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The spread of canine babesiosis and risk factors in the city of Poltava, Ukraine

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Babesia spp. are intracellular parasitic organisms that primarily infect the erythrocytes of most mammals, causing the disease known as babesiosis, and are transmitted by ticks. Canine babesiosis is a potentially fatal and major disease of dogs in Ukraine. The purpose of this study was to determine the spread of canine babesiosis based on the data of "Aibolyt" veterinary clinic in the city of Poltava and to identify risk factors associated with it. A total of 440 dogs were randomly selected from the clinic between June 2024 and May 2025. The information on sex, age, breed, and season of the year of sampling was recorded. The dogs were divided into the following age groups: group 1 – dogs under 12 months, group 2 (12–36 months), and group 3 (over 36 months). Breeds were classified as: purebred and mongrels. Blood smears were prepared, dyed with Leucodyph 200, and examined under a light microscope for the presence of *Babesia* spp. The study results showed a total of 30.2% babesiosis spreading. The share of positive cases among males was 35.5 % (77 out of 217), and among females – 25.1 % (56 out of 223). According to the analysis results, the males had by1.64 higher chances of a positive result, in comparison with the females (Odds Ratio = 1.64; 95 % CI: 1.09–2.47; P<0.05). The chi-square test also showed a statistically significant difference between the groups ($\chi^2 = 5.6$; P<0.05). The analysis using the Pearson xi-square criterion did not reveal a statistically significant difference between purebred dogs and mongrels in the frequency of positive results. The dogs aged 12–36 months (29.8 %) and older than 36 months (34.5 %) were most often affected. The risk of babesiosis infection increases with the age of dogs, and is particularly significant in the group older than 36 months (OR = 1.88; 95 % CI: 1.1–3.23; P<0.01).The highest level of the infection prevalence (EI) was recorded in spring – 39.3 %, as well as in autumn – 35.1 %, which significantly exceeded the corresponding indicators in the summer period (18.4 %). Therefore, the priority should be given to these factors when introducing the infection control measures.

Keywords: spread, *Babesia canis*, protozoa, prevalence of infection, risk factors.

Поширення бабезіозу собак та фактори ризику у місті Полтава, Україна

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Babesia spp. – це внутрішньоклітинні паразитичні організми, які вражають переважно еритроцити більшості ссавців, спричиняючи захворювання, відоме як бабезіоз, і передаються кліщами. Бабезіоз собак є потенційно смертельним та основним захворюванням собак в Україні. Метою цього дослідження було визначити поширення бабезіозу собак, базуючись на даних ветеринарної клініки «Айболить» у місті Полтава та виявити пов'язані з ним фактори ризику. Загалом у період з червня 2024 року по травень 2025 року було випадковим чином відібрано 440 собак, які поступали на клініку. Була записана інформація щодо статі, віку, породи та пори року відбору проб. Собак розподілили за такими віковими групами: група 1 – собаки (до 12 місяців), група 2 (12–36 місяців) та група 3 (старші 36 місяців). Породи були класифіковані як: породисті та метиси. Були підготовлені мазки крові, забарвлені барвником Лейкодиф 200 та досліджені під світловим мікроскопом на наявність *Babesia* spp. Результати досліджень показали загальне поширення бабезіозу 30,2 %. Частка позитивних випадків серед самців становила 35,5 % (77 із 217), а серед самок – 25,1 % (56 із 223). Згідно з результатами аналізу, самці мали в 1,64 раза вищі шанси на позитивний результат, порівняно з самками (Odds Ratio = 1,64; 95 % CI: 1,09–2,47; P<0,05). Хі-квадрат тест також показав статистично значущу відмінність між групами ($\chi^2 = 5,6$; P<0,05). Аналіз за критерієм хі-квадрат Пірсона не виявив статистично значущої різниці між породистими та метисами за частотою позитивних результатів. Найчастіше хворіли собаки 12–36-місячного віку (29,8 %) та старші 36 міс. (34,5 %). Ризик бабезіозної інфекції зростає з віком собак, і особливо значущий – у групі старше 36 місяців (OR = 1,88; 95 % ДІ: 1,1–3,23; P < 0,01). Найвищий рівень екстенсивності інвазії (EI) було зафіксовано навесні – 39,3 %, а також восени – 35,1 %, що суттєво перевищувало відповідні показники в літній період (18,4 %). Тому пріоритет слід надавати цим факторам під час впровадження заходів боротьби з інфекцією.

