Blood parasitic diseases of farm animals in Kyrgyzstan

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Abstract. This paper presents the results of a study of the distribution of ticks, carriers of transmissible infections, and types of blood-parasitic diseases recorded among domestic animals. Data from studies conducted on dogs and cats in Bishkek and adjacent areas are presented. Tick-infested domestic animals were also used for the study. Of the 135 tick-infested animals studied, 49 were found to have blood parasites, with the incidence of disease among domestic animals in Bishkek amounting to 36.3%. Six species of ticks were identified on the animals: *Rhipicephalus turanicus, Haemophysalis punctata, Rhipicephalus bursa, Ixodes ricinus, Hyalommas cupense, Dermacento rmarginatus*. Blood tests of tick-infested dogs and cats revealed the presence of pathogens causing blood-parasitic diseases, including ehrlichiosis, babesiosis, and anaplasmosis. In dogs infected with ehrlichiosis, inclusion bodies of *Ehrlichia canis* were detected in monocytes and *Ehrlichia ewingii* in granulocytes. Babesia gibsoni protozoa were detected in the blood of dogs infected with babesiosis. Anaplasma platys was detected in the blood of a sick animal with anaplasmosis. Previously, Kyrgyzstan had no cases of detection of the pathogens causing monocytic, granulocytic, and platelet ehrlichiosis, anaplasmosis caused by *Anaplasma platys*, or canine babesiosis caused by *Babesia gibsoni*.

1 Introduction

Blood-parasitic diseases are primarily transmitted by ticks, which carry infectious agents. Depending on the blood-parasitic pathogens carried by ticks, diseases can be caused by protozoa (babesiosis), rickettsia (anaplasmosis, ehrlichiosis), and mycoplasmas (hemobartonellosis).

Due to global climate change, tick habitats are shifting, which in turn is causing the spread of transmissible blood-parasitic infections in these new regions [1-3]. While a particular tick species was previously considered the characteristic carrier of a specific infectious agent in a given region, the specific tick species currently carrying the infection has changed [4-6]. Furthermore, the distribution range of infectious diseases has also shifted. Consequently, new types of transmissible infections previously not recorded in a

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given area are being identified worldwide [7-8]. All of this is associated with the formation of new natural foci of vector-borne infections and the habitat of ticks carrying the infectious agent.

In Kyrgyzstan, babesiosis, anaplasmosis, and haemobartonellosis are reported among domestic animals. In recent years, ehrlichiosis, which had not previously been recorded, has been detected among dogs and cats.

Therefore, the aim of this study was to determine the species composition of ticks that cause vector-borne infections and the types of diseases.

2 Materials and methods

Ticks parasitizing animals and the blood of sick domestic animals served as the study material. A total of 318 domestic animals were examined in a tick outbreak. Blood was collected from 135 tick-infested animals (dogs and cats) suspected of having blood parasitic diseases, and ticks were collected for laboratory testing. The species of the collected ticks was determined using the Atlas of Animal Blood Parasites and Ixodid Ticks [9]. Microscopy was performed using a Visi Scope TL3841 trinocular microscope and a Visi Cam 16 Plus camera. Blood smear preparations were stained with the Romanowsky-Giemsa method. The stained preparation was examined under a microscope using immersion oil (at 1000x magnification).

The bioassay was conducted on white laboratory mice, using 0.3 ml of sick animal blood intramuscularly.

3 Results

A study of 318 domestic animals (cats and dogs) revealed ticks on 135 animals, representing a 42.5% prevalence rate, indicating a high tick infestation rate. The tick infestation rate per individual infested host was 1.9. The average tick count per individual studied domestic animal (abundance index) was 0.8. These data indicate a high tick infestation rate. Table 1 presents data on the species composition and prevalence of ticks in Bishkek and the surrounding area.

Table 1. Types of ticks and their prevalence.

