DETECTION OF HEMOPARASITES AND ASSOCIATED RISK FACTORS IN BACKYARD CHICKENS IN KOTA BHARU, KELANTAN

By

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ABSTRACT

Avian hemoparasites are known to have detrimental effects on chickens, causing pathological changes and contributing to mortality. Various infections, including hemoparasites, can infect backyard chickens and affect poultry productivity or the economy. In this study, backyard chickens raised in Kota Bharu, Kelantan, were examined for the presence of hemoparasites and their association with several risk factors. In July to August 2025, a cross-sectional study was conducted. Blood samples were collected from the wing and jugular veins of a total of 40 chickens. Fresh thin blood smears were prepared, fixed with methanol and stained with Giemsa stain at the Parasitology Laboratory, University Malaysia Kelantan for the microscopic examination. Out of 40 samples analysed, 11 were positive, yielding an overall detection rate of 27.5%. *Leucocytozoon* sp. was the only genus of hemoparasites found and species identification was based on morphological characteristics. *Leucocytozoon* was identified by the presence of roundish host cells without spindle-like projections. Furthermore, no significant association ($p > 0.05$) was found between hemoparasite detection and age, sex or rearing types. This study confirms the presence of hemoparasite infection in backyard chickens within the study area. The findings also provide baseline information for future research and may assist in developing effective infection control strategies.

Keywords: Hemoparasite, *Leucocytozoon,* risk factors, backyard chickens, detection

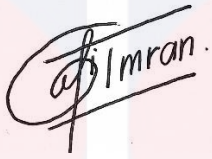
ABSTRAK

Hemoparasit dalam avian mampu menjejaskan kesihatan ayam dengan menyebabkan perubahan kepada patologi dan juga menyebabkan kematian. Pelbagai jangkitan termasuk hemoparasit boleh terjadi kepada ayam kampung dan mempengaruhi produktiviti serta memberi implikasi terhadap ekonomi, Kajian ini dijalankan untuk mengesan kewujudan jangkitan hemoparasit dalam ayam kampung dan untuk mengkaji kaitannya dengan faktor risiko yang tertentu di Kota Bharu, Kelantan. Satu kajian keratan rentas telah dilaksanakan dari Julai hingga Ogos 2025. Sebanyak 40 sampel darah telah diambil melalui vena sayap dan jugular. Filem darah nipis di atas slaid telah disediakan, difiks dengan metanol dan diwarnakan menggunakan pewarna Giemsa di Makmal Parasitologi, Universiti Malaysia Kelantan untuk pemeriksaan mikroskopik. Daripada 40 sampel yang dianalisis, 11 sampel didapati positif memberi kadar pengesanan keseluruhan sebanyak 27.5%. *Leucocytozoon* sp. merupakan satu-satunya genus hemoparasit yang dikesan dan penentuan spesies dibuat berdasarkan ciri morfologi. *Leucocytozoon* dikenal pasti melalui kehadiran sel perumah berbentuk bulat tanpa unjuran seperti ‘spindle’. Tiada perkaitan yang signifikan ($p > 0.05$) dijumpai antara pengesanan hemoparasit dengan umur, jantina atau jenis sistem ternakan. Kajian ini mengesahkan kewujudan jangkitan hemoparasit dalam ayam kampung di kawasan kajian. Maklumat ini dapat digunakan sebagai asas rujukan untuk penyelidikan lanjut serta membantu dalam perancangan strategi kawalan jangkitan yang lebih berkesan.

Kata kunci: Hemoparasit, *Leucocytozoon*, faktor risiko, ayam kampung, pengesanan

CERTIFICATION

It is hereby certified that we have read this research paper entitled of **"Detection of Hemoparasites and Associated Risk Factors in Backyard Chickens in Kota Bharu, Kelantan"**, by Nor Fazlinda binti Jafri and in our opinion, it is satisfactory in terms of scope, quality and presentation as partial fulfillment of the requirements for the course of DVT55204 - Research Project.



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LIST OF ABBREVIATIONS

DNA	-	Deoxyribonucleic acid
EDTA	-	Ethylenediaminetetraacetic acid
spp	-	species (plural)
FPV	-	Fakulti Perubatan Veterinar
GPS	-	Global Positioning System
ID	-	Identification
PCR	-	Polymerase Chain Reaction
SPSS	-	Statistical Package for the Social Sciences
UMK	-	Universiti Malaysia Kelantan
UPM	-	Universiti Putra Malaysia
WHO	-	World Health Organization

CHAPTER 1: INTRODUCTION

1.1 Research Background

In poultry production, overall performance and productivity are often impacted by various health issues, including outbreaks of parasitic, viral and bacterial diseases, as well as malnourishment, poor management and predation (Navqi *et al*., 2017). These issues can compromise production and result in significant economic losses. Among various poultry systems, backyard chicken production is vulnerable due to its low level of biosecurity, which increases the risk of disease exposure and transmission.

Despite these vulnerabilities, backyard chicken farming remains an important livelihood activity for many rural households due to its low initial investment and relatively high economic return. In many Southeast Asian nations, including Malaysia, Thailand, Indonesia and Vietnam, indigenous or local chicken breeds are commonly preferred for backyard systems. These breeds are favoured for their adaptability to the local environment, natural disease resistance and desirable flavour of their meat and eggs. As a result, backyard poultry contributes not only to food security but also to income generation, thereby enhancing purchasing power and supporting economic growth in rural communities (Das & Samanta, 2021).

Most backyard chickens are not confined and have free access to the outdoors, which increases the risk of exposure to vectors and consequently elevates the risk of hemoparasite infections. Furthermore, backyard flocks typically receive minimal veterinary attention, making them more susceptible to undetected and unmanaged parasitic diseases. Several hemoparasites have been identified, including *Plasmodium*, *Leucocytozoon*, *Trypanosoma* and *Hemoproteus*.

