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RESEARCH ARTICLE

# Prevalence of blood parasitic infections in raising dogs at Thaksin University area Phatthalung Campus

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## Abstract

Hematological values are important for assessing animal health, especially in asymptomatic animals. Hematological data for individuals can aid in differential diagnosis and reduce the spread of pathogens. This study delved into the prevalence of hematopoietic blood infestation in raising dogs in the Thaksin University area, Phatthalung Campus. It specifically focused on hematological values in 50 raised dogs, with samples taken for hematological analysis. The findings revealed that 34 % (17/50) of the dogs had anemia, 72 % (36/50) had thrombocytopenia, and 4 % (2/50) had leukopenia. These results indicate blood parasite infections. Further tests for blood parasites using thin blood smears showed the highest prevalence of *Ehrlichia* spp., together with *Anaplasma* spp. at 12 % (6/50), *Babesia* spp. at 8 % (4/50), and *Anaplasma* spp. at 2 % (1/50), respectively. Moreover, no parasites were found in 78 % (39/50) of the cases. Despite the absence of parasites, the examination of hematology values revealed that they were not within the normal range. There was a tendency to indicate blood parasite infection in 90 % (45/50) of the dogs, with only 10 % (5/50) at risk of not having blood parasite infection. The findings of the study revealed that dogs, despite showing no clinical sign, exhibited abnormal hematological test results. Therefore, disease surveillance in asymptomatic dogs is essential for animal health and prevention of zoonoses.

Keywords: Blood parasites, hematology, raising dog, zoonoses

## Introduction

Exogenous parasitic infections in the southern region are a significant and frequently encountered health concern in dogs. According to Muguiro et al. (2023), these infections, primarily caused by tick infestations, suck the blood in host animal. Their prevalence is notably pronounced during the period spanning from August to December annually (Sangmaneedet et al., 2002). Furthermore, it is worth noting that these parasitic infections are not limited to dogs; a relatively high incidence of blood parasite diseases has also been documented in dairy cattle (Kanchanatemiya et al., 2021). These infections can have debilitating effects on the host circulatory system, manifesting as symptoms such as lethargy and reduced appetite in afflicted animals (Aumarm et al., 1999).

Regarding the domestic situation of blood parasite infection, Wongsawang and Jeimthaweeboon (2019) found that in Kanchanaburi Province, the prevalence of blood parasite infection in dogs was 22.20 %. *Ehlrlichia canis* showed the highest prevalence (16.97 %), followed by *Hepatozoon canis* (4.01 %), *Babesia canis* (2.31 %), *Brugia* spp. (0.52 %), *Dirofilaria immitis* (0.21 %), and *Babesia gibsoni* (0.17 %), respectively. Wankaew and Asawapattanakul (2022) found that at the Animal Hospital for Teaching and Learning, Faculty of Veterinary Sciences, Mahasarakham University, infection with a single species had the highest prevalence with *Ehrlichia canis* (35.92 %), followed by mixed infections with *Anaplasma platys* and *E. canis* (14.18 %). The most common infections caused by parasites in the southern region are *E. canis,* followed by *H. canis* and *B. canis*. Hematology test results of animals infected with parasites indicate that conditions signaling a risk of blood parasite infection include anemia and low hemoglobin levels in the blood. Hematological changes in dogs infected with blood parasites are predominantly characterized by anemia. This observation is derived from the interpretation of the total red blood cell (RBC) count and packed cell volume (PCV), which serve as primary indicators of parasite infection in the dog's bloodstream. Anemia can have various causes, with a common factor being significant blood loss due to external parasites like ticks, fleas, and hookworms. Additionally, the infection with blood parasites can further contribute to the destruction of RBC. Symptoms indicating that pets may have anemia include a change in mucous membrane (MM) color from pink to pale, lethargy, depression, and loss of appetite (Zygner et al., 2023)