Ключові слова: поширення, *Babesia canis*, найпростіші, екстенсивність інвазії, фактори ризику.

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Introduction

Canine babesiosis, also known as "malignant jaundice", is a wide-spread and clinically significant disease caused by protozoan parasites of *Apicomplexa* Levine family, 1970 [15]. The disease is characterized by fever, splenomegaly, reduced appetite, weakness, indolence, generalized lymphadenopathy, anemia, hemoglobinuria, and collapse connected with intra- and extravascular hemolysis, hypoxic injury, systemic inflammation, thrombocytopenia, and pigmenturia [7]. The obligate intracellular apicomplexa protozoa of *Babesia* genus are classified by the morphological characteristics into large (3–5 μm in diameter) and small forms (1–3 μm in diameter) [4, 7]. Morphologically, *Babesia canis* is characterized as large (2.5–5.0 μm) [19].

According to meta-analysis data, the overall united spread of babesiosis in dogs made 12.0 % (95 % confidence interval [CI]: 9.7–14.6 %), with higher incidence rates observed among domestic dogs (13.9 %, 95 % CI: 10.4–18.0 %) in comparison with homeless or dogs in shelters (11.6 %, 95 % CI: 6.6–17.8 %). The cases of babesiosis in dogs were registered in 61 countries. The largest number of studies was conducted in India (29 publications). Among the countries, the highest pooled spread was found in Slovakia (90.8 %, 95 % CI: 82.6–94.8 %) and Bosnia and Herzegovina (82.5 %, 95 % CI: 73.0–89.7 %) [1].

Babesiosis is a potentially fatal and major disease of dogs in Nigeria. The results showed the overall spread of *Babesia canis* infection in 10.8 % of cases. Among the infected dogs, 13.7 % were female and 8.3 % were male. Dogs aged from 12 to 36 months had the highest (17.0 %) spread of infection, while dogs aged >60 months had the lowest one (4.5 %). Depending on the breed, the infection was more common among exotic dogs (12.9 %) than in crossbreeds (9.4 %). [13].

On the territory of Hong Kong, researchers found that in multi-measurable logistic regression, the dogs of mixed breeds were more likely to be infected than purebred ($P = 0.005$), while dogs > 10 years of age were less likely to be infected than younger dogs ($P = 0.019$) [11].

The spread of *Babesia* in dogs on the territory of Latin America and the Caribbean varied considerably depending on the parasite species and geographical location, with the values close to zero and made 26.2 % [14].

In Baghdad province (Iraq), the prevalence of infection caused by *Babesia canis* made 5.1 %. Higher rates of infestation were observed in male dogs and animals under three years of age. Husky dogs appeared to be more susceptible to the infection than other breeds. The peak spread was observed in April and June, with overall incidence rates higher in the spring and summer periods than in winter season [2].

On the whole, on the territory of Germany, 659 out of 20,914 dogs (3.2 %) were tested positive for *Babesia* spp. by PCR. Seasonality had a statistically significant effect on the test results: the share of positive cases was significantly higher in spring and autumn (4.7 %) in comparison with the summer and winter period (1.6 %), with peaks in April (5.2 %) and October (7.4 %) [$P<0.001$; odds ratio (OR) = 3.16]. There was also a significant effect of sex and age: among males, the detection frequency made 3.5 %, which was higher than among females (2.8 %) [$P = 0.012$; OR = 1.49], and in dogs under 7 years of age (4.0 %) the infection was recorded more frequently than in animals aged 7 years and older (2.3 %) [$P < 0.001$; OR = 1.76] [17].

