IDENTIFICATION OF BLOOD PARASITES IN CATTLE AND BUFFALOES IN VETERINARY TEACHING FARM, UMK BACHOK

By

NUR HANANI BINTI MOHAMED HARIS

A RESEARCH PAPER SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE DEGREE OF DOCTOR OF VETERINARY MEDICINE

FACULTY OF VETERINARY MEDICINE

UNIVERSITI MALAYSIA KELANTAN

2024

ORIGINAL LITERARY WORK DECLARATION

I hereby certify that the work embodied in this thesis is the result of the original research and has not been submitted for a higher degree to any other University or Institution.

☐	**OPEN ACCESS**	I agree that my thesis is to be made immediately available as hardcopy or online open access (full text).
☐	**EMBARGOES**	I agree that my thesis is to be made available as hardcopy or online (full text) for a period approved by the Post Graduate Committee. Dated from______until_____.
☐	**CONFIDENTAL**	(Contains confidential information under the Official Secret Act 1972)*
☐	**RESTRICTED**	(Contains restricted information as specified by the organization where research was done)*

I acknowledge that Universiti Malaysia Kelantan reserves the right as follows.

1. The thesis is the property of Universiti Malaysia Kelantan
2. The Library of Universiti Malaysia Kelantan has the right to make copies for the purpose of research only.
3. The library has the right to make copies of the thesis for academic exchange.

SIGNATURE OF CANDIDATE

SIGNATURE OF SUPERVISOR

NUR HANANI BINTI MOHAMED HARIS

NRIC/PASSPORT NO. 010406101186

DATE: 5/2/2025

DR. DR. BASRIPUZI NURUL HAYYAN BINTI HASSAN BASRI

NAME OF SUPERVISOR

DATE: 5/2/2025

Note: * If the thesis is CONFIDENTIAL OR RESTRICTED, please attach the letter from the organization stating the period and reasons for confidentiality and restriction.

ABSTRACT

An abstract of the research paper presented to the Faculty of Veterinary Medicine, Universiti Malaysia Kelantan, in partial requirement of the course DVT 55204 – Research Project.

Anaplasmosis, babesiosis, and theileriosis are major tick-borne diseases caused by blood parasites (*Anaplasma* spp., *Babesia* spp., and *Theileria* spp.), which affect cattle and buffaloes that contribute to subclinical infections. This study aimed to detect and identify the blood parasites at the Veterinary Teaching Farm, UMK Bachok, microscopically.

A total of 17 animals (16 cattle, one buffalo) were sampled for blood collection. Thin blood smears were prepared, stained, and examined under a microscope to identify *Anaplasma* spp., *Babesia* spp., and *Theileria* spp. Co-infections between the parasites have been observed, and packed cell volume (PCV) was measured to assess the relationship between parasitic infections and anemia. Data were analyzed using Spearman's Rank Correlation Coefficient.

The study found a high prevalence of *Anaplasma* spp. (86.7%), moderate prevalence of *Babesia* spp. (20%), and low prevalence of *Theileria* spp. (6.7%). Co-infections of *Anaplasma* spp. and *Babesia* spp. were observed in 20% of the animals. No significant correlations were found between blood parasites and PCV levels. Environmental factors such as the tropical climate and grazing practices likely contributed to the high prevalence of *Anaplasma* spp., while reduced vector activity during the sampling period may explain the lower prevalence of *Babesia* spp. and *Theileria* spp.

In conclusion, blood parasites are prevalent in the cattle and buffalo population at UMK Bachok, with *Anaplasma* spp. being the most common. Future studies should include larger sample sizes, explore host factors such as age and immunity, and incorporate molecular techniques like PCR to improve sensitivity and detection accuracy. Regular screening programs, along with monitoring health parameters like PCV, are recommended to enhance parasite control and improve herd health and productivity.

