.

For this purpose, the following specific objectives have been pursued:

1. To study the distribution and species composition of ticks, distributors of blood parasitic diseases, among animals in Kyrgyzstan.
2. Identify the types of blood parasitic diseases among animals in Kyrgyzstan transmitted by ticks.

The research was carried out as part of a research project with the Division of Vectors and Parasitic Diseases of the Republic of South Korea **"Epidemiological surveillance of arthropod-borne and parasitic diseases in Kyrgyzstan"** at the Laboratory of Microbiology and Molecular Biology of the Faculty of Veterinary Medicine of the Kyrgyz

National Agrarian University named after K.I. Scryabin. The material for the research was ticks that parasitize animals and the blood of a sick animal. The research was conducted in Bishkek and in the districts of various regions of the Kyrgyz Republic. Ticks were collected on animals and stored in 96° ethanol. Species belonging of the collected ticks was determined using the Atlas of blood parasites of animals and ticks ixodid by V.F. Kapustin [15]. Microscopy was performed using a trinocular microscope VisiScope TL3841 American company VWR and camera VisiCam 16 Plus. Preparations with blood smears fixed with ethanol were stained with a working solution of azur-eosin (1:10 on distilled water) and kept in athermostat at 37 °C for 40 minutes. The stained preparation was examined under a microscope using immersion oil at 1000x magnification.

3 Results

As a result of studies of ectoparasites (2021-2022) of domestic and farm animals, probable carriers of infectious diseases, 17 species of ectoparasites were found in animals. Of the identified insects, 12 species of ticks of the family Ixodidae, 2 – Argasidae (*A. Lahorensis and Argas persicus* is marked in blue in the table), 2 species (horse flies) of blood-sucking insects of Hippoboscidae family *(Hippobosca equina and Melophagus ovinus are* marked in yellow in the table*)* and one species of flea (*Ctenocephalides canis)*.

From Table No.1 it can be seen that ixode ticks are spread throughout all areas of the republic. The species of ticks *R.turanicus,* according to our research, is distributed in all regions except for the Issyk-Kul and Talas regions. Two species of argasides and Hippoboscidae are recorded throughout the republic. In the north of the republic, *D. marginatus* and *Hl. marginatum are often recorded.* In the south of the republic, only *Hyalomma marginatum* is more common.

During the two-year period of collection of ectoparasites, there were species that were rarely found and were confined to a certain territory. Thus, it was revealed that *H. sulcata* and *Hl. anatolicum* are found only in the Chui valley, and *D. ushakovae* is found on the territory of the Issyk-Kul valley only, *Hl. asiaticum* was identified in the Jalal-Abad region and two species found only in this region were identified in the Naryn region – *D.niveus, D. ushakovae.*

Studies have shown that ectoparasites have a certain type of host on which they parasitize. The widest range of hosts is, judging by the material provided, *Rhipicephalus turanicus* (6 species*),* as well as *Haemaphysalis punctata, Hyalomma marginatum, Hyalomma scupense* (4 species). Monoxenous are *Rhipicephalus sanguineus, Hyalomma asiaticum, Hyalomma anatolicum, Argas persicus, Melophagus ovinus, Hippobosca equina.* Among the captured stray dogs were collected fleas (*Pulicidae – Ctenocephalides canis).*

Table 1. List of ectoparasites of domestic and farm animals of the Kyrgyz Republic

No.	Ectoparasites	Regions	Hosts
1	*Rhipicephalus turanicus*	Bishkek, J-Abad, Naryn, Osh, Chui region	Dogs, cats, cattles, sheeps, goats, horses
2	*Rhipicephalus sanguineus*	Bishkek, Talas	Dogs, cattles
3	*Haemaphysalis punctata*	Bishkek, I-Kul, Naryn, Talas, Chui	Cattles, sheeps, goats, horses
4	*Haemaphysalis sulcata*	Chui	Cattles, sheeps
5	*Dermacentor marginatus*	I-Kul, Naryn, Chui	Cattles, sheeps, horses
6	*Dermacentor niveus*	Naryn	Dogs, cattles, sheeps
7	*Dermacentor ushakovae*	I-Kul, Naryn	Cattles, sheeps
8	*Hyalomma marginatum*	Batken, J-Abad, Osh, Talas, Chui	Dogs, cattles, sheeps, horses