Cases of co-infection in Ukraine were described in literature, in particular babesiosis and dirofilariasis. [12]. A comprehensive study of canine babesiosis in Vinnytsia region (Ukraine) showed that during the periods of increased activity of the *Dermacentor reticulatus* ixodid ticks (April-June and August-September), the infestation rate of dogs with *Babesia canis* and *Babesia gibsoni* reached 44.9 %, with the level of parasitemia varying from 0.5–2.5 % to 33 %. The highest parasitemia indicators (17–23 %) were registered in April-May among 154 dogs (16.5 %) with the clinical manifestations of moderate babesiosis. In animals with a mild form of the disease, parasitemia made 2.4–6 %. The cases of severe babesiosis were noted in 23 out of 1,326 dogs (1.73 %), with the level of parasitemia reaching 20–35 %. [9].

The analysis by breed showed that during the study period, the highest spread of babesiosis was observed among non-pedigree dogs (45.2 %). Among pedigree animals, the disease was most often detected in terriers (12.6 %), cocker spaniels (11.1 %), German shepherds (9.9 %), Central Asian shepherds (4.8 %) and assizes (2.1 %). The share of other breeds made 14.3 % [9].

The aim of the study

Therefore, the purpose of this study was to determine the spread of canine babesiosis based on the data from "Aibolyt" veterinary clinic in the city of Poltava and to identify the risk factors connected with it.

Materials and methods

The work was conducted during 2024–2025 in the laboratory of the Department of Parasitology and Veterinary and Sanitary Expert Examination of Poltava State Agrarian University and in the conditions of "Aibolyt" veterinary clinic in the city of Poltava, Ukraine.

All 440 dogs were randomly selected from the clinic from June 2024 to May 2025. The information on sex, age, breed, and season of the year of sampling was recorded. The dogs were divided into the following age groups: group 1 – dogs under 12 months, group 2 (12–36 months), and group 3 (over 36 months). The breeds were classified as: purebred and mongrels.

Parasitological analyses were conducted immediately after each sampling according to the standard method [5]. Thin smears were prepared from blood samples, air-dried, and fixed in methanol for 3-5 minutes. The smears were stained using the Leucodif 200 kit (Erba Lachema, Czech Republic). Subsequently, they were examined under immersion magnification (×1000) using a MICROmed XS-3330 LED microscope with a 5.0 Mpix digital CCD video camera (China) for the presence of intra-erythrocytic *Babesia* merozoites. Morphometric parameters were determined using the Micam 3.0.6 program.

The main indicator of animal infestation by protozoan pathogens was the prevalence of infection (EI, %). Statistical processing of the obtained data was carried out on a personal computer using the MedCalc Statistical Software version 20.216 software (MedCalc Software bvba, Ostend, Belgium). The results are expressed in percentages and presented in the form of tables. After that, chi-square analysis was conducted. To assess the risk factors concerning gender and breed, the odds ratio was used at 95 % confidence interval. For age and season, the calculation was conducted using the logistic regression method. $P < 0.05$ values were considered significant.

Results and discussion

Of the 440 dogs sampled 133 (30.2 %) were tested positive for *Babesia canis* in blood smears. *Babesia* species was identified morphologically as typical pear-shaped bodies in the erythrocytes of infected dogs (***Fig. 1***).

