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Epizootological aspects of pyroplasmids in cows (*Bos taurus*) in Azerbaijan

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Abstract. This article analyzes the epizootological situation of pyroplasmosis in cattle in Azerbaijan from the middle of the last century to the present. The blood samples were collected from cattle at private farms in Absheron Region, Azerbaijan. Total 87 cows were examined for blood parasite infection. Experiments were carried out from January to November 2021. Modified Romanovsky-Giemsa staining was used on peripheral blood smears of suspected cattle. Among 87 clinically suspected cattle examined, only 16 cows (18.4%) had *Babesia* infection — *Babesia* sp. The present study also analyzes the prevalence of blood protozoa in relation to season dynamics. The study results revealed that the prevalence of Babesiosis infection in cows in Absheron Region is much higher in spring (31.8%) and autumn (27.3%) than in summer (11.5%). Babesiosis was found in all seasons except winter.

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Keywords: piroplasmids, Babesiosis, ticks, cow, blood parasites, cattle, *Babesia*

Эпизоотологические аспекты пироплазмоза коров (*Bos taurus*) в Азербайджане

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Аннотация. В данной статье анализируется эпизоотологическая ситуация по пироплазмозу крупного рогатого скота в Азербайджане с середины прошлого века по настоящее время. Образцы крови были взяты у крупного рогатого скота из частных хозяйств Абшеронского района Азербайджана. Всего на зараженность кровепаразитами обследовано 87 коров. Эксперименты проводились с января 2021 г. по ноябрь 2021 г. Модифицированная краска по Романовскому-Гимзе использовалась для мазков периферической крови зараженного крупного рогатого скота. По результатам исследований среди 87 обследованных коров с подозрением на клинические проявления только 16 коров (18.4%) были инфицированы бабезиями — *Babesia* sp. В настоящем исследовании также изучалась зараженность простейшими крови в зависимости от сезонной динамики. Выявлено, что распространенность бабезиозной инфекции у коров Абшеронского района значительно выше весной (31.8%) и осенью (27.3%), чем в летний период (11.5%). Бабезиоз обнаруживали во все сезоны года, кроме зимы.

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Ключевые слова: пироплазмиды, бабезиоз, клещи, корова, кровепаразиты, крупный рогатый скот, бабезиоз

Introduction

The fauna of pyroplasmids is especially rich in countries with a hot climate, and therefore the most serious economic losses from pyroplasmidosis are borne by animal husbandry in Africa, South and Central America, Southern Europe and Asia (He et al. 2021). Outbreaks of the disease occur in spring, summer and sometimes fall.

Pyroplasmids cause colossal damage to agriculture. So, according to some reports, from 1907 to 1938 in the United States alone, more than 456 million dollars were spent on programs to combat tick vectors (Graham 1977). In Ireland, from 1.3 to 2.5% of the cattle population suffers from babesiosis every year, and more than 10% of the diseased animals die. In England and Scotland, the damage from babesiosis is about 450 thousand dollars annually, and in Northern Ireland — 320 thousand dollars (Gray, Harte 1985).

The family Babesiidae is the richest in quantity and quality. The wide distribution and variety of babesid species is observed largely due to their ability to circulate in many generations of carrier ticks, passing from generation to generation of invertebrate hosts transovarially. The family Babesiidae includes four genera: *Babesia*, *Piroplasma*, *Francaiella* and *Nuttallia*.

Pyroplasmids are single-celled protists with a complex development cycle, parasitic in representatives of all classes of vertebrates and in ticks of the superfamily Ixodoidea. The life cycle of pyroplasm takes place in the organisms of two hosts — an invertebrate host and tick vectors. Reproduction of pyroplasmas in the body of an invertebrate host occurs in the blood by simple division, and in the body of ticks — in tissues, hemolymph and eggs (Yokoyama 2018). In the body of an invertebrate host, they multiply first in the internal organs, and then in the peripheral blood.

Babesiosis is a tick-borne disease of economic importance in livestock caused by *Babesia* spp., which are hemoparasitic piroplasmids that target the host erythrocytes. Cat-

tle, dogs, small ruminants and wild ruminants are the species most commonly affected, while in cats, horses and pigs it is observed less frequently.

