



UDC: 636.709:616.99

## PECULIARITIES OF DOG BABESIOSIS DISTRIBUTION IN KYIV CITY

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**Peculiarities of Dog Babesiosis Distribution in Kyiv City. Semenko, O. V., Galat, M. V., Shcherbak, O. V., Galat, V. F., Shulga, I. V.** — The article presents the information on the distribution of babesiosis among domestic dogs of Kyiv City investigated in 2014–first half of 2017. Among 2030 examined dogs, *Babesia canis* was found in 416 animals. The prevalence of infection was 20.5 %. Dependence of the number of cases of babesiosis on the temperature of the environment was indicated. The highest level of dogs' infection was observed in spring (April–May) and autumn (September–October). Ixodid tick *Dermacentor reticulatus* was found to be the most common vector of dog babesiosis in Kyiv.  
Key words: *Babesia canis*, babesiosis, dogs, ticks, distribution, Kyiv.

### Introduction

In recent years, the problem of the spreading of transmissible diseases has become acute. It is contributed by lots of factors, such as climate changes, increases of transport links, higher rates of migration and relocation of dogs, the existence of large numbers of stray animals, insufficient quantity of monitoring studies on the spreading of disease carriers (Prus, Semenko, 2011; Svidersky, Roshchina, 2001; Tishchenko, Martsinchik, 2011; Tiškina, Jokelainen, 2017). One of such diseases is the babesiosis of dogs, which is widespread in Ukraine. According to M. P. Prus (2000, 2001), the city of Kyiv and Kyiv Region is one of the enzootic centers of this protozooisis. In the city, the disease is caused by *Babesia canis canis* (Piano et Galli-Valerio, 1895), which has been proved by PCR methods (Hamel et al., 2013).

The purpose of our work was to investigate the peculiarities of the situation on the babesiosis of dogs in Kyiv City. In particular, we studied the dynamics of the disease, seasonal and age characteristics of dog infection by *Babesia canis*, and the local fauna of ixodid ticks, potential vectors of babesiosis.

### Material and methods

The researches were conducted at the Department of Parasitology and Tropical Veterinary Medicine, the Educational and Production Department Clinical Center “Vetmedservis” of the National University of Life and Environmental Sciences of Ukraine and the Municipal Enterprise “Kyiv City Hospital of Veterinary Medicine” from 2014 — to the first half of 2017.

For the diagnosis of animals, a drop of blood was taken from the ear. The drop was thereafter placed on a skimmed glass and a smear was prepared. After drying it was fixed with the Nikiforov’s mixture and stained by the Romanovsky-Gimza method; alternatively, a non-fixed smear was stained by the Papanheim method (Prus, Semenko, 2011; Willard et al., 2004). We also used a kit for fast staining “Leykodiff-200”. Stained and dried blood smears were examined under a light microscope using the maximum magnification. In the investigated blood smears, the degree of parasitaemia was determined, i. e. the ratio of the number of erythrocytes affected by *Babesia* to the total number of counted cells (not less than 100 cells), expressed as a percentage. The agents of babesiosis were identified based on their morphological characteristics, using parasitological atlases and identification keys (Cicco, Birkenheuer, 2012; Kapustin, 1955; Krylov, 1996). In each case, we examined at least 200 fields of view of the microscope.

Standard statistical package “Data analysis” for Excel was used for the analysis of the data obtained. Identification of ixodid ticks was carried out using identification keys by E. Emchuk (1960), I. Akimov, I. Nebogatkin (2016) and N. Filippova (1997).

### Results

In the blood smears, *Babesia canis* had varying shape. However, pear-like forms (fig. 1) dominated at the acute phase, when the cells were connected sharp edges at an acute angle. In each infected erythrocyte, usually 2–4 specimens of *Babesia* were detected, however, at high intensity of the infection, up to 8, and some times up to 16–18 parasites were detected in one erythrocyte. Also, in the acute course, large oval forms of babesia cells were detected. That confirmed the active division of *Babesia* cells by longitudinal division or budding (fig. 2).