Types of ticks	Number of animals studied		Ticks collected	Extensiveness, %	Abundance index	Intensity
	Total	Tick-ridden				
Rhipicephalus turanicus, Haemophysalis punctata, Rhipicephalus bursa, Hyalomma scupense, Ixodes ricinus, Dermacentor marginatus	318	135	261	42.5	0.8	1.9

Source: [Compiled by the authors.]

Six species of ticks carrying diseases were identified on animals: *Rhipicephalus turanicus* (Pomerantsev 1936), *Haemaphysalis punctata* (Canestrini & Fanzago, 1878), *Rhipicephalus bursa* (Canestrini & Fanzago, 1878), *Ixodes ricinus* (Linnaeus, 1758), *Hyalomma scupense* (Schulze, 1919), and *Dermacentor marginatus* (Sulze, 1770).

Of the 135 tick-infested animals, 49 were found to be infected with blood parasites. The incidence of blood parasites in domestic animals in Bishkek was 36.3%, indicating the city's epizootic status for this disease group.

Table 2 presents information on tick species and the diseases they transmit. Studies have revealed a predominance of vector-borne infections among *Dermacentor marginatus* ticks, with this species causing morbidity at a rate of 5.03% of all animals studied. High rates of carriage of blood-parasitic pathogens were also found among *Rhipicephalus turanicus* (3.46%), *Haemophysalis punctata* (2.51%), *Rhipicephalus bursa* (1.89%), and *Hyalomma scupense* (1.26%) ticks. Ixodes ricinus ticks posed the least risk in the studies, with only two animals tested positive for babesiosis.

Table 2. Types of ticks that carry diseases.

Type of ticks	Name of the disease	Number of infected animals	Number of sick animals	Morbidity (%)
Dermacentor marginatus	Anaplasmosis	8	16	5.03
	Babesiosis	6		
	Ehrlichiosis	2		
Rhipicephalus turanicus	Babesiosis	7	11	3.46
	Ehrlichiosis	4		
Rhipicephalus bursa	Babesiosis	6	6	1.89
	Anaplasmosis	6		
Hemophysalis punctata	Ehrlichiosis	8	8	2.51
	Anaplasmosis	8		
Hyalomma scupense	Babesiosis	4	4	1.26
Ixodes ricinus	Babesiosis	2	2	0.63

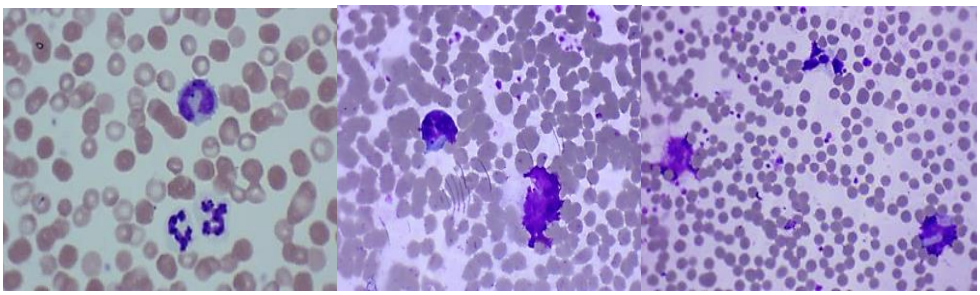
Source: [Compiled by the authors]

Table 2 shows that anaplasmosis is caused by ticks of the species *Dermacentor marginatus, Rhipicephalus bursa*, and *Hemophysalis punctate*. The incidence of anaplasmosis among all animals studied was 10.1%. Babesiosis is caused by ticks of the species *Dermacentor marginatus, Rhipicephalus turanicus, Rhipicephalus bursa, Hyalomma scupense,* and *Ixodes ricinus* - that is, all tick species identified except Hemophysalis punctate. The incidence of babesiosis was 7.9%. Ehrlichiosis was caused by ticks of the species *Dermacentor marginatus, Rhipicephalus turanicus,* and *Hemophysalis punctate*. The incidence of babesiosis was 4.4% of susceptible animals. Of the transmissible ticks we discovered, *Dermacentor marginatus* posed the greatest danger. It can transmit all types of blood-parasitic diseases reported in domestic animals and can cause high disease prevalence among these animals.