A study examining the impact of blood parasite infections, specifically *Ehrlichia canis* and *Anaplasma platys*, on dogs observed an elevation in serum creatinine levels at the Animal Hospital, Faculty of Veterinary Medicine (Seerintra et al., 2022). This increase in creatinine levels may concomitantly coincide with leukopenia, a condition characterized by a low white blood cell (WBC) count resulting from bacterial destruction of WBC transmitted by ticks. Dogs can contract the ehrlichiosis pathogen through brown tick bites or blood transfusions. *Ehrlichia canis* infiltrates WBC, thereby compromising the immune system and circulating through the bloodstream, leading to symptoms such as fever, weight loss, reduced platelet count, purpura, abnormal bleeding, pale MM, and weakness (Vetdiags, 2020). Thrombocytopenia, characterized by reduced platelet levels, is also frequently observed. Several factors, including diseases like leukemia and lymphoma, significant blood loss, specific viral infections, and platelet destruction, can contribute to thrombocytopenia, diminishing the body's ability to produce platelets efficiently (Pet-AZ, 2020; Thongsahuan et al., 2020).

Evaluating the health of animals, particularly dogs holds paramount importance as a foundational component of disease surveillance in the domain of pet care. Such assessments play a pivotal role in averting severe illnesses and fatalities in affected animals. Notably, diseases stemming from blood parasite infections often originate from external parasites. Effectively curbing the incidence of these diseases entails the implementation of rigorous parasite control measures and vigilance in their elimination. Timely detection is of utmost significance, as treatment efficacy significantly diminishes when pets exhibit no symptoms or are diagnosed at an advanced stage of the disease, thus elevating the risk of fatal outcomes. Consequently, regular health monitoring of pets becomes

an indispensable practice. Gathering essential information, including the animal's medical history and body condition score (BCS), facilitates in-depth analysis of individual health issues and informs decisions regarding dietary management and disease prevention (Chun et al., 2019). Moreover, this practice assumes added significance as it serves to minimize the transmission of pathogens from animals to humans such as rabies (Ministry of Public Health, Department of Disease Control, 2023). The primary objective of this research endeavor is to ascertain the prevalence of blood parasitic infections in dogs raised at Thaksin University, Phatthalung Campus.

## Materials and Methods

The research methodology involved the collection of samples from Thai breed dogs, encompassing both males and females aged 3 months and older, which could be restrained for blood sampling without the need for sedation. A total of 50 dogs were selected as participants in this study, and the sample collection process was executed as outlined below:

1) Physical Examination (PE): The initial step in the data collection process involved conducting a comprehensive PE of each dog. This examination was carried out following a structured data record protocol. The data record involved general animal information and specific measurements and observations. The specific measurements and observations included: (1) Fecal condition score (FCS), referring to the fecal scoring chart (Figure 1), (2) body temperature (BT), measured by inserting a digital thermometer into the animal's rectum, waiting for the number to stabilize, then reading and recording the value on a data recording form, (3) MM, observed by opening the animal's upper lip to view the color of the mucous membrane and recording the results, (4) capillary refill time (CRT), determined by pressing on the animal's MM, releasing it, then counting the time it took for the mucosa to return to its normal color and recording the time, (5) degree of dehydration, assessed by pulling the skin on the back of the neck and recording the time it took for the skin to return to normal after release, and (6) BCS, evaluated by visual inspection of the external animal's shape and assessment of muscle condition at the back, middle, and front, based on the BCS chart. BCS chart determined through the utilization of a BCS chart (with a 5-point scale) (Figure 2), Eastland-Jones et al. (2014), and an assessment of the dog's vaccination history from the system recording the history of receiving rabies vaccination from the Department of Livestock Development at Thaksin University.

Any aberrations or abnormal symptoms observable with the naked eye, relating to the skin, circulatory system, muscular system, and respiratory system, were documented in the data record sheet.