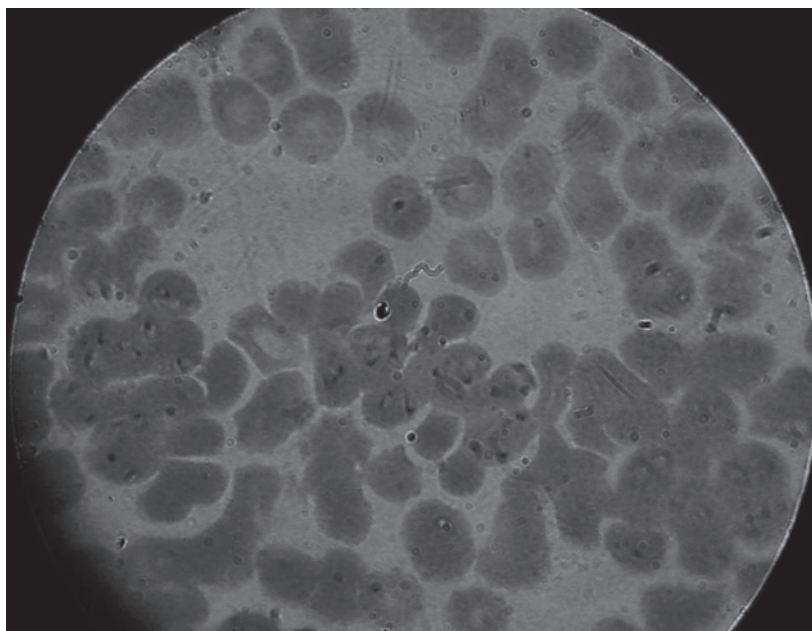


Fig. 1. *Babesia canis* in blood smear from a dog with acute form of babesiosis (stained according to the Romanovsky-Gimza method, magnification 1500×); note predominantly paired pear-shaped form.

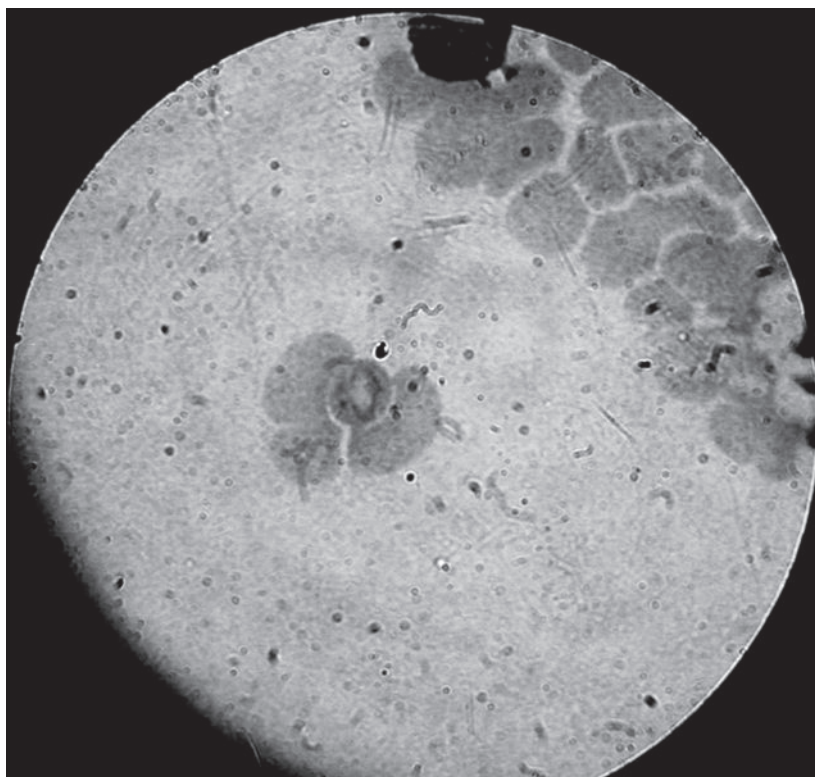


Fig. 2. *Babesia canis* in blood smear from a dog with acute form of babesiosis (stained according to the Romanovsky-Gimza method, magnification 1500 $\times$ ), babesia in division process by budding.

At the subacute and chronic form of babesiosis, *B. canis* cells were polymorphic, they had elongate, cigar-like, rounded forms.

During the period from 2014 — to the first half of 2017, we investigated 10 000 dogs with different pathologies. Among all those animals, 2030 dogs were with clinical signs similar to the babesiosis infection. The number of visits of dog owners with animals was different depending on the season. Laboratory diagnosis of babesiosis was confirmed in 416 out of 2030 investigated dogs (table 1).

In 2014, 588 dogs was examined. *Babesia* species was found in 120 animals (20.4 %). In 2015, the diagnosis of babesiosis was confirmed in 112 (20.4 %) of 550 dogs, in 2016 — in 114 (20.6 %) out of 554 examined dogs. From January to June of 2017, the babesiosis was detected in 70 out of 338 examined animals (20.7 %).

Our studies demonstrated that the annual percentage of dogs infected with *B. canis* varied within narrow range, with average prevalence of infection  $20.5 \pm 0.125$  %, and with an approximately equal number of dogs: 564 on average (table 1). The infection was significantly higher in the spring months (a paired two-sample t-test for  $t = 0.6$  at 95 confidence level), although it differed by years and months. The maximum percentage of dogs infected in spring was in March (the estimated yield of ixodid ticks from diapause) in 2014 and 2017, 46.9 % and 35.7 %, respectively. The peak of infection was in May in 2015 and 2016 years, but the percentage of dogs infected in May was approximately the same in all analyzed years and composed  $28.15 \pm 1.38$  %. Single cases of infection of dogs with babesiosis in January and December were also recorded.