Bovine babesiosis is a tick-borne disease of cattle caused by protozoan parasites of the genus *Babesia*, order Piroplasmida, phylum Apicomplexa. The principal species of *Babesia* that cause Bovine babesiosis are *Babesia bovis*, *Babesia bigemina* and *Babesia divergens*. *B. major*, *B. ovata*,

B. occultans and *B. jakimovi* also can infect cattle (Thompson, Goodrich 2018).

B. bigemina is the major large species that causes the disease called Texas fever, and *B. bovis* is the major small species (Thompson, Goodrich 2018). The parasite — *B. bovis* — positions itself as single, multiple, or paired complexes within erythrocytes (Fig. 1). Erythrocytes infected with *B. bovis* are harder to identify than in *B. bigemina* infection. *B. bovis* can change the structure and the function of erythrocytes leading to fatal cerebral babesiosis.

The aim of this research was to study the infection of cows with pyroplasmids from private farms in Absheron Region of Azerbaijan.

Materials and methods

Experiments were carried out in the Protozoology Laboratory of the Institute of Zoology of ANAS from January to November

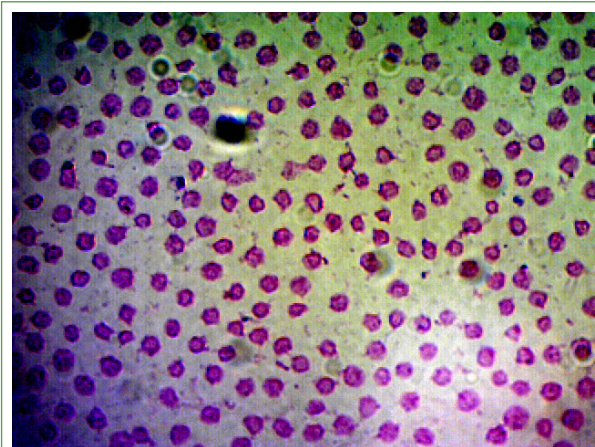


Fig. 1. Erythrocytes of cows infected with *Babesia* sp.

Рис. 1. Эритроциты коров, инфицированных *Babesia* sp.

2021. Materials constitute the fauna of blood parasites in the examined 87 cows from private farms in Absheron Region of Azerbaijan.

Microscopic detection of *Babesia* was performed using the microscope with a video camera (Carl Zeiss Axio Scope A1) with oil immersion (400× and 1000×) for hemoparasites, the genus of which was identified based on their morphology. The number of parasites observed in 100 optic fields was recorded. Blood smears were made on site, air dried, fixed with 100% methanol and then stained using a modified Romanovsky-Giemsa staining technique (Marshall 1978). The genus and species of parasites found in the blood were determined upon their morphological traits (Krylov 1996, Uilenberg 1995).

All animals from private farms in Absheron were studied for the extensiveness of invasion by pyroplasmids in all seasons of the year, calculated for 50 fields of view of the microscope. Data from the study were entered in Ms Excel; the IBM SPSS Statistics 20 statistical program was used for statistical processing of the results.

Results

Table 1 presents the obtained morphological data as well as the data on parasites studied throughout the republic in 2013–2014. The table indicates comparative morphological characteristics of three species of pyroplasmids.

The obtained data indicates the sizes and shapes of the *Babesia* sp. parasites found in the erythrocytes of the cows from Absheron Region of Azerbaijan.

The larger form of *Babesia* sp. has the sizes of 4.0–5.3 mkm × 2.1–3.5 mkm. The parasites are characteristically oval shaped. *Babesia* sp. has a smaller form (3.5–4.7 mkm × 2.0–3.6 mkm). These parasites are characteristically figure eight shaped.

In this study we investigated seasonal prevalence of *Babesia* infection in 87 cows (Table 2). Among 87 clinically suspected cattle examined, only 16 cows (18.4%) had *Babesia* infection.

Table 2 indicates that the prevalence of Babesiosis infection in cows in Absheron Re-

gion is much higher in spring (31.8%) and autumn (27.3%) than in summer (11.5%). Babesiosis was found in all seasons except winter.

Discussion

Blood parasitic diseases (piroplasmosis) are widespread in Azerbaijan, mainly in the subtropics. Cattle and other animals are affected. In the southern zone of Azerbaijan, natural and climatic conditions and the relief are favorable for the development and spread of various species of ticks — carriers of blood-parasitic diseases of domestic animals — over the vast territory. This zone is also rich in species composition of pyroplasmidosis in cattle. The study established strong infestation of ticks collected from cattle in this area by *Babesia* sp. parasites (Mirzabekov, Mamedova 2014).