2) Hematology test: The dog was forced to lie on its side, and a blood sample was collected from the cephalic vein at the dog's forelimb (Figure 3) by wiping the area and surrounding area with alcohol into the vein to be injected. Use your non-dominant hand to hold the paw of the dog on the side where blood will be drawn to stretch the tip of the dog's foot, and then a blood sample was taken using a 1-inch size 22 needle and 3 mL syringe. After collecting a blood sample, hemostasis was ensured by using dry cotton wool and applying a conforming stretch bandage. One milliliter of blood was collected into a blood sampling tube containing the anticoagulant: ethylenediaminetetraacetic acid (EDTA). Subsequently, the samples were gently mixed by inversion (Vijarnsorn et al., 2018) and sent to a laboratory for analysis of blood samples within 24 hours. The examination was conducted using Complete Blood Cell (CBC) analyzer (Figure 4) to assess the RBC Count , Hemoglobin (Hb), Hematocrit (HCT), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC), White Blood Cell (WBC) Count, and Platelet Hematocrit (PLT). The types of WBC, including neutrophils, eosinophils, basophils, monocytes, and lymphocytes, were counted. Additionally, a check for parasites in the blood was conducted using a thin blood smear stained with Giemsa (Rucksaken, 2019). The smear was then examined under a microscope at 40× and 100× magnifications to detect blood parasites, including *Babesia* spp., *Ehrlichia* spp., and *Anaplasma* spp.




Figure 1. Fecal scoring chart

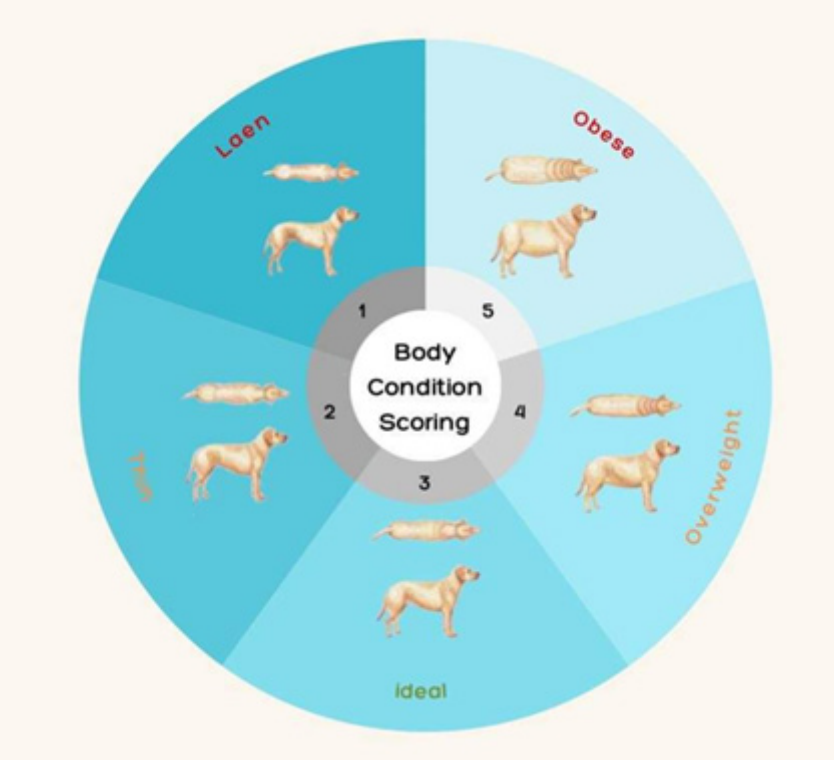



Figure 2. Body condition scoring



**Figure 3** Blood collection from the cephalic vein (arrow indicates the marking on the front right leg)

3) The data collection area encompassed both inside and around Thaksin University, Phatthalung Campus, Baan Prao Sub-District, and Papayom District, Phatthalung. The area covers 5.4864 $km^2$, and the research was conducted between January 27, 2023, and March 12, 2023.