Furthermore, it was observed that *Dermacentor marginatus, Rhipicephalus turanicus, Rhipicephalus bursa,* and *Hemophysalis punctuate* ticks frequently caused coinfections, meaning that the pathogens of two or more blood-parasitic diseases were detected simultaneously in sick animals.

Microscopic examination of the blood of sick animals revealed pathological changes in blood cells characteristic of these diseases.

Leukocyte damage was observed, manifested as destructive changes in immune cells, increased nuclear segmentation, cytoplasmic vacuolization, and lysis of the nucleus and cell wall, indicating the toxic effects of pathogenic factors on the body caused by the pathogens during the infectious process (Fig. 1).



a) b) c)

Figure 1. Lesions of leukocytes. a) hypersegmentation of the leukocyte nucleus. b) destructive changes in the leukocyte. c) lysis of two blood leukocytes. Magnification 1000. Stained according to Romanovsky-Giemsa.

Clusters of elementary particles of the pathogen (Ehrlichia rickettsia) were observed in monocytes and leukocytes as intracellular inclusions (morulae), 0.5–1.5 μm in size, which is characteristic of canine ehrlichiosis (Fig. 2). Morulae were also observed in monocytes, indicating the presence of the causative agent of monocytic ehrlichiosis, Ehrlichia canis, which parasitizes monocyte plasma as morulae and stains blue using the Romanovsky-Giemsa method. In Figure 2, a morula in a monocyte is indicated by an arrow.

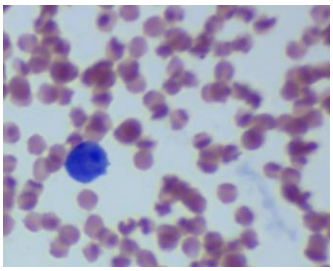


Figure 2. Morula in a dog blood monocyte. Magnification 1000. Stained according to Romanovsky-Giemsa.

Furthermore, morulae were also observed in granulocytic leukocytes in this same animal, indicating the presence of the granulocytic ehrlichiosis pathogen, *Ehrlichia ewingii*. In Figure 3, morulae in granulocytes are marked with a red arrow.

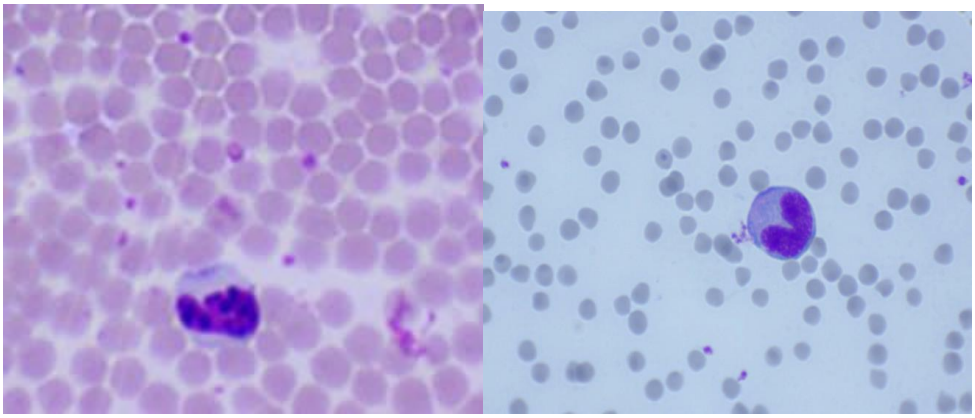
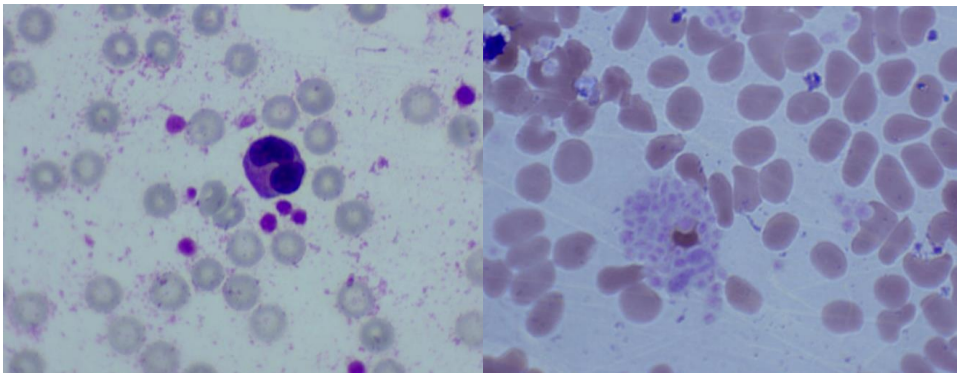