Keywords: Anaplasmosis, babesiosis, theileriosis, tick-borne diseases, blood parasites, *Anaplasma* spp., *Babesia* spp., *Theileria* spp., cattle, buffalo, co-infections, packed cell volume (PCV), prevalence

ABSTRAK

Satu abstrak bagi kertas penyelidikan yang dibentangkan kepada Fakulti Perubatan Veterinar, Universiti Malaysia Kelantan, sebagai sebahagian daripada keperluan kursus DVT 55204 – Projek Penyelidikan.

Anaplasmosis, babesiosis, dan theileriosis adalah penyakit utama yang disebarkan oleh kutu dan disebabkan oleh parasit darah (*Anaplasma* spp., *Babesia* spp., dan *Theileria* spp.), yang mempengaruhi lembu dan kerbau serta menyumbang kepada jangkitan subklinikal. Kajian ini bertujuan untuk mengesan dan mengenal pasti kehadiran parasit darah ini di Ladang Pengajaran Veterinar, UMK Bachok, secara mikroskopik.

Sejumlah 17 haiwan (16 lembu, satu kerbau) telah diambil sampel untuk pengambilan darah. Slaid darah nipis disediakan, diwarnakan, dan diperiksa di bawah mikroskop untuk mengenal pasti *Anaplasma* spp., *Babesia* spp., dan *Theileria* spp. Ko-infeksi juga direkodkan, dan jumlah sel pekat (PCV) diukur untuk menilai hubungan antara jangkitan parasit dan anemia. Data dianalisis menggunakan Koefisien Korelasi Peringkat Spearman.

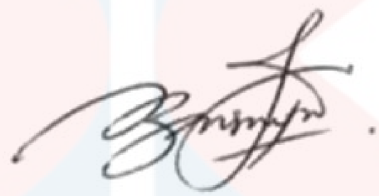
Kajian ini mendapati prevalens yang tinggi bagi *Anaplasma* spp. (86.7%), prevalens sederhana bagi *Babesia* spp. (20%), dan prevalens rendah bagi *Theileria* spp. (6.7%). Ko-infeksi antara *Anaplasma* spp. dan *Babesia* spp. ditemui dalam 20% haiwan yang dikaji. Tiada korelasi yang signifikan dijumpai antara parasit darah dan tahap PCV. Faktor persekitaran seperti iklim tropika dan amalan meragut mungkin menyumbang kepada prevalens tinggi *Anaplasma* spp., sementara pengurangan aktiviti vektor semasa tempoh pengambilan sampel mungkin menerangkan prevalens yang lebih rendah bagi *Babesia* spp. dan *Theileria* spp.

Kesimpulannya, parasit darah adalah lazim dalam populasi lembu dan kerbau di UMK Bachok, dengan *Anaplasma* spp. sebagai yang paling lazim. Kajian akan datang harus melibatkan saiz sampel yang lebih besar, meneroka faktor hos seperti umur dan imuniti, serta menggabungkan teknik molekul seperti PCR untuk meningkatkan sensitiviti dan ketepatan pengesanan. Program saringan berkala, bersama dengan pemantauan parameter kesihatan seperti PCV, disyorkan untuk meningkatkan kawalan parasit dan memperbaiki kesihatan serta produktiviti kumpulan ternakan.

Kata kunci: Anaplasmosis, babesiosis, theileriosis, penyakit bawaan kutu, parasit darah, *Anaplasma* spp., *Babesia* spp., *Theileria* spp., lembu, kerbau, jangkitan bersama, volum sel pekat (PCV), prevalens

CERTIFICATION

This is to certify that we have read this research paper entitled **'Identification of Blood Parasites In Cattle And Buffaloes In Veterinary Teaching Farm, UMK Bachok'** by **Nur Hanani Binti Mohamed Haris**, and in our opinion, it is satisfactory in terms of scope, quality, and presentation as partial fulfilment of the requirements for the course DVT 55204 – Research Project.