Instruments employed in this research encompassed a CBC analyzer , alongside a designed data collection form created to document the health status of each individual animal. The analytical approach pursued in this study aimed to ascertain the prevalence rate and acquire an understanding of the general characteristics of the animal population through descriptive statistical techniques, specifically frequency and percentage analyses. It is imperative to note that this research proposal underwent a thorough review process and received formal approval from the Institutional Animal Care and Use Committee of Thaksin University approved. This approval was granted under the project code TSU 2023-003, ensuring adherence to ethical and research standards in animal studies.

## Results and Discussion

In a study of PE in raising dogs at Thaksin University, Phatthalung Campus, a total of 50 animals were examined. The FCS index is to assess whether the animal has diarrhea or normal bowel movements. A loose stool score in an animal typically indicates gastrointestinal infection, while cracked stool suggests abnormal physical health possibly due to a tendency toward constipation. The findings revealed that the majority were at the normal level, with an FCS value of 2.5 observed in 52 % (26/50) of the dogs. Moist stool with no cracks has an FCS value of 3, accounting for 26 % of the dogs (13/50). Liquid stool with minimal consistency has an FCS value of 3.5, observed in 12 % (6/50) of the dogs. Dogs with entirely liquid stools, having an FCS value of 4.5, make up 10 % (5/50). These FCS values serve as indicators of the dogs health. According to the PE, the MM test results revealed that 48 % (24/50) of the dogs exhibited pale MM, which is likely indicative of anemia. This finding aligns with the studies of Ortuño et al. (2023) and Salakij et al. (1999), who reported that animals

infected with blood parasites displayed a pale MM. This aligns with the findings of Wongsawang and Jeimthaweeboo (2019), which identified common blood parasitic infections in pets in Thailand caused by protozoa or rickettsia groups. These included species such as *Babesia* spp., *Ehrlichia canis*, *Anaplasma* spp., or *Mycoplasma* spp., with transmission primarily occurring through vectors from external parasites. Such infections persist in the body for extended periods before symptoms manifest. For instance, pets might contract the infection in an outbreak area, and symptoms may emerge in the subsequent year in different regions. Symptoms of blood parasitic infections encompass fatigue, decreased energy, loss of appetite, fever, anemia, muscle pain, coughing, and jaundice. Some pets might experience additional symptoms such as enlarged lymph nodes, spleen enlargement, and body edema. Other signs included bleeding spots, vomiting blood, blood in the stool, difficulty in stopping bleeding, pale MM, and compromised immune function.

The study found that the MM values were within the normal range for over 52 % (26/50) of the dogs. The assessment of dehydration indicated normal levels in 92 % (46/50) of the cases. Regarding the BCS, it was observed that 6 % (3/50) of the dogs were very thin with a BCS value of 1, 16 % (8/50) were underweight with a BCS value of 2, 18 % (9/50) were overweight with a BCS value of 4, 20 % (10/50) were obese with a BCS value of 5, and 40 % (20/50) were considered to have an ideal BCS with a value of 3, respectively. However, during the initial assessment, it was observed that the circulatory systems of all the dogs, accounting for 100 % (50/50), exhibited readings within the normal range. Furthermore, the evaluation of capillary refill time (CRT) in the dogs consistently indicated CRT values of less than 2 seconds. Moreover, the assessment of body temperature demonstrated that the dogs maintained normal temperature ranges, spanning from 101 to 102.9 °F (Table 1)

The prevalence of blood parasitic infections in the population of raised dogs was investigated. Among the 50 dogs examined, 8 % (4/50) were identified as being infected with *Babesia* spp. These infections were specifically observed in dogs numbered 5, 9, 23, and 24. Furthermore, 12 % (6/50) of the dogs exhibited concurrent infections involving more than one type of blood parasite, namely *Ehrlichia* spp. and *Anaplasma* spp. This dual infection was noted in dogs numbered 16, 17, 19, 20, 21, and 22. Additionally, a single case, constituting 2 % (1/50), was found to be infected solely with *Anaplasma* spp., with dog number 31 being the affected individual. It is noteworthy that a significant portion of the dogs underwent microscopic examination of stained blood smears, revealing that the majority, 78 % (39/50), were free from blood parasitic infections, as outlined in (Table 2).