Figure 3. Morulae in a granulocyte of dog blood. Magnification 1000. Stained according to Romanovsky-Giemsa.

There have been cases of platelet damage by blood parasite pathogens, manifesting as morulae, which appear as blue inclusions within platelets. This type of platelet damage is characteristic of platelet-mediated anaplasmosis, caused by *Anaplasma platys*. Figure 4(a) shows a photograph of affected platelets, with morulae visible in the granulocyte, indicating coinfection with granulocytic anaplasmosis, caused by *Anaplasma phagocytophilum*. Figure 4(b) shows platelet aggregation and clumping, which is likely related to the body's response to the effect of *Anaplasma platys*, the pathogen that causes platelet-mediated anaplasmosis, on platelet cells.



a) b)

Figure 4. Affected platelets. Magnification 1000. Stained according to Romanovsky-Giemsa: a) damaged platelets and granulocytes b) platelet accumulation

Cases of red blood cell damage, with symptoms characteristic of canine babesiosis, were very frequently recorded. These dogs exhibited fever, jaundice, impaired movement, and one case of hindlimb paresis. Microscopic examination of blood smears from these animals revealed pyriform protozoa, characteristically paired within red blood cells, reaching 4-5 µm in size (Fig. 5). This pathogen is characteristic of the species *Babesia canis*.

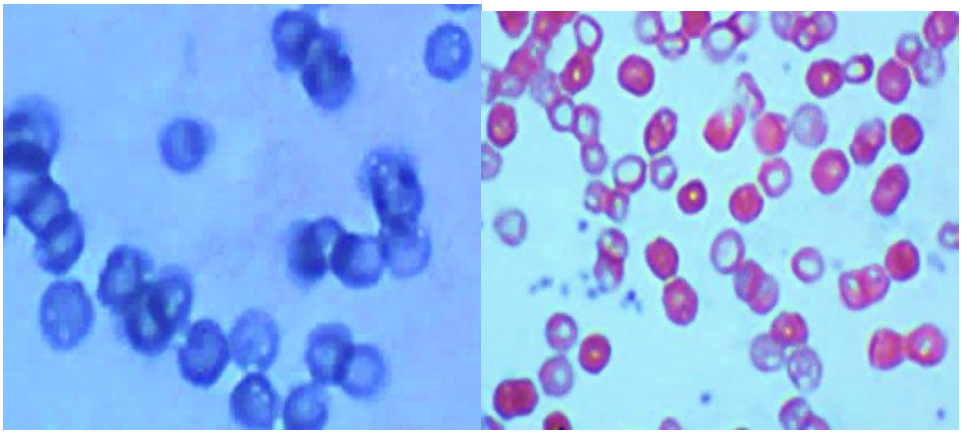


Figure 5. *Babesia canis* in dog erythrocytes. Magnification 1000. Stained according to Romanovsky-Giemsa.

In addition, there were cases of detection of the pathogen species *Babesia gibsoni*, which had the appearance of small round or oval protozoa (Fig. 6).