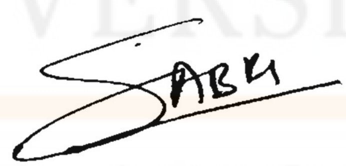
DR. BASRIPUZI NURUL HAYYAN BINTI HASSAN BASRI

DKHP (UPM), DVM (UPM), MVSc (UPM), PhD (University of Glasgow) Senior Lecturer

Faculty of Veterinary Medicine

Universiti Malaysia Kelantan

(Supervisor)



DR. MOHAMMAD SABRI BIN ABDUL RAHMAN

DVM (UPM), MVSc (UPM), PhD (UPM)

Senior Lecturer

Faculty of Veterinary Medicine

Universiti Malaysia Kelantan

(Co-supervisor)

ACKNOWLEDGEMENT

Special thanks to those who have given their support, guidance, advice, and aid for the completion of this project paper:

DR. BASRIPUZI NURUL HAYYAN BINTI HASSAN BASRI

DR. MOHAMMAD SABRI BIN ABDUL RAHMAN

DING TIAN YANG

MY FAMILY

LAB ASSISTANTS OF FPV UMK

DVM 5 CLASS OF 2020/2025

Thank You

DEDICATIONS

First and foremost, I would like to express my deepest gratitude for the health and strength granted to me by Allah SWT throughout this research project, allowing me to successfully complete it without any major challenges.

I would like to extend my heartfelt thanks to my beloved parents, Norlela Binti Abu Mansor and Mohamed Haris Bin Yahya, for their unwavering support, love, and encouragement throughout this journey. Their sacrifices have been fundamental to my success.

I sincerely appreciate the guidance and constant support from my supervisor, Dr. Basripuzi Nurul Hayyan Binti Hassan Basri, and co-supervisor, Mohammad Sabri Bin Abdul Rahman. Special thanks to Dr. Hayyan for providing the necessary resources and for patiently guiding me throughout this research process. This would not be possible without her as my mentor.

I am also grateful to Ding Tian Yang for his assistance with blood collection and laboratory works, which were important parts to my research project.

Additionally, I would like to thank the lab assistants of FPV UMK for their support and assistance throughout the research project and laboratory works.

Finally, I dedicate this work to my classmates from the DVM 5 Class of 2020/2025, who have been by my side, offering endless support and encouragement throughout the project.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

spp.	-	species (plural)
DVS	-	Department of Veterinary Services
FPV	-	Fakulti Perubatan Veterinar
PCR	-	Polymerase Chain Reaction
PCV	-	Packed Cell Volume
SPSS	-	Statistical Package for the Social Sciences
UMK	-	Universiti Malaysia Kelantan

CHAPTER 1

1.0 INTRODUCTION

Anaplasmosis, babesiosis, and theileriosis are significant tick-borne diseases that cause remarkable health threats to cattle populations worldwide (Adjou-Moumouni et al., 2015). These diseases are caused by blood parasites that are often associated with inapparent infections, that contribute to a large reservoir of subclinical carriers. These carriers then play a crucial role in maintaining the transmission cycle, serving as ongoing sources of infection to susceptible animals within herds (Bell-Sakyi et al., 2004). Previous studies have highlighted the presence of various blood parasites in cattle across Peninsular Malaysia, including species such as *Anaplasma* spp., *Babesia* spp., *Mycoplasma* spp., *Theileria* spp., and *Trypanosoma* spp. (Agina et al., 2021). Among these, *Anaplasma marginale* is widely recognized as endemic in Malaysia (Tay et al., 2014).

Anaplasmosis, historically known as gall sickness, is primarily caused by the bacteria *Anaplasma* spp., which are obligate intraerythrocytic bacteria that infect red blood cells. This disease mainly affects ruminants such as cattle, buffaloes, goats, and sheep. The primary vectors for the transmission of *Anaplasma* spp. are ticks, although biting flies can also contribute to the spread of infection. Cattle in endemic regions often develop partial immunity over time, but they still can remain asymptomatic carriers, which can cause harder disease management (Aiello et al., 2016). The pathogenesis of anaplasmosis typically involves an acute febrile response coupled with severe hemolytic anemia, often resulting in the destruction of up to 70% of erythrocytes within a short span of one week. Clinically, affected animals exhibit signs of anemia, including depression, weakness, fever, dyspnea, inappetence, dehydration, constipation, and jaundice, with disease severity typically increasing with the age of the animal (Taylor et al., 2016).