Intriguingly, the infected dogs included both those displaying clinical manifestations and those without. Wang et al. (2023) conducted a study on the outbreak of *Babesia* spp. in dogs in China, and their research indicated that *B. vogeli* and *Babesia* spp. were present in dogs devoid of any clinical manifestations, suggesting the potential for widespread asymptomatic infections. It is essential to underscore that the diagnosis of blood parasite infections was carried out through the staining of blood smears under a microscope, representing a fundamental diagnostic approach. However, it is critical to acknowledge that this method has inherent limitations, such as low sensitivity, which can lead to instances where infections may remain undetected (Faculty of Veterinary Technology, Kasetsart University, 2021). Diagnosis blood parasite infections can be achieved through diverse elements, including pet owner-provided history, observed symptoms, and laboratory tests. Laboratory tests typically entail collecting blood samples from sick animals for testing complete blood count (CBC) and blood chemistry. Crucially, blood smear examination under a microscope allows for the visualization of blood cell characteristics and the identification of parasites within the blood. Examination under a microscope, involving sampling from only 1 drop of blood, exhibits low sensitivity but high specificity. However, if parasites are detected, it can confirm their presence of blood parasite infection. Blood parasitic detection through microscopy involves collecting a blood sample of approximately 0.5 - 1 mL from the animal, placing it in a tube containing ethyl-

Table 1. Physical examination of raising dogs

| Data | Criterion score | Raising Dogs | Percentage (%) |
|---|---|---|---|
| Fecal condition score | 2.5 | 26 | 52 |
| | 3 | 13 | 26 |
| | 3.5 | 6 | 12 |
| | 4.5 | 5 | 10 |
| Temperature | 101 - 102.9 °F | 50 | 100 |
| Mucous membrane | Pink | 26 | 52 |
| | Pale | 24 | 48 |
| Capillary refill time | < 2 second | 50 | 100 |
| Dehydration | < 5 % hydration | 46 | 92 |
| | > 5 % dehydration | 4 | 8 |
| Body condition score | Very thin (1) | 3 | 6 |
| | Underweight (2) | 8 | 16 |
| | Ideal (3) | 20 | 40 |
| | Overweight (4) | 9 | 18 |
| | Obese (5) | 10 | 20 |

Table 2. Prevalence of parasite infection

| Type of parasites infection | Blood parasite in raising dog | |
|---|---|---|
| | No. of Infection/total | Prevalence (%) |
| *Babesia* spp. | 4/50 | 8 |
| *Anaplasma* spp. | 1/50 | 2 |
| *Ehrichia* spp.+ *Anaplasma* spp. | 6/50 | 12 |

enediaminetetraacetic acid (EDTA), and preparing both a blood smear and a buffy coat smear. The process includes air drying the blood film slide, fixing slides in methanol for 1-2 minutes, and staining with Modified Wright's Giemsa. After preparation, the blood film is examined under an optical microscope with 1000x magnification to check for 4 types of blood parasites: *Babesia* spp., *H. canis*, *A. platys*, and *E. canis* (Wankaew & Asawapattanakul, 2022). In addition to laboratory testing methods, an antibody test kit is available to identify antibodies against blood parasites. To increase sensitivity and specificity, molecular techniques such as Polymerase Chain Reaction (PCR) are adopted to detect the genetic material of parasites in the blood. However, in early infection stages, the animal may produce insufficient antibodies for detection by the test kit. Therefore, a microscopic examination or PCR test is essential to confirm results (Suppakarn, 2023).