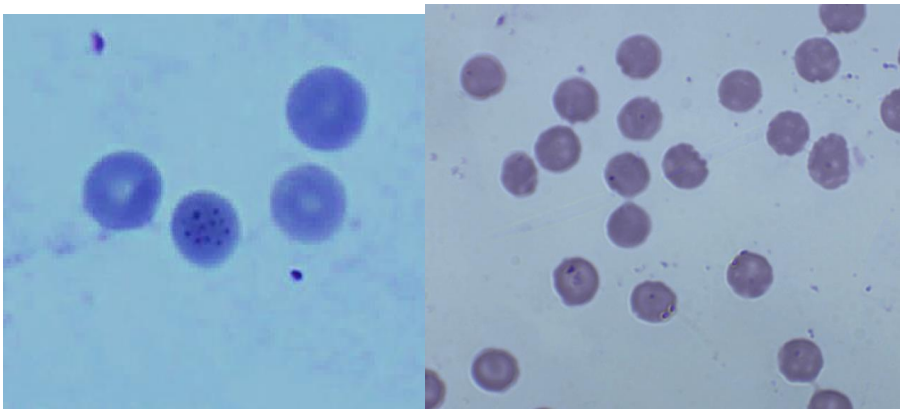


Figure 6. *Babesia gibsoni* in canine erythrocytes. Magnification 1000. Stained according to Romanovsky-Giemsa.

Bioassay on laboratory mice.

To study the susceptibility of laboratory mice to blood parasites, they were inoculated with blood from sick animals with obvious clinical signs confirmed by microscopic examination. The infection was administered intramuscularly with a volume of 0.3 ml.

The mice showed no clinical changes for two weeks. Euthanasia was performed on the third week; no changes were detected in the internal organs. Blood microscopy revealed the presence of the pathogen in the formed elements of the blood. Figure 7 shows morulae in a monocyte, and vacuolization and granularity of the leukocyte cytoplasm are noticeable, indicating a toxic effect in immune cells.

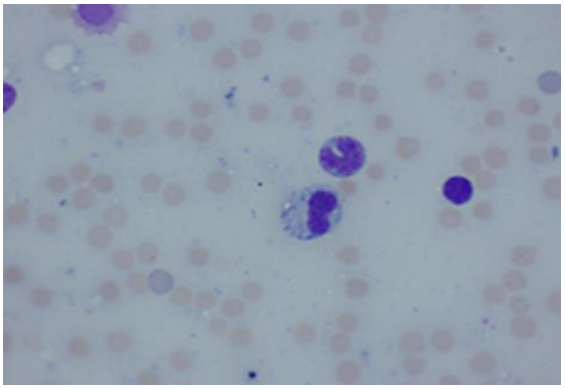
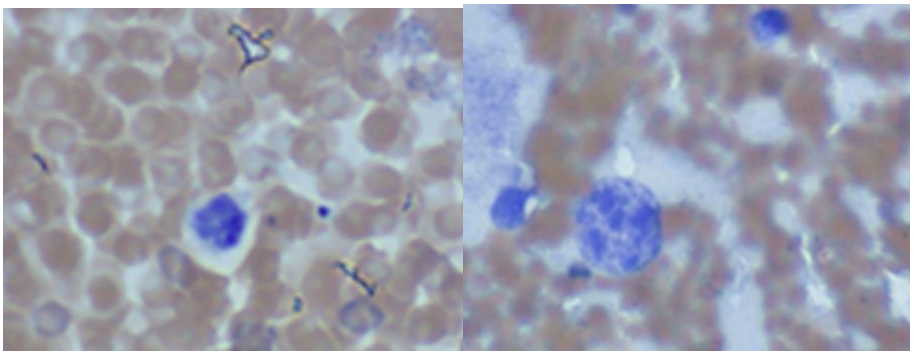


Figure 7. Microscopy of mouse blood (monocytes show morulae and vacuolization, neutrophils show vacuolization and granularity). Magnification 1000. Stained according to Romanovsky-Giemsa.