Ramgopal L. et al. (2015) recorded that *B. bigemina* parasites are large and characteristically pear shaped. Round (2–3 μm in diameter) oval or irregularly shaped forms of *Babesia* may also be found (4.5 μm × 2.0 μm). *B. bovis* parasites are a small form of *Babesia* (2.0 μm × 1.5 μm). Slightly larger than *B. divergens*, vacuolated signet ring forms are particularly common (Laha et al. 2015). *B. divergens* is a small form (1.5 μm × 0.4 μm) of *Babesia*. Parasites generally remain as a paired form, superficially lie on the RBC; stout and pyriform or circular forms may be found. *B. major* is a large form (3.2 μm × 1.5 μm) of *Babesia*. Pyriform bodies, the angle between the organism is < 90. Round forms are with a diameter of about 1.8 μm (Laha et al. 2015).

Piroplasmosis, the biology and ecology of ixodid ticks, carriers of their pathogens, and the epizootological situation of blood-parasitic diseases of animals in Azerbaijan have been investigated since the 1940s. At the beginning of the first half of the last century, 24 species of ixodes ticks belonging to six genera and 15 species of pyroplasmids were registered in Azerbaijan (Mirzabekov, Mamedova 2014).

The first detection of blood-parasitic diseases of cattle in Azerbaijan was described at the beginning of the 20th century. In 1903 E. P. Dz-hunkovskiy and I. M. Luz found the causative agent of theileriosis — *Theileria annulata* — in

Table 1

Comparative morphological characteristics of three species of pyroplasmids

Таблица 1

Сравнительная морфологическая характеристика 3-х видов пироплазмид

Shape of parasite	<i>Francaella colchica</i> (mkm) (by K. D. Mirzabekov, G. R. Mamedova)	<i>Babesia bigemina</i> (mkm) (by K. D. Mirzabekov, G. R. Mamedova)	<i>Babesia</i> sp. (mkm) (by K. D. Mirzabekov, G. R. Mamedova)	<i>Babesia</i> sp. (mkm) (the obtained data)
pear	2.8–3.9x1.4–1.9	3.7–5.6x2.1–2.7	—	—
oval	2.4–2.6x1.8–2.0	3.6–4.0x2.3–2.4	4.2–5.7x2.3–3.8	4.0–5.3 × 2.1–3.5
cigar-shaped	3.2–3.6x1.7–1.8	3.6–4.2x1.9–2.0	—	—
rod-shaped	2.1–2.3x0.9–1.1	—	3.4–5.5x1.2–1.7	—
figure eight	—	—	3.6–4.9x1.8–2.5	3.5–4.7 × 2.0–3.6
amoeboid form	5.2–5.3	—	3.6–4.2x1.7–2.7	—
banana shape	—	—	4.2–5.6x1.9–2.9	—
sickle shape	—	—	2.8–4.2x1.0–2.4	—

cattle for the first time on the territory of Azerbaijan (Goy-Gel Region). In the subsequent years these researchers described piroplasmidosis of cattle and small ruminants in Azerbaijan. In 2010–2014, 11 species of ticks of the family Ixodidae related to all four genera were recorded in the southern part of Azerbaijan and 16 species of ticks were registered throughout the republic of Azerbaijan. The species composition in the southern part of Azerbaijan is also rich in cattle pyroplasmids. In addition to the pyroplasmids specific for this zone — *B. bigemina*, *Fr. colchica*, *Anaplasma marginale* and *Th. annulata* — three new species of pyroplasmids were also reported. In addition, new species for the southern

zone — *Fr. caucasica* and *Th. mutans* — were identified. *Th. mutans* was identified in 20 regions of the republic of Azerbaijan (Mirzabekov, Mamedova 2014).

The obtained results of the study concerning pyroplasmids in Azerbaijan by seasons are comparable to other studies. Seasonal prevalence of *Babesia* infection recorded in this study also supports other authors' reports (Miah Y. et al. 2008). No babesiosis was found during the rainy season (Nath et al. 2013).

Based on the results of our research, it is necessary to continue epizootic monitoring of cattle babesiosis in order to reduce the economic damage from blood parasitic diseases.

Table 2

Season-wise prevalence of Babesiosis infection

Таблица 2

Сезонная распространенность инфекцией Babesiosis

Season	Number of animals	Babesiosis	
Winter	17	0	0 %
Spring	22	7	31.8%
Summer	26	3	11.5%
Autumn	22	6	27.3%
Total	87	16	18.4%

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