Among these, *Plasmodium* and *Leucocytozoon* are known to have the most significant influence on poultry productivity, while the other hemoparasites still require further investigation (Boonchuay *et al*., 2023, Navqi et al., 2017).

Avian plasmodiasis is an apicomplexan protozoan parasite caused by *Plasmodium* spp. It has become an important subject of study due to its potential to cause avian malaria. There are two well-known pathogenic species, which are *Plasmodium gallinaceum* and *Plasmodium juxtanucleare*. This disease is transmitted by dipteran insects that feed on blood that belong to the Culicidae and Ceratopogonidae families. The primary vectors include mosquitoes of the genera *Culex*, *Aedes*, *Culiseta*, *Mansonia*, *Aedeomya*, and *Coquillettinidia* (Nourani *et al*., 2020). Clinical signs of *Plasmodium* spp. infections in chickens include greenish feces, anemia, depression, reduced weight gain, fluffed-out feathers, and frequently cause death (Boonchuay *et al*., 2023). In addition, *Plasmodium* infection poses a significant threat to the poultry industry because of its potential to cause significant economic losses.

Leucocytozoonosis includes over 100 species, but only three are known to infect chickens, which are *Leucocytozoon caulleryi*, *Leucocytozoon sabrazesis*, and *Leucocytozoon schoutedeni* (Win *et al*., 2020). Among these, the most pathogenic is *Leucozytoon caulleryi*, which causes a lethal hemorrhagic disease. The primary vectors are several species of black flies (Diptera: Simuliidae). Clinical signs of Leucocytozoonosis in chickens vary depending on the chicken's age and health status. In young chicks, the disease is typically more severe and progresses rapidly. Signs include loss of appetite, listlessness, rapid breathing, weakness, and sometimes death within 24 hours. In adult chickens, symptoms tend to develop gradually and are generally milder compared to those in young chicks. Similar to *Plasmodium* infections, controlling vectors is an important preventive measure.

Trypanosoma spp. on the other hand, are flagellated protozoa that can occasionally be found in backyard chickens. This species is known to have no harmful effects on domestic chickens and wild birds. Although there are over 100 species of avian trypanosomes, they remain poorly understood (Boonchuay *et al*., 2023). Furthermore, *Hemoproteus* spp. are commonly found in wild birds. They are transmitted by biting midges (*Culicoides* spp.) and louse flies. Infections in chickens are rare to absent and typically subclinical but in some cases, they may contribute to anemia, weakness or decreased productivity, especially when co-infections are present (Valkiūnas & Iezhova, 2022).

1.2 Research Problems

Hemoparasites such as *Plasmodium* and *Leucocytozoon* are well-known to infect poultry and cause significant health problems and reduced productivity. *Trypanosoma* and *Hemoproteus* infections are less common in chickens in which they are typically occur in wild birds, but they can still infect chickens occasionally. While a study on hemoparasites in both wild birds and domestic poultry had been conducted in Selangor (Gimba *et al*., 2014), there is currently no recent published research focusing specifically on backyard chickens in Malaysia, particularly in Kelantan. In Kelantan, more than 70% of poultry flocks consist of backyard chickens, highlighting the importance of maintaining their health in backyard settings to achieve economic efficiency and promote animal welfare (Wan Norulhuda *et al*., 2017). The state's hot and humid climate also provides ideal conditions for insect vectors that transmit hemoparasites. Therefore, this study aims to identify the existence of hemoparasites in backyard chickens in Kota Bharu, Kelantan. Identifying these hemoparasites can help in understanding the disease burden, improving poultry health management and guiding future surveillance and control strategies.

1.3 Research Questions

1. Is there any presence of hemoparasites among the backyard chickens in Kota Bharu, Kelantan?
2. What are the genera of hemoparasites that can be identified from the blood samples of backyard chickens in Kota Bharu, Kelantan?
3. What are the risk factors associated with hemoparasite infections in backyard chickens in Kota Bharu, Kelantan?

1.4 Research Hypothesis

1. Hemoparasites are present in backyard chickens in Kota Bharu, Kelantan.
2. *Plasmodium*, *Leucocytozoon*, *Trypanosoma* and *Hemoproteus* are the hemoparasites that can be identified from the blood samples among the backyard chickens in Kota Bharu, Kelantan.
3. The age, sex and the rearing system (intensive, semi-intensive, and extensive) are the associated risk factors in backyard chickens.

1.5 Research Objectives

1. To detect the presence of hemoparasites in backyard chickens in Kota Bharu, Kelantan.
2. To identify the genera of hemoparasites found in blood samples collected from the backyard chickens in Kota Bharu, Kelantan.
3. To determine the risk factors associated with hemoparasite infections in backyard chickens.

CHAPTER 2: LITERATURE REVIEW

2.1 Overview of Backyard Chicken Rearing Systems

Backyard poultry rearing is typically a low-cost or no-cost activity requiring minimal resources. It primarily serves as a household supplementary activity rather than a main source of income. Furthermore, backyard chickens are also one of the low biosecurity poultry productions and they are at high risk of being exposed to diseases. Typically, each household raises 5 to 10 native or mixed-breed chickens. Rearing backyard chickens often involves indigenous night shelters, hens naturally hatching chicks without an artificial incubator, and a scavenging system where chickens forage for food naturally, so specialized feeding is usually not necessary. Sometimes, owners provide small amounts of grain twice daily to supplement their foraging. As a result, productivity is generally low, characterized by limited egg production and slow weight gain. However, interventions such as supplementation can boost their growth, egg output, and survivability (Sonkar *et al*., 2020).

Backyard chicken rearing is categorized into three types, which are extensive free-range, semi-intensive, and intensive. Free-range systems allow chickens to roam and forage freely. Semi-intensive combines free ranging with night-time confinement. Intensive systems are less common but present in Malaysia due to urban demand, involve full confinement, and have been practiced by medium to large-scale enterprises. Overall, backyard chicken farming is essential to rural livelihood by offering affordable protein income with low capital investment, despite challenges in productivity and health management.