Microscopy of smears of internal organ imprints revealed phagocytic activity. Figure 8 shows an increase in phagocytes in the lung (Fig. 8a) and an increase in the number and volume of phagocytes in liver imprint smears (Fig. 8b).



a) b)

Figure 8. Phagocytic activity in parenchymatous organs. Magnification 1000. Stained according to Romanovsky-Giemsa: a) lung b) liver.

Spleen smears show high phagocytic activity of immune cells. Figure 9 shows an increase in leukocyte cells, particularly lymphoid cells and macrophages. Parasites are also visible within the phagocytic cells as cellular inclusions, as well as the process of parasite phagocytosis.

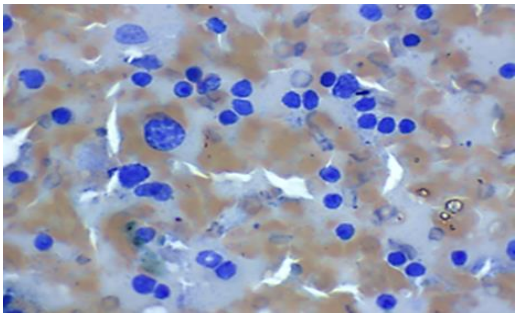


Figure 9. Phagocytic activity in the spleen. Magnification 1000. Stained according to Romanovsky-Giemsa.

Research has shown a high tick infestation rate among domestic animals in Bishkek, identifying six species of transmissible ticks (*Rhipicephalus turanicus, Haemophysalis punctata, Rhipicephalus bursa, Hyalomma scupense, Ixodes ricinus, and Dermacentor marginatus*) that cause blood parasitic diseases. Research has also determined that anaplasmosis is caused by ticks of the species *Dermacentor marginatus, Rhipicephalus bursa,* and *Hemophysalis punctata*. The incidence of anaplasmosis among all animals studied was 10.1%. Babesiosis is caused by ticks *Dermacentor marginatus, Rhipicephalus turanicus, Rhipicephalus bursa, Hyalomma scupense, and Ixodes ricinus* - that is, all identified tick species except *Hemophysali spunctata*. The incidence of babesiosis was 7.9%. Ehrlichiosis was caused by ticks *Dermacentor marginatus, Rhipicephalus turanicus,* and *Hemophysalis punctata*. The incidence of ehrlichiosis was 4.4% of susceptible animals.

Bioassays conducted on white mice did not produce the characteristic pathological changes observed in domestic animals; however, an immunological response to the pathogen was detected.

4 Discussion

Studies show that the incidence of blood-parasitic diseases among domestic animals in Bishkek and surrounding areas was 36.3%, indicating the city's epizootic status for animal blood-parasitic diseases. The prevalence of infestation was 42.5%, indicating a high prevalence of tick infestation.

Microscopic studies to determine the type of transmissible infection revealed the presence of blood-parasitic pathogens such as anaplasmosis, babesiosis, and ehrlichiosis in the blood. Anaplasmosis was found to be caused by ticks of the species *Dermacentor marginatus, Rhipicephalus bursa,* and *Hemophysalis punctata*. The incidence of anaplasmosis among all animals studied was 10.1%. Babesiosis is caused by ticks of the species *Dermacentor marginatus, Rhipicephalus turanicus, Rhipicephalus bursa, Hyalomma scupense,* and *Ixodes ricinus* - that is, all identified tick species except *Hemophysalis punctate*. The incidence of babesiosis was 7.9%. Ehrlichiosis was caused by ticks of the species *Dermacentor marginatus, Rhipicephalus turanicus,* and *Hemophysalis punctuate*. The incidence of ehrlichiosis was 4.4% of susceptible animals.

Of the transmissible ticks we identified, *Dermacentor marginatus* poses the greatest danger. It can transmit all types of blood-borne diseases reported in domestic animals and can cause high disease prevalence among these animals.

Furthermore, *Dermacentor marginatus, Rhipicephalus turanicus, Rhipicephalus bursa,* and *Hemophysalis punctata* ticks were observed to frequently cause coinfections, meaning that infected animals were simultaneously infected with the pathogens of two or more blood-borne diseases.