Similarly, *Babesia bigemina*, a protozoan parasite from the *Babesia* genus, is another significant pathogen affecting cattle, particularly in tropical regions. *Babesia bigemina* belongs to the Apicomplexa phylum and is a part of a larger group that includes more than 100 species, many of which can infect ruminants (Hunfeld et al., 2008; Svensson et al., 2019). Bovine babesiosis caused by *Babesia* spp., is manifested in cattle through a variety of clinical symptoms including anemia, hemoglobinemia, fever, and hemoglobinuria (Martins et al., 2008; Tembue et al., 2011). As one of the key tick-borne diseases, babesiosis causes a significant health concern for cattle, causing reduced productivity and economic losses (Rehman et al., 2017; Pupin et al., 2019; Svensson et al., 2019).

Theileriosis caused by *Theileria* protozoa is another major parasitic disease transmitted by ticks and is a significant concern for cattle health in tropical and subtropical regions. One of the *Theileria* species which is known as *Theileria annulata* are carried by ticks belonging to the *Rhipicephalus* genus (Hammer et al., 2015). Infected cattle experience a range of symptoms, including fever, anemia, and enlarged lymph nodes, due to the infection and subsequent transformation of host leukocytes. The disease can result in severe economic losses through decreased milk production, weight loss, and even mortality in affected cattle. The use of tick control methods and vaccination programs have been proposed as effective strategies for mitigating the impact of this disease on livestock populations (Kaba et al., 2014).

In Malaysia, these tick-borne diseases continue to affect cattle farming, with remarkable consequences for both the health of the animals and the economic stability of the industry. The high prevalence of blood parasites such as *Anaplasma* spp., *Babesia* spp., and *Theileria* spp. in cattle herds across the region highlights the importance of early diagnosis and management strategies. As the cattle farming industry is crucial to the agricultural economy of the region, improving herd health through the early detection of these infections can contribute to enhanced productivity and reduced losses (Agina et al., 2021).

The primary goal of this study is to identify blood parasite infections among cattle and buffaloes in the Veterinary Teaching Farm at UMK Bachok using microscopic method. Specifically, this study aims to identify the presence of *Anaplasma* spp., *Babesia* spp., and *Theileria* spp. within the herd. By improving diagnostic capabilities, this research seeks to provide valuable information for managing and controlling these parasitic infections, ultimately improving herd health and productivity in the farm.

1.1 RESEARCH PROBLEM STATEMENT

Tick-borne diseases cause a significant threat to farm animals' health and productivity worldwide. Among these, anaplasmosis, babesiosis, and theileriosis are particularly problematic to livestock industry. At the Veterinary Teaching Farm, UMK Bachok, there is no previous documentation on the presence and impact of *Anaplasma* spp., *Babesia* spp., and *Theileria* spp. infections in cattle and buffaloes. This knowledge gap hinders effective management and control strategies.

Solving this issue is essential since these illnesses may have a detrimental impact on farm animals' health and generate large production losses (Koonyosying et al., 2022), due to clinical presentations such as anemia, fever, and even death. Moreover, the health of animals at the Veterinary Teaching Farm indirectly impacts the learning process of veterinary students who rely on healthy animals for practical training and clinical studies. Infections by these blood parasites can disrupt the availability of healthy animals for hands-on learning experiences, thereby compromising the quality of veterinary education. Understanding the presence of these pathogens will help in developing targeted interventions to improve animal health and farm productivity, ensuring that veterinary students receive the best possible training environment.

The study focuses on cattle and buffaloes at the UMK Bachok farm, a representative setting for similar agricultural enterprises in the region. By identifying the presence of these pathogens, the research can inform broader regional control strategies.