2.2 Overview of Common Hemoparasites in Backyard Chickens

Backyard chickens are particularly vulnerable to hemoparasite infections due to low biosecurity and exposure to insect vectors. Several hemoparasites have been identified in poultry, but among them, *Plasmodium* and *Leucocytozoon* are considered the most significant in terms of pathogenicity and economic impact (Boonchuay *et al*., 2023, Navqi *et al*., 2017).

Avian plasmodiasis or avian malaria is a protozoan disease caused by *Plasmodium* spp. that affects various vertebrate hosts, including chickens. Among the numerous species, *Plasmodium gallinaceum* and *Plasmodium juxtanucleare* are the two most well-known pathogenic species in poultry. Transmission primarily occurs via mosquitoes from several genera, including *Culex*, *Aedes*, *Culiseta*, *Mansonia*, *Aedeomya*, and *Coquillettinidia* (Nourani *et al*., 2020), all of which play a critical role in spreading the infection. The life cycle of *Plasmodium* spp. requires both a vertebrate host and an insect vector, typically a mosquito. There are many stages in the life cycle of *Plasmodium*. Infection begins when an infected mosquito takes a blood meal and injects the sporozoites into the host's bloodstream. These sporozoites migrate to the liver cells, where they multiply before invading erythrocytes. This invasion leads to hemolysis, contributing to clinical signs such as anemia, which is typically observed as pale combs and wattles. Common clinical signs of avian plasmodiasis include fluffed-out feathers, decreased weight gain, depression, anemia, greenish diarrhea and frequently death (Boonchuay *et al*., 2023). According to Vaisusuk *et al*. (2022), *Plasmodium gallinaeum* is particularly widespread in Asia and Africa, while *Plasmodium juxtanucleare* is endemic in Asia, Africa, and South America.

Leucocytozoonosis is a malaria-like disease caused by protozoan parasites of the genus Leucocytozoon, which belong to the order Haemosporida within the phylum Apicomplexa (van

Wettere, 2016). Although more than 100 species have been identified, only three are known to infect chickens, which are *Leucocytozoon caulleryi*, *Leucocytozoon sabrazesis*, and *Leucocytozoon schoutedeni* (Win *et al*., 2020). From these three, *Leucocytozoon caulleryi* is the most pathogenic, known to cause severe hemorrhagic disease in young chickens. While chickens are the primary host, other avian species such as turkeys and ducks can also be affected. The disease is transmitted by biting insects, primarily black flies (*Simulium* species) and occasionally biting midges (*Culicoides* species). The parasite has a complex life cycle involving a vertebrate host, which is a chicken and an insect vector. Infection begins when an infected vector bites the host and injects sporozoites, which invade the liver and other tissues, undergo schizogony to produce merozoites, which then infect blood cells. Gametocytes form in the blood, are ingested by another vector and undergo sexual reproduction in the insect, eventually producing infective sporozoites in the salivary glands, completing the cycle. Clinical signs are often subclinical but can become severe, particularly in young chicks, where rapid onset may result in death within 24 hours. Common signs include anorexia, lethargy, fast breathing, and weakness. In adult chickens, symptoms tend to develop gradually and are generally mild. This disease has been reported in Malaysia (Gimba *et al*., 2014) and southern Thailand, particularly in backyard chicken populations (Boonchuay *et al*., 2023).

While less commonly associated with severe disease, other blood parasites, such as *Trypanosoma* spp. are flagellated protozoa that can occasionally be found in backyard chickens. They are generally considered non-pathogenic in poultry. According to a report, avian trypanosomes are not harmful to domestic chickens and their effects on wild birds remain poorly documented and understudied. In addition, there are about 100 species of avian trypanosomes that have been described but they remain poorly understood (Boonchuay *et al*., 2023). Furthermore,

Hemoproteus spp. are occasionally observed in chickens and are commonly found in wild birds. They are transmitted by louse flies and biting midges (*Culicoides* spp.). Infections in chickens are rare to absent and typically subclinical but in some cases, they may contribute to anemia, weakness or decreased productivity, especially when co-infections are present (Valkiūnas & Iezhova, 2022).

2.3 Diagnostic Techniques in Hemoparasite Detection

Several diagnostic techniques are available for the detection of hemoparasites in poultry. One of the most widely used methods in blood smear examination, which includes thin, thick and wet blood smears. Thin blood smears are useful for identifying hemoparasites such as *Plasmodium, Leucocytozoon, Hemoproteus* and *Trypanosoma,* as the morphology of erythrocytes and parasites can be clearly visualized. Thick blood smears although sensitive in detecting mild or chronic trypansome infections when parasite numbers are low but are not recommended for chickens due to the presence of nucleated erythrocytes. Wet blood smears, on the other hand, are a simple and rapid method for detecting live *Trypanosoma* species, as well as microfilariae (Ketema, 2025). Another commonly used diagnostic method is hematocrit centrifugation, in which blood in capillary tubes is centrifuged and the buffy coat layer is expressed onto a glass slide and examined under low-light microscopy to detect motile organisms such as microfilariae (Wettere, 2025). Impression smears of organs, including heart, spleen, liver and lungs, may also be prepared to detect hemoparasites present in tissues (Chandrawathani *et al*., 2019). In addition, molecular techniques such as polymerase chain reaction (PCR), provide high sensitivity and are capable of detecting infections even when parasite levels are too low to be observed in blood or tissue smears (Kumar *et al*., 2024).

However, microscopic examination of a thin blood smear remains the cornerstone of hemoparasite detection in avian species, particularly in field-based and resource-limited settings

such as backyard poultry systems. In addition, it is also considered a cheap, fast and gold standard for observing the morphology of the hemoparasite via Giemsa stain (Surya, 2025). Despite advances in molecular techniques, which are often praised for their higher sensitivity, microscopy examination continues to be widely used due to its practical advantages and diagnostic reliability.