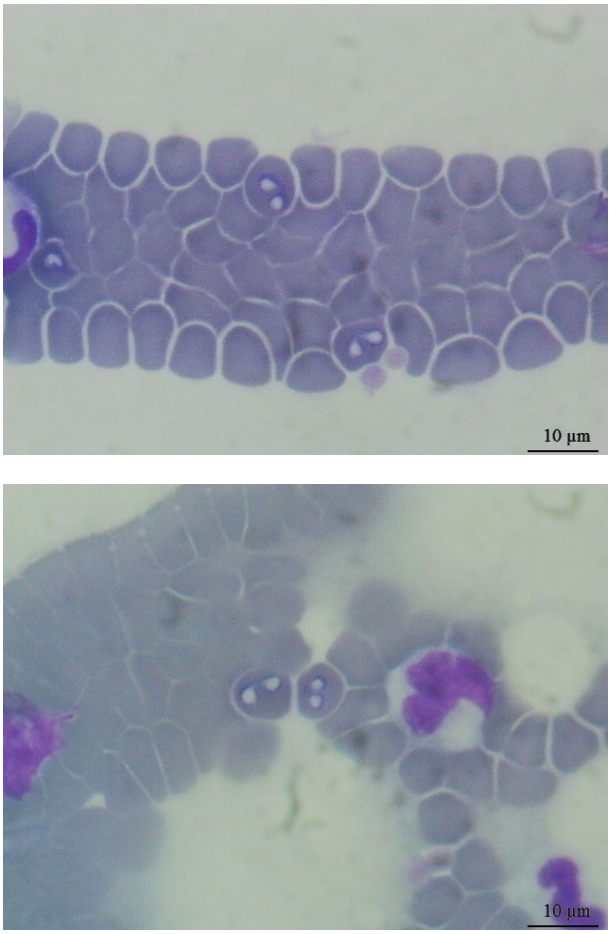

Fig. 1. Intra-erythrocytic merozoite of *Babesia* in smears stained with Leucodyph 200, showing pear-shaped bodies with basophilic cytoplasm, from dogs

According to ***Table 1***, as to comparing the chances of obtaining a positive result depending on the gender (males and females) the share of positive cases among males made 35.5 % (77 out of 217), and among females – 25.1 % (56 out of 223). According to the results of the analysis, the males had by 1.64 times higher chances of a positive result in comparison with the females (Odds Ratio = 1.64; 95 % CI: 1.09-2.47; $P<0.05$). The chi-square test also showed a statistically significant difference between the groups ($\chi^2 = 5.6$; $P<0.05$).

Table 1
Spread of babesiosis invasion among dogs depending on gender

Sex	Number of dogs (n=440)	Positive	EI, %	Odds ratio	95% CI
Males	217	77	35.5	1.64	1.09–2.47
Females	223	56	25.1		
$\chi^2 = 5.6$ DF=1, P<0.05					

The analysis according to the criterion of Pearson's chi-square test did not reveal any statistically significant difference between purebred dogs and mongrels in the frequency of positive results ($\chi^2 = 0.261$; $P>0.05$). There was also no statistical difference in the odds ratio indicator (***Table 2***).

Table 2
Spread of *babesiosis* invasion among dogs depending on breed

Breed	Number of dogs (n=440)	Positive	EI, %	Odds ratio	95% CI
Pedigree	200	58	29.0	0.9	0.6–1.35
Mongrels	240	75	31.2		
$\chi^2 = 0.261$ DF=1, P>0.05					

According to the obtained results (***Table 3***), dogs older than 36 months have by 88 % higher chances of infestation in comparison with young animals (up to 12 months). The share of positive cases among the dogs in the age group older than 3 years was 34.5 % (70 out of 203), and the lowest level was among the youngest animals under 12 months of age – 21.9 % (21 out of 96). The chi-square test also showed a statistically significant difference between the groups ($\chi^2 = 4.91$; $P<0.05$).

Table 3
The spread of babesiosis invasion among dogs depending on age

Age	Number of dogs (n=440)	Positive	EI, %	Odds ratio	95% CI
Up to 12 months	96	21	21.9	–	–
12–36 months	141	42	29.8	1.51	0.83–2.77
Older than 36 months	203	70	34.5	1.88	1.1-3.23
$\chi^2 = 4.91$ DF=1, P<0.05					

According to the results of the analysis (***Table 4***), a statistically significant dependence was found between the season of the year and the probability of obtaining a positive result ($\chi^2 = 10.6$; DF = 2; $P<0.01$). The highest level of the prevalence of infection (EI) was recorded in spring – 39.3 %, and in autumn – 35.1 %, which significantly exceeded the corresponding indicators in the

summer period (18.4%). In autumn, the chances of obtaining a positive result were 2.4 times higher in comparison with summer (OR = 2.4; 95 % CI: 1.23–4.66; P<0.01). In spring, this indicator was even higher – by 2.88 times in comparison with the summer period (OR = 2.88; 95 % CI: 1.53-5.42; P<0.01).