Consequently, this could result in false-negative

outcomes, making the confirmation of diagnoses challenging. As a result, it is strongly recommended to verify these results employing more specific and sensitive diagnostic methods. Nevertheless, for a comprehensive and accurate interpretation, it is imperative to integrate hematology values into the diagnostic process for blood parasitic examinations. The findings from this study closely align with the outcomes of a research study conducted by Thongsahuan et al. (2013), underscoring the importance of combining blood sampling for the assessment of blood parasite infection, hematology tests, and the observation of clinical manifestations to attain precise and reliable diagnostic results.

The investigation into hematological changes in the study revealed that a significant portion of the dogs exhibited low levels of Red Blood Cells (RBC), Hematocrit (Hct), and Hemoglobin (Hb), with 34 % (17/50) showing signs of anemia. Moreover, 72 % (36/50) of the dogs displayed thrombocytopenia, indicating a parasitic infection in their blood. A smaller proportion, 4 % (2/50), exhibited low WBC counts, referred to as leukopenia, while 20 % (10/50) displayed leukocytosis, characterized by higher-than-normal white blood cell counts, a condition often associated with sepsis. These hematological findings accentuate the pivotal role of hematological values in evaluating the health of animals and diagnosing and prognosing blood parasite infections in dogs. This aligns with the observations made by Wongsawang and Jeimthaweeboon (2019), who identified hematological abnormalities in dogs afflicted with blood parasite infections, including leukopenia, thrombocytosis, hypohemoglobinemia, and anemia. Specifically, dogs infected with *H. canis* were noted to exhibit anemia, hypohemoglobinemia, and thrombocytopenia, while those infected with *B. vogeli* displayed anemia, hypohemoglobinemia, thrombocytopenia, and leukopenia.

Furthermore, the correlation between hematological values and blood parasitic infections in dogs indicates that dogs with values below the normal range for RBC, PCV, Hb, and PLT are more likely to be afflicted with blood parasitic infections compared to dogs with values within the normal range. These findings align with the research conducted by Su et al. (2023), which identified primary blood abnormalities in animals infected with *Babesia gibsoni,* characterized by thrombocytopenia and anemia. Additionally, they concur with the observations of Wongsawang and Jeimthaweeboon (2018), who found that hematological changes in dogs infected with blood parasites were primarily indicative of anemia, as evidenced by RBC and PCV values, which serve as crucial indicators of blood parasitic infections in dogs. The study also revealed that 4.16 % of the dogs exhibited leukopenia, a condition linked to bacteria that actively damage white blood cells. Ticks serve as the vector for this condition, with the underlying cause being identified as Ehrlichiosis (EH) disease. Dogs contract *Ehrlichia canis* when bitten by brown ticks carrying EH in their bloodstream. This infection infiltrates the dog's bloodstream, targeting white blood cells and triggering them to attack other blood cell tissues and the immune system, resulting in various symptoms such as fever, weight loss, low platelet count, purpura, abnormal bleeding, and pale mucous membrane (Vetdiag, 2020) (Table 3).

The results of the hematology tests on dogs revealed that 78 % (39/50) of the dogs had normal color and size. Anemia was observed in 6 % (3/50) of the dogs, with less color than normal (hypochromia) and a normal size of red blood cells. Additionally, anemia was observed in 8 % (4/50) of the dogs with less color than normal (hypochromia) and large red blood cells (macrocytes). Another 8 % (4/50) of the dogs also had anemia, with an increase in the intensity of red blood cell color (hyperchromic) and the normal size of RBC. The abnormal color of RBC indicates nutritional deficiencies such as iron and low RBC counts due to chronic diseases. The abnormal RBC size indicates increased destruction of RBC; for example, RBC may be more fragile than usual due to immunodeficiency and blood parasite diseases, as shown in Table 4.

The insights gained from this study will subsequently be applied as a valuable guideline for mitigating diseases attributed to parasitic infestations in dogs.