One of the key strengths of microscopy is its ability to provide direct visualization of parasite morphology and developmental stages, allowing for species-level identification and confirmation of mixed infections. This is especially important for parasites such as *Plasmodium*, *Leucocytozoon* and *Hemoproteus*, which can present similar features but differ in pathogenicity and epidemiology. In contrast, PCR often fails to detect co-infections accurately due to selective DNA amplification (Valkiūnas *et al*., 2008).

In a comparative study by Valkiūnas *et al*. (2008), the sensitivity of microscopy was found to be comparable to PCR when performed using high-quality smears and by experienced examiners. The overall prevalence detected by microscopy (53.6%) closely matched PCR results (54.2%), and in some cases, microscopy was even slightly more sensitive.

In addition, microscopy has a lower risk of false positives, as parasite identification is based on visual confirmation. In contrast, PCR is vulnerable to DNA contamination and may yield inaccurate results if protocols are not carefully controlled. Furthermore, microscopy is capable of identifying mixed infections and a broader range of blood parasites without the need for multiple primers or protocols. On the other hand, PCR typically requires multiple primer sets and reactions to identify different parasites (Valkiūnas *et al*., 2008). Therefore, the use of microscopy in this study is justified and molecular techniques may be considered in future work to complement and confirm microscopic findings.

2.4 Prevention and Control Strategies

Some hemoparasite treatments are not effective and are mainly symptomatic. Therefore, to decrease infection rates, biosecurity and vector control measures need to be strengthened and prioritized.

Farmers can reduce mosquito and midge exposure by using insecticidal methods such as ULV fogging and residual sprays on coop walls, as well as physical barriers. In addition, a very low-cost method is installing mosquito nets, which help reduce hemoparasite transmission, such as *Plasmodium* (Gimba *et al*., 2014). Elimination of standing water through drainage and larvicide is crucial to control mosquito breeding sites and break the mosquito life cycle (WHO, 2020).

Currently, there is no widely available commercial vaccine for hemoparasites in chickens. However, a recombinant subunit vaccine targeting the R7 protein of *L. caulleryi* has been developed and brought to market in some countries (Umali *et. al*., 2014). Studies have demonstrated that this vaccine induces a strong humoral immune response, providing protective immunity against *L. caulleryi* infections in chickens. In the event of outbreaks, antiprotozoal drugs may be used prophylactically to limit the spread of infection. Historically, chloroquine and related antimalarials were administered to chickens to suppress *Plasmodium* infections (Gimba *et al*., 2014). However, due to variable efficacy and the emergence of drug resistance, supportive treatment such as improving nutrition and managing secondary infections remains an essential part of managing the affected flock.

CHAPTER 3: RESEARCH METHODOLOGY

3.1 Ethical considerations

The ethical approval for the use and handling of animals in this study was obtained from the Animal Ethics Committee of University Malaysia Kelantan under the code (UMK/FPV/ACUE/FYP/015/2025).

3.2 Methodology

3.2.1 Study Area

This study was carried out in Kota Bharu, Kelantan. A total of eight households with backyard chickens within this district were included based on accessibility and owner consent. The exact location of each household was also recorded using GPS coordinates to ensure precise geolocation. The addresses and coordinates for the selected households are as in Table 1. The sample processing was carried out in the Parasitology Laboratory of University Malaysia Kelantan, Pengkalan Chepa, Kelantan.

Table 1: Selected backyard chickens' households in Kota Bharu, Kelantan

Household	Latitude (°N)	Longitude (°E)
1	6.1679322	102.3147579
2	6.1679462	102.3146727
3	6.1684184	102.3133508
4	6.1684935	102.3129951
5	6.1370165	102.3039288
6	6.1376868	102.3043053
7	6.1367301	102.304051
8	6.1360585	102.3051539

3.2.2 Study Design

A cross-sectional study was conducted to determine the presence and diversity of blood parasite infection in backyard chickens in Kota Bharu, Kelantan.

3.2.3 Study Population

A total of forty chickens (n=40) were chosen by purposive sampling. The study population in this research consists of backyard chickens reared in eight selected households in Kota Bharu, Kelantan. The number of chickens sampled from each household was determined based on availability and feasibility during the study period, as shown in Table 2.

Table 2: Number of samples collected from selected backyard chickens' households in Kota Bharu, Kelantan

Household	Latitude (°N)	Longitude (°E)	No. of Samples
1	6.1679322	102.3147579	16
2	6.1679462	102.3146727	2
3	6.1684184	102.3133508	4
4	6.1684935	102.3129951	9
5	6.1370165	102.3039288	4
6	6.1376868	102.3043053	1
7	6.1367301	102.304051	2
8	6.1360585	102.3051539	2
Total			40

3.3 Study Criteria

3.3.1 Inclusion Criteria

The inclusion criteria for the samples focused on backyard chickens from small-scale reared primarily for household consumption or limited local sale. Owner consent was obtained before sampling. The study included any breed of chicken from both sexes and various age groups, specifically chicks (< 2 months), growers (2 to 8 months), and adults (more than 8 months), as shown in Table 3.

Table 3: The age variable converted into categorical data

Backyard chickens	
Chicks	Less than 2 months
Grower	Between 2 to 8 months
Adults	More than 8 months

3.3.2 Exclusion Criteria

The exclusion criteria of the sample were chickens raised in commercial farms with structured housing systems.

3.4 Sampling Method

3.4.1 Animal Profile

The history of the backyard chickens was collected from the owners, and a physical examination of every selected backyard chicken was performed. The patient signalment was recorded in an animal profile form before blood sampling, as in Table 4.