Noteworthy, in January 2015, 2016 and 2017 cases of dog babesiosis were not recorded, and the average monthly temperature ranged from  $-2$  °C to  $-5$  °C. In 2014, the average monthly temperature of January was up to  $+5$  °C and 2 cases of babesiosis

were recorded. In March, the average monthly temperature was +3+6 °C, and the number of cases of babesiosis in dogs increased. In the summer months, there was a decrease in the prevalence infection of dogs with babesiosis due to a decrease in the biological activity of ticks caused by an increasing environmental temperature up to +20+ 25 °C (even up to + 35 °C on some days). The next highest level of infection falls on the autumn months and is related to a decrease in average daily temperatures. In November, the average monthly temperatures ranged from +3 to + 6°C, the number of infected dogs in 2014 was 10, in 2015 — 7, and in 2016 — 3. In 2014–2016, December was characterized by a decrease in temperature to below zero values, and no cases of babesiosis were registered. In December 2015, anomalously high temperatures (up to +10 °C) were recorded, and 2 dogs were found infected.

The most susceptible to the parasites were animals aged from 2 to 5 years old — 61.7 %. At the same time, males were affected more often (72.7 %) than females (27.3 %). However, the latter data cannot be considered reliable, since male dogs are more popular as pets than females.

In general, the seasonal dynamics plot had the shape of a two-vertex curve with a clearly defined peak of the infection in April–May and less conspicuous increase in the autumn months. In March and in the beginning of April, a slight degree of parasitaemia was noted — 1–2 % of the affected red blood cells, manifested by unexpressed clinical signs (no fever and icterus of the mucous membranes). With the increase in average monthly temperatures, the degree of parasitaemia comprised 2 to 4 % with pronounced symptoms of the disease (fever, anemia, and subsequently jaundice of the mucous membranes, hemoglobinuria).

**Table 1.** Number of dogs infected with *Babesia canis* investigated in 2014–2017; n = 2030

No.	2014			2015			2016			2017		
	Number of dogs examined	Number of dogs infected	Prevalence of infection, %	Number of dogs examined	Number of dogs infected	Prevalence of infection, %	Number of dogs examined	Number of dogs infected	Prevalence of infection, %	Number of dogs examined	Number of dogs infected	Prevalence of infection, %
01	10	2	20	12	0	0	8	0	0	8	0	0
02	9	2	22.2	15	4	26.7	13	2	15.4	8	1	12.5
03	32	15	46.9	62	12	19.4	51	12	23.5	42	15	35.7
04	130	14	10.8	94	22	23.4	106	27	25.5	124	22	17.7
05	136	38	27.9	107	28	26.2	94	29	30.9	98	27	27.6
06	56	4	7.1	47	4	8.5	52	6	11.5	38	5	13.2
07	19	2	10.5	23	2	8.7	25	4	16	-	-	-
08	17	4	23.5	19	2	10.5	19	2	10.5	-	-	-
09	50	13	26.0	48	16	33.3	79	16	20.3	-	-	-
10	76	16	21.1	77	14	18.2	68	12	17.6	-	-	-
11	45	10	22.2	37	6	16.2	30	4	13.3	-	-	-
12	8	0	0	9	2	22.2	9	0	0	-	-	-
Total	588	120	20.4	550	112	20.4	554	114	20.6	338	70	20.7

During the autumn-spring period in 2014–2017, 114 mites of the Ixodidae family were collected from 86 dogs. The largest number of ticks were *Dermacentor reticulatus* (Fabricius, 1794) 76 out of 114 individuals (66.7 %), of which 41 females and 35 males. The species *Ixodes ricinus* (Linnaeus, 1758) was represented by 38 individuals (33.3 %), including 31 females and 7 males. Owners of all examined dogs lived in Kyiv City. The animals were walking in park and forest-park areas of the city. Seventy-four dogs (86.1 %) infected with ticks were found to be infected also with *B. canis* during clinical and laboratory researches.

## Conclusions

Babesiosis of dogs is widely distributed among the population of domestic dogs in Kyiv and is revealed in  $20.5 \pm 0.125$  % of the dogs examined. Prevalence and intensity of dogs' infection by *B. canis* increased during the warm season. The highest prevalence of the infection was detected in the April–May months, which is associated with the biological activity of ticks — the vectors of the disease, which depends on the temperature of the environment. It is revealed that the intensity of infection also depends on the season.

The age and gender of dogs was important in the pathology of clinical babesiosis in Kyiv. Seasonal dynamics of *B. canis* infection reaches peaks in spring and autumn, which is directly related to the activity of ixodid ticks as vectors. As the average monthly temperatures increased in the spring, the number of dogs with babesiosis increased, as well as the degree of parasitemia with a maximum of 2–4 % in May. Two species of ticks, *I. ricinus* and *D. reticulatus*, were found to infect dogs in the city. The latter species was predominating with prevalence of infection 66.7 %. It is, therefore, considered to be the main vector of *B. canis* in the studied region.

We express our sincere gratitude for the assistance in conducting researches to the chief doctor of the Communal enterprise “Kyiv City Hospital of Veterinary Medicine” I. I. Teplyuk.

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Received 4 September 2017

Accepted 24 October 2017