9	*Hyalomma scupense*	J-Abad, Chui	Dogs, cattles, sheeps, horses
10	*Hyalomma asiaticum*	J-Abad	Cattles
11	*Hyalomma anatolicum*	Chui	Cattles
12	*Boophilus annulatus*	Talas, Chui	Cattles, horses
13	*Alveonasus lahorensis*	Batken, J-Abad, Osh, Talas, Chui	sheeps, cattles
14	*Argas persicus*	J-Abad, Osh, Chui	chickens
15	*Melophagus ovinus*	Bishkek, Batken, I-Kul, Naryn, Osh, Chui	sheeps
16	*Hippobosca equina*	Chui	Horses
17	*Ctenocephalides canis*	Chui	Dogs

To study vectors of arthropod-borne infections, ticks from clinically ill animals were selected. As a result, 6 types of disease vector ticks were identified: *Dermacentor marginatus, Rhipicephalus turanicus, Rhipicephalus sanguineus, Hemophysalis punctate, Hyalomma scupense, Hyalomma marginatum*.

Table 2 provides information on the types of ticks and the diseases they carry. Studies have revealed the advantage of carrying arthropod-borne infections among *Dermacentor marginatusticks*, while this type of tick causes a morbidity of 5% in relation to all animals studied. High rates of carriage of pathogens of blood parasitic diseases were also found among ticks of the species *Rhipicephalus turanicus* (4%), *Rhipicephalus sanguineus* (3%), *Hemophysalis punctate* (3%).

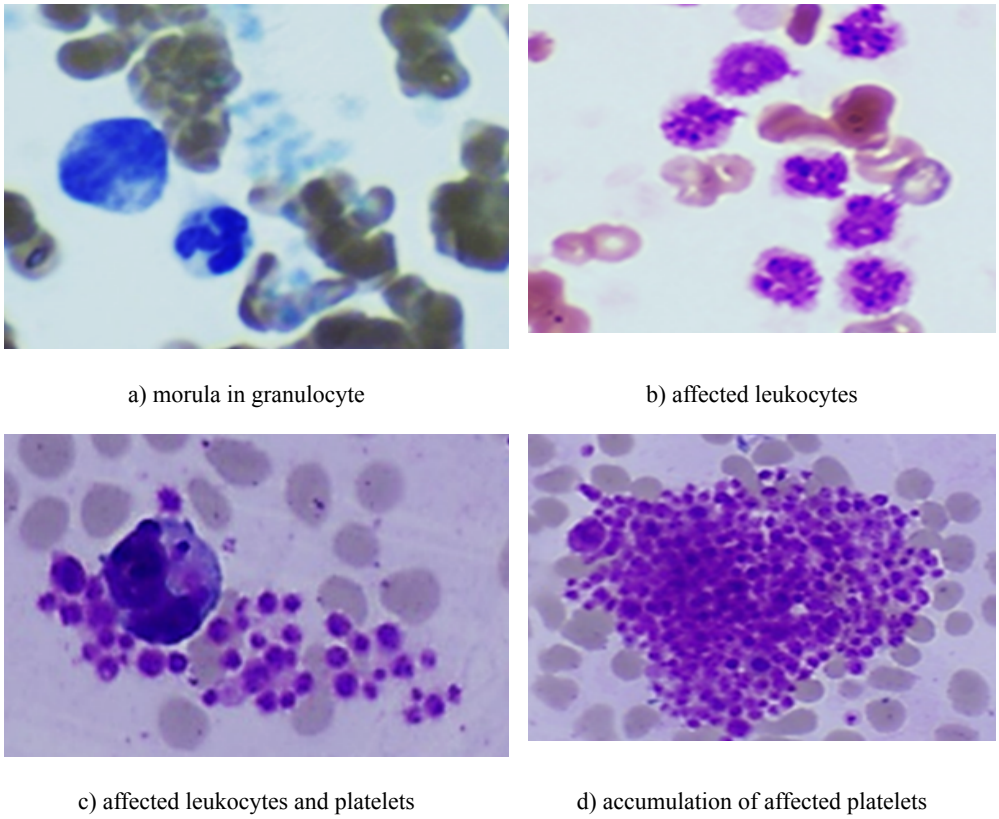
Table 2. Species of blood parasitic disease vector ticks