Table 4: Animal Profile Form

Chicken ID	Date	Location	Sex	Age	Rearing System

3.4.2 Sample Collection

Backyard chickens were captured using a hand-held catching net. This method was selected especially for the backyard chickens raised under extensive and semi-intensive systems as they were alert, difficult to approach and highly responsive to human presence. The catching process required careful handling, patience and proper technique to safely capture the chickens without causing stress or injury. Furthermore, the brachial or jugular veins of the 40 backyard chickens were used to draw blood using 25G needles and 3 mL disposable syringes. Each chicken was restrained using a two-hand body restraint technique to limit movement and to reduce the risk of injury to both the chicken and the handler during the procedure.

For brachial vein sampling, lateral recumbency was performed and the wing was lifted with one hand. Small feathers were plucked to allow better visualization of the vein. The area was swabbed with alcohol. The needle was positioned at a slight angle, bevel up and gently inserted into the vein. Approximately 3mL of blood was withdrawn slowly.

For jugular vein sampling, the chicken was placed laterally and the neck and feathers were stretched with one hand to expose the vein. The vein was palpated and swabbed with alcohol. The needle was then inserted at a slight angle, bevel up and blood was withdrawn slowly.

The collected blood was transferred into a sterile ethylenediaminetetraacetic acid (EDTA) blood collecting tube as an anticoagulant. All tubes were labeled using a permanent marker with their respective sample IDs. During transit, the samples were stored in an icebox to maintain an appropriate temperature until processing in the laboratory. Upon arrival at the laboratory, the samples were stored at 2–8 °C until further processing.

3.4.3 Sample Processing

3.4.3.1 Thin blood smear

A thin blood smear was prepared on a glass slide labelled with the respective sample ID. The smear was air-dried, fixed with methanol (HmbG Chemicals, Germany*)* for 5 minutes, and stained with Giemsa (R&M Chemicals, United Kingdom) for 30 minutes. After staining, the slide was rinsed with tap water, air-dried again, and examined for the presence of blood parasites under a light microscope (Olympus CX22LED microscope, Japan) using the 100x oil immersion magnification. In addition, a duplication smear was prepared for each sample ID to ensure accuracy and verification of results.

3.5 Data Collection

All data and relevant information for each sample chicken, such as location of house, age, sex, rearing system, sample collection date, and laboratory results for blood parasites (microscopic findings), were systematically recorded in Microsoft Excel.

3.6 Data Analysis

The IBM SPSS software version 27 was used to evaluate the collected data. Descriptive statistic, such as percentages, was used to express the detection rate, while the Fisher Exact test was employed to compare the association of hemoparasite infections with different risk factors such as age, sex and rearing types. A 95% confidence level and 0.05 absolute precision errors were considered. Statistical significance was defined as a p-value < 0.05.

CHAPTER 4: RESULTS AND DISCUSSION

4.1 Detection of Hemoparasites

A total of 40 backyard chickens from eight selected households in Kota Bharu, Kelantan, were examined for hemoparasite infections. The samples were analyzed to determine the relationship between risk factors and hemoparasite infection including age, sex and rearing type. Regarding sex, 13 backyard chickens (32.5%) were male while 27 (67.5%) were female. For age, the backyard chickens were categorized into three groups which were chicks, growers and adults. There were 2 chicks (5%), 11 growers (27.5%) and 27 adults (67.5%). Lastly, for rearing types, the samples included 12 backyard chickens raised in extensive systems (30%), 9 in semi-intensive systems (22.5%) and 19 in intensive systems (47.5%).

Table 5 shows the occurrence of hemoparasites across the eight selected backyard households. Hemoparasite-positive samples were detected in three households (Household 1, 5 and 6), with infection percentages ranging from 50% to 100%. The remaining five households recorded no hemoparasite-positive chickens.

Table 5: Hemoparasite occurrence in selected backyard households

Household	No of samples	Positive Hemoparasites	Negative hemoparasites	Percentage (%)
1	16	8	8	50
2	2	0	2	0
3	4	0	4	0
4	9	0	9	0
5	4	2	2	50

6	1	1	0	100
7	2	0	2	0
8	2	0	2	0

In the three hemoparasite-positive households, most chickens appeared clinically healthy and did not exhibit classical clinical signs of hemoparasite infection such as anemia, anorexia, weakness or difficulty breathing (McDougald *et al*., 2019). Several factors may contribute to the absence of clinical signs including parasite species pathogenicity, host adaptability and the age of the chickens. For example, *Plasmodium* infections are known to cause severe clinical signs in chickens, whereas *Trypanosoma* or *Hemoproteus* are generally harmless even when parasite burden is present (Valkiūnas & Iezhova, 2022). A previous study reported that *Leucocytozoon* is generally non-pathogenic in naturally adapted hosts but may cause disease when infecting new or immunologically naïve hosts (Elshad Ahmadov, 2019). Furthermore, the clinical signs in grower and adult chickens infected with *Leucocytozoon* are typically minimal or absent (Zahid, 2016).

Despite the absence of clinical signs, the presence of hemoparasites can still affect chicken performance and welfare. Subclinical infections may cause measurable alterations in hematological parameters, particularly reductions in leucocyte and erythrocyte counts, which indicate underlying physiological stress even when external signs are not apparent (Wamboi *et al*., 2020). A decrease in leucocytes can suppress humoral immunity, predisposing chickens to opportunistic infections and indirectly reducing productivity including egg production and growth performance which leads to economic losses for backyard owners. On the other hand, erythrocytes are vital for oxygen transport and carbon dioxide removal. Therefore, a reduction in erythrocyte

counts can impair physiological function and contribute to anemia. In more severe or prolonged cases, such impairment may progress to life-threatening conditions (Vinakpon *et al*., 2025).