Table 3. Hematology in raising dogs

| Parameters | Reference ranges | Raising dogs (n = 50) | | |
|---|---|---|---|---|
| | | Low | High | Normal |
| White blood cell | 6 - 17×10$^3$/uL | 4 (2/50) | 20 (10/50) | 76 (38/50) |
| Neutrophil | 60 - 75 % | 58 (29/50) | 6 (3/50) | 36 (18/50) |
| Blast cell | 0 - 4 % | 0 (0/50) | 0 (0/50) | 0 (0/50) |
| Lymphocyte | 12 - 30 % | 2 (1/50) | 54 (27/50) | 44 (22/50) |
| Monocyte | 3 - 10 % | 4 (2/50) | 70 (35/50) | 26 (13/50) |
| Eosinophil | 2 - 10 % | 76 (38/50) | 0 (0/50) | 24 (12/50) |
| Basophil | 0 - 1 % | 0 (0/50) | 2 (1/50) | 98 (49/50) |
| Red blood cell | 5.5 - 8.5 % | 42 (21/50) | 2 (1/50) | 56 (28/50) |
| Hemoglobin | 12.0 - 18.0 % | 34 (17/50) | 2 (1/50) | 64 (32/50) |
| Hematocrit | 37 - 55 % | 40 (20/50) | 0 (0/50) | 60 (30/50) |
| Mean cell volume | 60 - 77 fL | 0 (0/50) | 4 (2/50) | 96 (48/50) |
| Mean cell hemoglobin | 20 - 25 pg | 8 (4/50) | 12 (6/50) | 80 (40/50) |
| Mean corpuscular hemoglobin concentration | 32 - 36 g/dL | 8 (4/50) | 14 (7/50) | 78 (39/50) |
| Red blood cell distribution width | 12 - 15 % | 4 (2/50) | 44 (22/50) | 52 (26/50) |
| Platelets | 200 - 500×10$^3$/uL | 72 (36/50) | 0 (0/50) | 28 (14/50) |

## Conclusions

The findings from this study, derived from the examination of blood smears for diagnosing blood parasites, revealed varying prevalence rates of different parasitic infections among the examined dogs. *Ehrlichia* spp. in conjunction with *Anaplasma* spp. exhibited the highest prevalence, affecting 12 % (6/50) of the canine population. *Babesia* spp. infections were observed in 8 % (4/50) of the dogs, while *Anaplasma* spp. infections were identified in 2 % (1/50) of the cases. A substantial proportion, 78 % (39/50) of dogs, showed no evidence of parasitic infestations through blood smears. However, it is noteworthy that despite the absence of observable parasites, hematological values were often outside the normal range. This discrepancy suggested a strong inclination toward potential

Table 4. Red blood cell morphology in raising dogs.

| Red blood cell morphology | | Raising dogs % |
|---|---|---|
| Abnormal color | Abnormal size | |
| Normochromic | Normocytic | 78 (39/50) |
| Hypochromia | Normocytic | 6 (3/50) |
| Hypochromia | Microcyte | 0 (0/50) |
| Hypochromia | Macrocyte | 8 (4/50) |
| Hyperchromic | Normocytic | 8 (4/50) |
| Hyperchromic | Microcyte | 0 (0/50) |
| Hyperchromic | Macrocyte | 0 (0/50) |

blood parasite infections in a significant portion of the dogs, with 90 % (45/50) displaying hematological profiles indicative of such infections, while only 10 % (5/50) were at lower risk of having blood parasite infections.

These findings emphasize the crucial role that hematological values play in evaluating animal health and in diagnosis and forecasting blood parasite infections in dogs. When dogs exhibit hematological values below the normal range for parameters such as RBC, PCV, HB, and PLT values, they are more likely to be afflicted with blood parasitic infections compared to dogs with hematological values within the normal range. This highlights the significance of hematological assessments as a valuable tool in the early detection and management of blood parasite infections in dogs. To enhance testing efficiency, if budget permits, PCR testing should be employed to ensure the provision of accurate test data.

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