	Species of tick	Occurrence (%)
1	*Dermacentor marginatus*	5
2	*Rhipicephalus turanicus*	4
3	*Rhipicephalus sanguineus*	3
4	*Hemophysalis punctate*	3
5	*Hyalomma scupense*	1
6	*Hyalomma marginatum*	1

As a result of microscopic studies of blood smears of sick animals, pathogens of blood parasitic diseases were discovered: anaplasmosis, babesiosis, theileriosis, nuttaliosis, ehrlichiosis, hemobartonellosis and toxoplasmosis.

The causative agent of anaplasmosis was recorded among cattle, horses, dogs, and cats and has a high prevalence in Kyrgyzstan. Animals affected by anaplasmosis had characteristic signs: high temperature, anemia of the mucous membranes, lack of appetite, there were cases of convulsions. In anaplasmosis, there is often a lesion of granulocyte leukocytes (neutrophils), the causative agent of which is *Anaplasma phagocytophilum*, while it is possible to observe morules (inclusions) in granulocytes or an erased picture of the morphology of a leukocyte cell. In addition, there is a spread of platelet anaplasmosis, observed in platelets in the form of morules, which look like blue dots in the blood plates. This type of platelet damage is characteristic of platelet anaplasmosis, the causative agent of which is *Anaplasma platys*. Figure 1 shows the forms of damage to blood cells in the form of an affected granulocyte with the morula viewed in it (Fig.1 a, c), the destroyed

nucleus of the cell (Fig.1 b) and platelet accumulation and clumping can be seen (Fig.1 d), which is associated apparently as a response of the body to the effect of the causative agent of platelet anaplasmosis *Anaplasma platys* on platelet cells. Figure 1b shows an increase in leukocytes (granulocytic leukocytosis), toxic granulation in the nucleus and vacuolation in the cell cytoplasm due to anaplasmosis.



a) morula in granulocyte

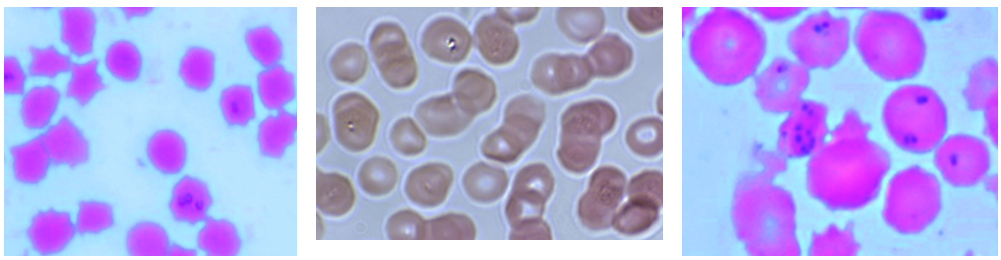
b) affected leukocytes

c) affected leukocytes and platelets

d) accumulation of affected platelets

Fig. 1. Affected granulocytes and platelets in anaplasmosis. Magn. 1000. Giemsa stained.

Very often, cases of erythrocyte damage were recorded, with symptoms characteristic of animal babesiosis. In such animals, clinical signs characteristic of this disease were observed in the form of anemia and jaundice, fever, urine yellow to blood red. During microscopy of a blood smear from such animals, pear-shaped protozoa with a characteristic paired presence inside erythrocytes were detected, and their size reaches 4-5 μm (Fig. 2).



a) Babesiosis in cow b) Babesiosis in dog c) Babesiosis and Theileriosis

Fig. 2. Erythrocytes affected by the causative agent of babesiosis. Magn. 1000. Giemsa stained.

Figure 2 shows the affected red blood cells in the blood of a cow and a dog (Fig.2.a, b) the causative agent of babesiosis, on which paired protozoa are visible. Also, a photo of the affected cow with babesiosis and theileriosis is given, as a co-infection occurring in the animal's body (Fig.2.c).