4.2 Hemoparasites Identification

Table 6 presents the type of hemoparasite species in the examined backyard chickens. Out of the 40 chickens sampled from eight selected households, 11 blood smears were positive for hemoparasite infection. Only *Leucocytozoon* sp. (27.5%) was identified based on its characteristic morphological features and the positive samples originated from three households. No other hemoparasites, including *Plasmodium*, *Hemoproteus* or *Trypanosoma,* were detected in any of the examined samples. The presence of *Leucocytozoon* suggests the existence of suitable vectors in the area, particularly biting midges (*Culicoides* spp.), which are known to transmit this parasite. *Leucocytozoon* is an obligately heteroxenous parasite, requiring more than one host species for its development, with part of its lifecycle occurring within blood-sucking vectors and the remainder in avian hosts after exposure to infected vectors (Srikacha *et al*., 2025).

In addition, the detection rate of *Leucocytozoon* was considerably higher than previously reported in Malaysia, where the occurrence was only 0.6% from 2 out of 728 samples over a two-year sampling period in commercial chickens and was absent in backyard flocks in Selangor. In contrast, the higher detection in the present study may be attributed to several factors such as the greater likelihood of vector exposure in backyard management systems and the local environmental conditions in Kota Bharu, Kelantan. Differences in *Leucocytozoon* occurrence between studies are often influenced by variations in sampling effort, geographical location, chicken's immune status, seasonality and the density of arthropod vectors involved in parasite

transmission (Gimba *et al*., 2014). These factors together may explain the higher detection observed in this study.

Table 6: Types of hemoparasites with the number of backyard chickens infected and the percentage

Type of hemoparasites	Number of infected	Percentage (%)
Leucocytozoon sp.	11	27.5
Other hemoparasites	0	0
Total	11	27.5

Leucocytozoon parasites were easily distinguishable from other avian hemoparasites based on several characteristic features. First, they possessed the unique capacity to fully break down hemoglobin which causes pigment granules to be absent in all blood stages. Second, since only gametocytes develop inside blood cells, they did not experience asexual multiplication in the blood. Third, they had a significant impact on the nucleus of the host cells, causing pronounced deformation. As *Leucocytozoon* gametocytes mature, the host cell nucleus undergoes rapid hypertrophy and distortion, often becoming displaced to the periphery and tightly appressed to the parasite. This nuclear deformation was a key diagnostic feature across all *Leucocytozoon* species. Gametocytes, which consist of microgametocytes and macrogametocytes, can be differentiated based on their morphology in a thin blood smear. Microgametocytes (male) possess cytoplasm that is more pink, larger in size, have poorly defined nuclei and lack prominent nucleoli (Figure 1). In contrast, macrogametocytes (female) have cytoplasm that stains intensely with Giemsa and their

nuclei are compact with clear nucleoli and sharply defined nuclear boundaries. The parasite also appears denser and more well-defined in macrogametocytes (Figure 2) (Gediminas Valkiūnas & Iezhova, 2023).

Species identification could be achieved through careful examination of gametocyte morphology, particularly the shape of the host cells they occupied. Based on host cell morphology, *Leucocytozoon* species were categorized into three groups. The first group was species that exclusively develop in roundish host cells, such as blood cells. The most common species were *L. caulleryi* and *L. schoutedeni.* In roundish host cells, they did not develop into spindle-like cytoplasmic projections. The second group included species like *L. sabrazesis*, which produced only in fusiform host cells and further formed distinct cytoplasmic projections. The last group was species that were capable of developing in both fusiform and roundish host cells (Srikacha *et al.*, 2025).

The examination of thin blood smears revealed gametocytes enclosed exclusively within roundish host cells, mainly in the erythrocytes, with no evidence of spindle-like cytoplasmic projections. This morphological feature strongly supported the identification of the *Leucocytozoon caulleryi* and *Leucocytozoon schoutedeni.* However, we were more towards the *L. caulleryi* as it was the most common to be found in Southeast Asian (McDougald *et al*., 2019) compared to *L. schoutedeni,* found in parts of Africa (Danisile Tembe *et al.*, 2023). In addition, the cytoplasm of the host cell became less evident as the gametocyte grew and can be seen a remnant of host cell nuclei and it became completely obscured when the parasite reached full maturity. The nucleus of

the host cell was displaced toward the periphery as a result of the expanding gametocyte (Figure 3).