Table 4

The spread of *babesiosis* invasion among dogs depending on the season

Season	Number of dogs (n=440)	Positive	EI, %	Odds ratio	95% CI
Summer	87	16	18.4	–	–
Autumn	114	40	35.1	2.4	1.23–4.66
Winter	89	18	20.2	1.12	0.53–2.38
Spring	150	59	39.3	2.88	1.53–5.42
$\chi^2 = 10.6$ DF=2, P<0.01					

Thus, spring and autumn seasons of the year are associated with a significantly higher risk of a positive result, which may indicate seasonal peculiarities of the pathogen circulation. This is coordinated with tick biology – the peak activity often occurs in spring and autumn.

Global climate changes in the recent decades have caused an increase in the activity and spread of many diseases, in particular, canine babesiosis [3]. The key risk factors for infecting with large species of *Babesia* spp. are living in rural areas, animals being kept in kennels or shelters, staying in regions endemic for the infection, seasonality (which is caused by the increased tick activity), the presence of ticks on the animal body, and the lack of acaricidal treatment [20]. In our studies, the most significant risk factors were: sex, age of animals and seasonality. Our data are similar to those ones of scientists who analyzed the incidence of babesiosis in animals taking into account sex. It was proven that the infestation rate in males was by 30.6 % higher than in females [16]. Among the dogs infected with babesiosis, males predominated over females by 20 %. The highest incidence rate was found in animals aged from 2 to 3 years (11.1–16 %). With the age, there is a steady trend towards a decrease in susceptibility to the infection. Concerning the breed, non-pedigree dogs (20.9 %), as well as German shepherds (13.9 %) and Rottweilers (7.5 %) are most often affected [10].

Babesiosis is a wide-spread disease among domestic dogs in Kyiv, detected in 20.5 % of the examined animals. The incidence and level of infesting dogs with *Babesia canis* pathogen increased in the warm season. The largest number of the invasion cases is registered in April-May, which is due to the increased biological activity of ticks – the main carriers of the disease, the activity of which depends on the environmental temperature. It has also been determined that the level of infestation varies depending on the season. The seasonal dynamics of *B. canis* is characterized by the peak values in spring and autumn, which is directly connected with the periods of increased activity of ixodid ticks [18]. The similar results were obtained in the process of the study, but the EI was somewhat higher (30.2 %).

Based on the analysis of the epizootic situation regarding canine babesiosis in the city of Zhytomyr during 2018–2022, the trend towards an increase in the number of cases of the disease was registered. In particular, in 2018, 526 diseased animals (23.1 %) were detected among 2,278 examined dogs, and in 2019 – 700 infected dogs (24.9 %) out of 2,812 animals. The similar incidence rates were recorded in the following years. On the average, over a five-year period, babesiosis was detected in 25.2 % of dogs out of a total number of 13,886 examined [6].

The spring sharp increase in the disease incidence in the city of Poltava over the analyzed years falls on April (29 %) and May (23.7 %), while in the summer season only 8.7 % of cases are recorded. The autumn maximum of the disease is noted in September (14.5 %) and October (8.1 %) [8]. In our studies, the EI was the highest in autumn (35.1 %) and spring (39.3 %). According to the obtained results, the highest risk of dogs being infested with *Babesia canis* is in spring and autumn in comparison with summer and winter months.

Conclusions

Canine babesiosis in the city of Poltava is quite a wide-spread disease (30.2 % of the total number of all examined animals). Dogs aged 12–36 months (29.8 %) and older than 36 months (34.5 %) are most often affected. The risk of babesiosis infection increases with the age of dogs, and is especially significant in the group older than 36 months (OR = 1.88; 95 % CI: 1.1–3.23; P<0.01). The males were by 27.3 % more likely to get ill in comparison with the females (OR = 1.64; 95 % CI: 1.09–2.47; P<0.05). The highest level of the prevalence of infection (EI) was recorded in spring – 39.3 %, as well as in autumn – 35.1 %, which considerably exceeded the corresponding indicators in the summer period (18.4 %). Thus, when introducing measures to control babesiosis, these factors should be prioritized.

Prospects for further research. In our further research, we plan to study the clinical signs as well as morphological and biochemical changes in the blood of dogs infected with babesiosis.

Conflict of interest

The authors state that there is no conflict of interest.

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