1.2 RESEARCH QUESTIONS

- Is there any presence of blood parasite infections among the cattle and buffaloes at the Veterinary Teaching Farm, UMK Bachok?
- What are the genus of blood parasites that can be identified from the blood sample of cattle and buffaloes in the Veterinary Teaching Farm, UMK Bachok?

1.3 RESEARCH HYPOTHESIS

- Blood parasite infections are present among the cattle and buffaloes at the Veterinary Teaching Farm, UMK Bachok.

- *Anaplasma* spp., *Babesia* spp. and *Theileria* spp. are the blood parasites that can be identified from the blood sample among the cattle and buffaloes in the farm.

1.4 RESEARCH OBJECTIVES

- To detect the presence of blood parasites among cattle and buffaloes at the Veterinary Teaching Farm, UMK Bachok using microscopic method.
- To identify the genus of blood parasites from the blood samples of cattle and buffaloes in the Veterinary Teaching Farm, UMK Bachok.

CHAPTER 2

2.0 LITERATURE REVIEW

The purpose of this literature review is to explore the prevalence and diagnosis of blood parasites such as *Anaplasma* spp., *Babesia* spp., and *Theileria* spp., in cattle and buffaloes at the Veterinary Teaching Farm, UMK Bachok. This review encompasses studies from diverse geographical regions in Malaysia over the past twenty years to provide a comprehensive understanding of the topic.

2.1 OVERVIEW OF ANAPLASMOSIS, BABESIOSIS AND THEILERIOSIS

Anaplasmosis, babesiosis, and theileriosis are significant vector-borne diseases that primarily affect livestock, posing substantial economic and health challenges globally. These diseases are caused by protozoan parasites and bacteria transmitted by ticks, leading to severe clinical manifestations and high morbidity and mortality rates among affected animals (Sawitri et al., 2022).

The bacteria of the genus *Anaplasma* are the cause of anaplasmosis, and the most common species that infects cattle is *A. marginale*. Fever, anemia, jaundice, and weight loss are the main symptoms of the condition, which is mostly brought on by the bacteria's destruction of red blood cells. Transmission occurs mainly through tick bites, but it can also happen via contaminated needles, dehorning, and castration equipment (Kocan et al., 2004). Anaplasmosis can impact the economy by reducing milk production, poor growth rates, and increased veterinary costs. Control measures focus on tick control, vaccination, and antibiotic treatment, though resistance to antibiotics is an emerging concern (Radostits et al., 2007).

Babesia spp. are protozoan parasites causing babesiosis, in which the most prevalent species infecting the cattle are *Babesia bovis* and *B. bigemina*. The disease manifests with fever, hemolytic anemia, hemoglobinuria, and in severe cases, death. Ticks in the *Rhipicephalus* genus are the primary vectors for *Babesia* spp., facilitating the transmission of the parasite during blood meals (Bock et al., 2004). Babesiosis poses significant economic threats due to cattle mortality, decreased productivity, and the costs associated with treatment and tick control. Integrated management strategies, including acaricide application, and vaccination are crucial for controlling the disease (Radostits et al., 2007).

Theileriosis which is caused by the protozoan parasite *Theileria* spp. has a significant threat to livestock populations in many regions particularly in tropical and subtropical areas. The disease is primarily transmitted by tick vectors of the genus *Hyalomma* (Morrison, 2015), which play a crucial role in its epidemiology. Infection with *Theileria* spp. results in various clinical manifestations, including fever, anemia, and lymphadenopathy, ultimately leading to substantial economic losses in affected livestock. The parasite has a complex life cycle that include both vertebrate hosts, predominantly cattle, and invertebrate hosts, the tick vectors (Kaba et al., 2014). Control measures for theileriosis focus on managing tick populations through acaricide use and environmental management, alongside monitoring and treating affected animals with appropriate antiprotozoal drugs (Jenkins et al., 2015; Watts et al., 2016).