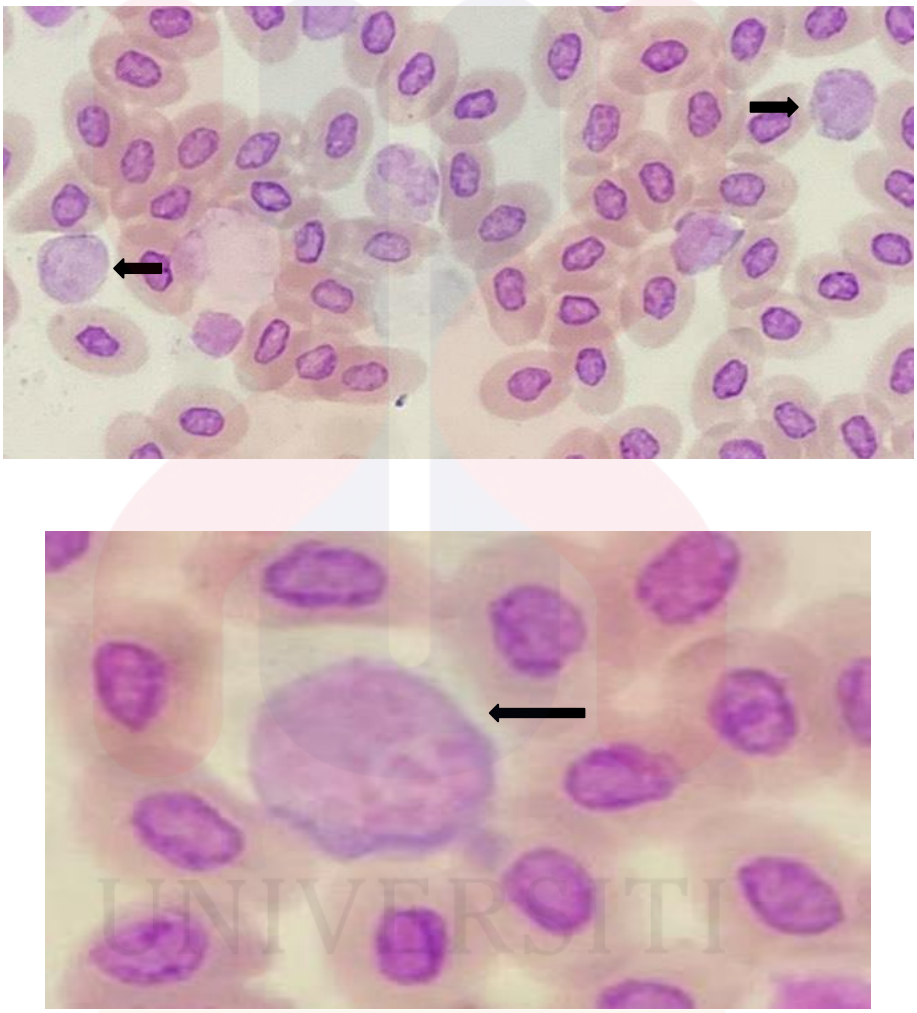

Figure 1: Microgametocytes of *Leucocytozoon* sp. with pinkish cytoplasm and poorly defined nucleus shown by black arrows

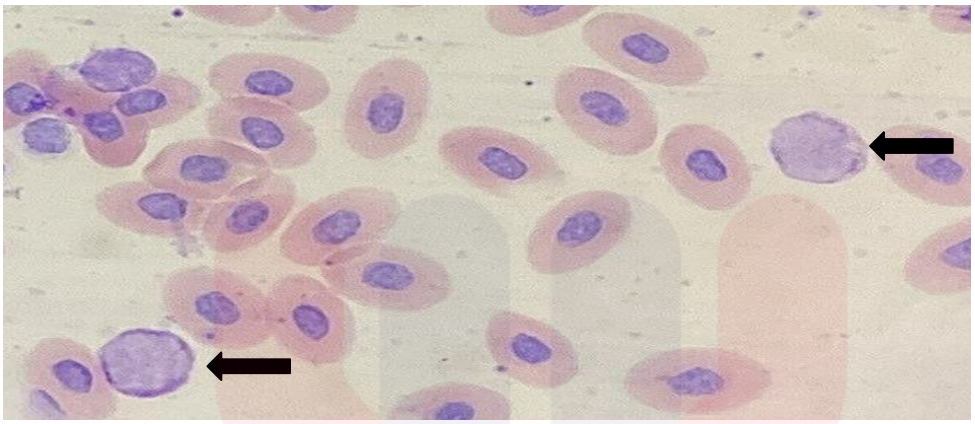

Figure 2: Macrogametocytes of *Leucocytozoon* sp. shown by black arrows

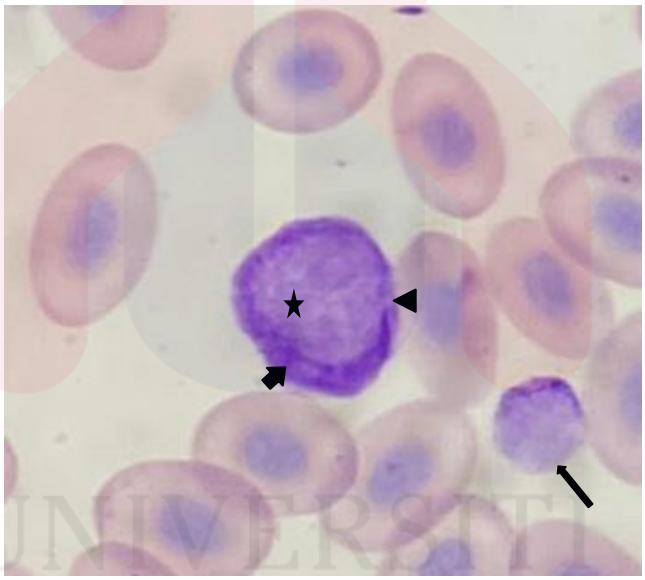

Figure 3: The macrogametocytes with a short arrow indicate the host cell nucleus at the periphery. The triangular arrowhead shows the remnants of the host cell cytoplasm, while the star-shaped shows the cytoplasm of the *Leucocytozoon* sp. with a defined nucleus. Another long arrow on the right side of the image points to the growing gametocytes.

4.3 Association of hemoparasites and risk factors

Table 7 summarizes the occurrence of hemoparasite infections across different age groups, sexes and rearing systems along with the statistical assessment of their associations. In this study, no infections were detected in chicks (0%), while growers and adults showed infection rates of 18.2% and 33.3% respectively. Although adults exhibited the highest percentage of hemoparasite infection, followed by growers, the association between age group and *Leucocytozoon* infection in backyard chickens was not statistically significant (Fisher's Exact Test, $p = 0.588$; $p > 0.05$).

The absence of hemoparasite infection in chicks and the relatively lower detection in growers in this study may be influenced by the small and uneven sample sizes among age groups. Only two chicks and 11 growers were included compared to 27 adult chickens, which reduces the likelihood of detecting infectious in these smaller subgroups even if the parasite is present in the population. Small sample sizes limit statistical power and may result in non-detection of low-prevalence infections, a phenomenon commonly observed in parasitology surveys (Volodimir Sarabeev *et al*., 2025). Therefore, the lack of detected infections in chicks does not necessarily indicate resistance or absence of exposure but rather reflects the limited representation of these age groups in the sample. Future studies should aim to include larger and more evenly distributed samples across all age groups to improve accuracy.