In dogs, there were cases in monocytes of accumulations of elementary particles of the causative agent of ehrlichiosis in the form of intracellular inclusions (morula), 0.5-1.5 μm in size. At the same time, morula was observed in monocytes, which indicates the presence of the causative agent of monocytic ehrlichiosis *Ehrlichia canis,* which parasitize in the plasma of monocytes in the form of morula and are Giemsa-stained blue (Fig. 3).

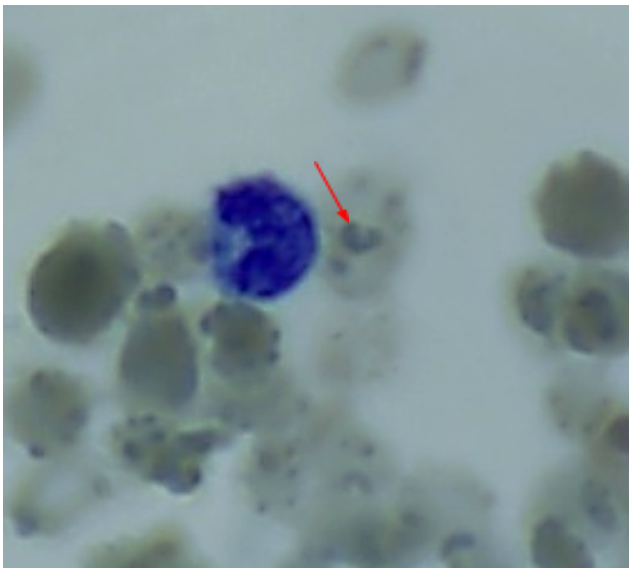
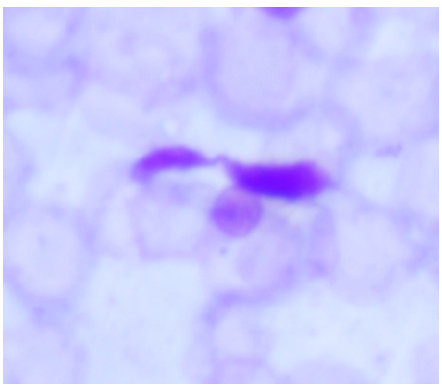


Fig. 3. Morula in a dog's blood monocyte. Magn. 1000. Giemsa stained.

When examining the blood of cattle in a farm affected by ectoparasites (ticks, bloodsuckers), toxoplasma trophozoites were found in blood smears, which were 4-7 microns long and up to 4 microns wide (Fig.4).



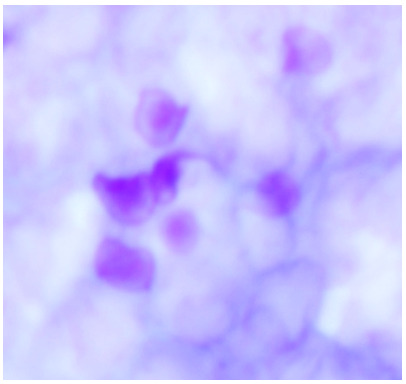


Fig. 4. Trophozoites of the causative agent of toxoplasmosis to the blood of a cow. Magn. 1000. Giemsa stained.

When examining the blood of horses affected by ectoparasites in erythrocytes, pathogens of horses' nuttaliosis, oval and pear-shaped, parallel in erythrocytes, were found, that is, there is a polymorphism of the pathogen, which is characteristic of nuttaliosis (Fig. 5).

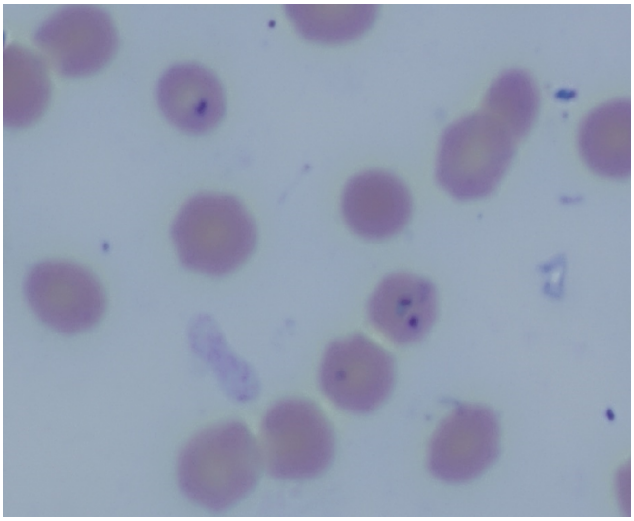


Fig. 5. Affected horse erythrocytes. Magn. 1000. Giemsa stained.