In studies, the pathogens that cause platelet-borne anaplasmosis, *Anaplasma platys*, and granulocytic anaplasmosis, *Anaplasma phagocytophilum*, were detected in dogs infested with *Dermacentor marginatus, Rhipicephalus turanicus,* and *Haemophysalis punctata* ticks. Previously, there were reports that anaplasmosis in dogs was caused primarily by ticks of the species Ixodes persulcatus. However, recently, information has emerged on the species diversity of ticks that carry anaplasmosis in animals, including *Dermacentor reticulatus, Ripicephalus turanicus, Ixodes spp., and Haemaphysalis spp.* [10]. However, the available literature did not contain information on *Dermacentor marginatus* as the primary vector of infection.

Studies have shown that babesiosis is transmitted by *Dermacentor marginatus, Rhipicephalus turanicus, Rhipicephalus bursa, Hyalomma scupense,* and *Ixodes ricinus* ticks - all tick species found in dogs except *Hemophysalis punctata* - indicating the diversity of vectors for this infection. Animals infected with babesiosis exhibited

characteristic clinical signs, including fever, jaundice, impaired movement, and one case of hindlimb paresis, which was important for targeted differential diagnosis. Microscopic examination of blood smears from these animals revealed pyriform protozoa with characteristic paired erythrocytes, reaching 4-5 μm in size. This pathogen is characteristic of the *Babesia canis* species, which is widespread worldwide. In addition, studies have identified cases of the causative agent *Babesia gibsoni*, which appeared as smaller round or oval protozoa. This species, which causes canine babesiosis, is considered a new pathogen and has become widespread worldwide in recent years [11]. This species was identified for the first time in Kyrgyzstan. There are reports that the carriers of *Babesia canis* babesiosis are the ticks *Dermacentor reticulatus, Babesi agibsoni – Haemaphysalis ssp. and Rhipicephalus ssp.* [12].

In addition, studies conducted in monocytes and leukocytes revealed the presence of erlichiosis pathogens, which appeared microscopically as intracellular inclusions (morulae) measuring 0.5–1.5 μm. Morulae were observed predominantly in monocytes, indicating the presence of the monocytic ehrlichiosis pathogen, *Ehrlichia canis*. Morulae parasitize monocyte plasma as morulae and stain blue according to the Romanovsky-Giemsa method.

Morulae were also observed in granulocytic leukocytes, indicating the presence of the granulocytic ehrlichiosis pathogen, Ehrlichia ewingii. This marks the first recorded case of this ehrlichiosis pathogen circulating among animals in Kyrgyzstan.

Ehrlichiosis in dogs is caused by three species of Ehrlichia: *Ehrlichia canis*, *E. ewingii*, and *E. chaffeensis*, which infect platelets, monocytes, and granulocytes. According to the Centers for Disease Control and Prevention (CDC), these species of Ehrlichia also cause disease in humans [13, 14].

Ehrlichiosis is caused by ticks of the species *Dermacentor marginatus, Rhipicephalus turanicus,* and *Hemophysalis punctata*.

Mice are naturally occurring carriers of blood parasite pathogens. Therefore, in acute experiments, no clinical changes were observed when animals were infected with the blood of animals infected with blood parasites. However, abnormalities in blood cells and phagocytic cell activity in parenchymal organs were detected.

5 Conclusion

The following findings were obtained from the study:

1. The studies revealed a high incidence of blood parasitic diseases in domestic animals (36.3%).

2. Cases of ehrlichiosis in dogs were identified, with inclusions of *Ehrlichia canis* in monocytes *and Ehrlichia ewingii* in granulocytes.

3. The first case of infection with the protozoan Babesia gibsoni in dogs in Kyrgyzstan, causing clinical signs of babesiosis, was identified.

4. Cases of circulation of the causative agent of platelet-mediated anaplasmosis, *Anaplasma platys*, in the blood of dogs were identified.

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