Furthermore, for sex groups, female chickens (33.3%) showed a higher proportion of infection compared to males (15.4%), although the calculated odds ratio (OR = 0.364; 95% CI: 0.066 - 2.002) and p-value ($p = 0.588$) indicate no significant association between sex and *Leucocytozoon* infection in backyard chickens.

On the other hand, among the rearing systems, the intensive system showed the highest occurrence (42.1%), followed by extensive (25%), whereas no infections were detected in semi-intensive systems. This contrasts with the general expectation that extensively reared chickens have higher exposure to vectors due to outdoor scavenging (Tawatchai Pohuang *et al*., 2021). A possible explanation is that several intensive flocks in this study were housed in shade, poorly managed with improper waste management, stagnant water, discarded tyres and damp surroundings. This favors vector breeding. In addition, *Culicoides* midges are not homogenously distributed in the environment as they tend to aggregate around hosts to obtain blood meals. In contrast, chickens in extensive systems ranged widely, dispersing vectors and reducing the likelihood of repeated bites, which may explain the lower infection rate observed in these flocks (Armin & Gonzales, 2023). In addition, no statistically significant correlation ($p > 0.05$) was found between rearing type and *Leucocytozoon* infection (Fisher's Exact Test, $p = 0.055$).

Table 7: Statistical analysis of the associated risk factors among hemoparasite-infected chickens

Risk factors		Tested (n)	Positive (n)	Percentage (%)	Odd ratio	95% CI	P-value
Age	Chicks	2	0	0	-	-	0.588
	Grower	11	2	18.2			
	Adult	27	9	33.3			
Sex	Male	13	2	15.4	0.364	0.066-2.002	0.588
	Female	27	9	33.3			
Rearing system	Extensive	12	3	25.0	-	-	0.055
	Semi-intensive	9	0	0			

	Intensive	19	8	42.1			

During the study, the sampling was conducted from July to August 2025, when the weather was warm (31-32°C) with substantial rainfall (averaging 155-172 mm) and high relative humidity (86%) (AccuWeather, 2025). These conditions are favourable for midges, which are most active in warm and humid environments. In fact, Malaysia generally experiences relatively consistent tropical conditions throughout the year, making these months a peak period for midge activity. Consistently, other studies have reported that Leucocytozoonosis is commonly detected from the summer to the beginning of the wet season (Suprihati and Yuniarti, 2017).

Several limitations may have affected the accuracy and representativeness of the findings. The sample size was small and only a limited number of households were included, which may reduce the statistical power and hinder the real associations. In addition, the study was confined to a single district, and therefore, the results may not represent all backyard chickens in Kelantan.

CHAPTER 5

5.1 Conclusion

This study revealed the presence of hemoparasites in backyard chickens within the study area. Although only one parasite species which is *Leucocytozoon* sp., was detected, its presence indicates active transmission within the area. With respect to age groups, adults had the highest detection rate (33.3%), followed by growers (18.2%) while no infections were detected in chicks. Among the sex groups, females showed a higher detection rate (33.3%) compared to males (15.4%). For the rearing types, the highest detection occurred in intensive systems (42.1%), followed by extensive systems (15.4%) and no infections were found in semi-intensive systems. No significant associations were observed between hemoparasite infection and the assessed risk factors, likely due to the limited study area and relatively small sample size.

Despite these limitations, the findings demonstrate that hemoparasite exposure is occurring in backyard flocks. Therefore, this study provides important baseline information on the current situation of hemoparasite infection in backyard chickens in Kota Bharu, Kelantan.

5.2 Recommendation

Based on the results obtained, future studies should include a larger sample size and cover more districts within the state to improve the likelihood of detecting hemoparasite infections, particularly when the overall detection rate is low. Furthermore, while microscopic examination allows species identification, combining it with molecular diagnostic techniques like PCR is recommended to increase the accuracy and sensitivity of hemoparasite detection.

APPENDICES

Figure 4: The captured chickens were provided with feed

Figure 5: Blood sample collection of the backyard chickens

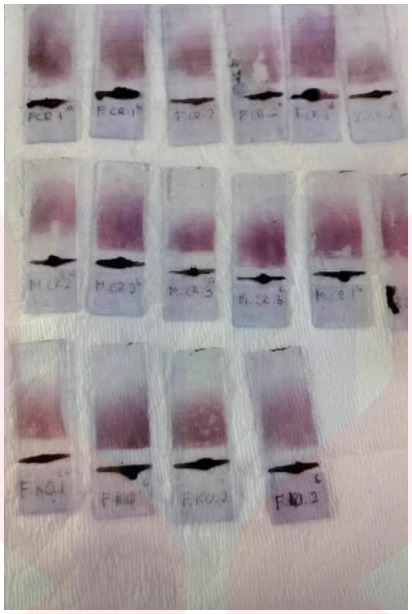

Figure 6: Thin blood smears fixed with methanol and stained with Giemsa stain

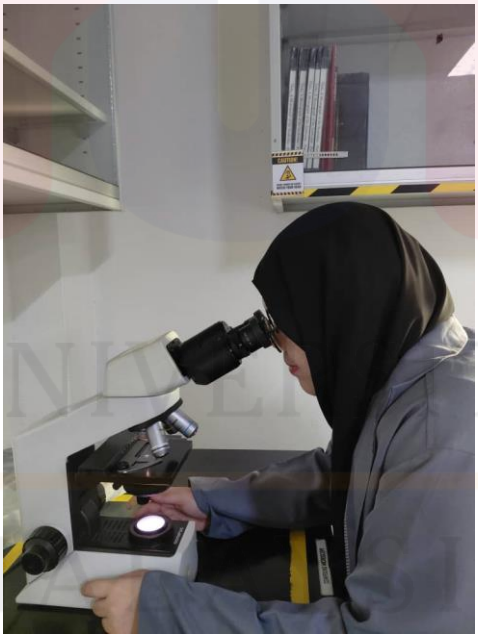

Figure 7: Microscopic examination at Parasitology Laboratory, FPV UMK

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