4 Discussion

Climate change is among the most important problems worldwide, affecting the geographical distribution of vectors and pathogens and causing losses in livestock production. In this regard, Roberta Marques [2] points to the potential spread of ticks around the world as an important vector of arthropod-borne animal diseases. Marquez et al. described the current situation of blood parasitic diseases as a potential threat to its geography.

In addition, climate change causes a number of biological changes in vector biology and, consequently, in the incidence of pathogens, which can lead to shifts in the distribution of diseases [3]. This phenomenon is reflected in the studies conducted to identify exotic types of pathogens of blood parasitic diseases for Kyrgyzstan. To date, no animal ehrlichiosis has been reported in the country. Studies have shown the presence of the causative agent of ehrlichiosis among dogs, carriers of which are identified ticks *Dermacentor marginatus* and *Rhipicephalus turanicus.* This type of disease (ehrlichiosis of dogs) was previously believed to be characteristic only for dogs. Currently, the disease is considered zoonotic [9, 10], which increases the risk of the disease and requires special attention and careful research among other species of animals.

The identified causative agent of platelet anaplasmosis *Anaplasma platys* and granulocyte anaplasmosis *Anaplasma phagocytophilum* is an obligate intracellular rickettsial pathogen that affects platelets and granulocytes of animals, forming basophilic intracellular morules [7-9]. In our studies, dogs were infected with anaplasmosis ticks *Dermacentor marginatus, Rhiphicephalus turanicus* and *Haemophisalis punctata.* According to the literature [7-9] *A. platys* was found in ticks *Ixodes persulcatus Ixodes*

ricinus, Dermacentor reticulatus, Rhipicephalus sanguineus, Rhipicephalus turanicus, Haemaphysalis spp. and *Ixodes ricinus.* This fact confirms the change in the biology of vector ticks.

According to some researchers [8, 9], the method of direct detection in a blood smear seems to be more sensitive to the detection of *A. phagocytophilum* than to the detection of *E. canis* in dogs. It is noted that morula can be observed in neutrophils up to 60% of clinical cases. However, morules cannot be distinguished from *Ehrlichia*morules, which infect neutrophils. At the same time, it is noted that the method of detection of morulae in platelets during infection with *A. platys* in some studies show that it may have a higher sensitivity at an early stage of infection [9]. Moreover, studies show the observation of morules in monocytes, which is observed only with infection with *E. canis.*

Many authors [2, 7-9, 16-18] note that *Rhipicephalus (Boophulus) microplu.* is an important vector of babesiosis and anaplasmosis worldwide. In our studies among animals affected by ticks *Dermacentor marginatus, Rhipicephalus sanguineus,* Hemophysalis *punctata revealed circulation in the* blood of animals of the causative agent of babesiosis*,* which probably has to do with changing the biology of tick-borne diseases. Babesia infection refers to a tick-borne infection caused by protozoan parasites. Typical symptoms include fever, anemia, pallor, jaundice, hemoglobinuria, splenomegaly, and weakness [11, 12]. The animals in our studies had clinical signs characteristic of babesiosis and were predominantly affected by *Dermacentor marginatus ticks.* Babesiosis is one of the most important protozoan diseases of life-threatening animals and humans, which is currently recorded worldwide [11, 12].

5 Conclusion

1. Studies have shown a high incidence of pets with blood parasitic diseases.
2. The studies revealed the carrier of vector-borne infections in ticks: *Dermacentor marginatus, Rhipicephalus turanicus, Rhipicephalus sanguineus, Hemophysalis punctate, Hyalomma scupense, Hyalomma marginatum.*
3. The types of blood parasitic diseases circulating on the territory of Kyrgyzstan have been identified: anaplasmosis, babesiosis, theileriosis, nutaliasis, ehrlichiosis, hemobartonellosis and toxoplasmosis.

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