While anaplasmosis, babesiosis, and theileriosis differ in their causative agents and specific vectors, they share similarities in their transmission through ticks and the resultant economic and health impacts on livestock. The management of these diseases relies heavily on effective tick control, vaccination, and chemotherapeutic interventions. However, each disease presents unique challenges, such as the development of drug resistance in anaplasmosis (Kocan et al., 2004), the necessity for integrated management in babesiosis (Bock et al., 2004), and vaccine efficacy issues in theileriosis (Radostits et al., 2007).

2.2 PREVALENCE OF BLOOD PARASITES

Anaplasmosis is a disease condition caused by *Anaplasma* spp.has been documented in various parts of Malaysia. Studies indicate a relatively high prevalence of this disease in cattle, with reports suggesting seroprevalence rates ranging from 30% to 70% in different states. For instance, a study conducted in Perak and Kelantan reported an average seroprevalence of approximately 45% (Rahman et al., 2010). The high prevalence is attributed to the widespread presence of tick vectors, primarily *Rhipicephalus (Boophilus) microplus*, which facilitate the transmission of *A. marginale*.

Babesiosis caused by *B. bovis* and *B. bigemina*, is also prevalent in Malaysian cattle. A study conducted in Selangor and Pahang found a seroprevalence of 17% for *B. bovis* and 16% for *B. bigemina* (Rahman et al. 2010). Factors such as tick density, cattle management practices, and environmental conditions can influence the variation in prevalence. *Rhipicephalus (Boophilus) microplus* is the primary vector, and its widespread distribution in Malaysia contributes to the high prevalence of babesiosis.

Theileria annulata exhibits varying prevalence rates across different regions, with high endemicity reported in countries like Turkey and Egypt, where prevalence rates can reach up to 50% among cattle populations (Aktas, 2014; El-Ashker et al., 2017). The parasite is primarily transmitted through the bite of infected ticks, particularly *Rhipicephalus* species, which act as biological vectors (Shahnawaz et al., 2019). Control efforts often focus on managing tick populations and implementing strict biosecurity measures to curb transmission.

2.3 DIAGNOSTIC METHODS

Packed cell volume (PCV) determination is a widely used diagnostic tool in veterinary parasitology to assess the severity of anemia, which is a common clinical sign of hemoparasitic infections such as anaplasmosis, babesiosis, and theileriosis. PCV measures the percentage of the blood volume representing the red blood cells, providing a quick and straightforward indication of the haematological status of the animal. Decreased PCV levels are indicative of haemolytic anemia, a hallmark of these parasitic diseases (Desta et al., 2020).

Microscopic examination of thin blood smears is a traditional and fundamental diagnostic method for detecting blood parasites. Using this method, a small drop of blood is smeared onto a microscope slide, stained (usually with Giemsa dye), then examined under a microscope to check for blood parasites among the red blood cells. Thin blood smears allow for the identification of the morphological characteristics of parasites, which is crucial for differentiating between species of *Anaplasma* spp., *Babesia* spp., and *Theileria* spp. (Hadi et al., 2012). While this method is cost-effective and widely accessible, its sensitivity and specificity are highly dependent on the skill of the technician and the parasitaemia level (Noaman & Shayan, 2010).

2.4 MICROSCOPIC EXAMINATION AS THE GOLD STANDARD FOR BLOOD PARASITE DETECTION

Microscopic examination of stained blood smears has long been considered the gold standard for diagnosing blood parasites due to its simplicity, accessibility, and affordability (Noaman & Shayan, 2010). Staining techniques, such as Giemsa, allow for the direct visualization of parasites within erythrocytes, making it a reliable method for identifying various blood parasites (Hammed & Albadrani, 2021). This technique provides immediate results and is widely used in both field studies and laboratories where advanced diagnostic tools may not be available (Sawitri et al., 2022).

The primary advantages of microscopy include its ability to visually confirm parasitic infection and its cost-effectiveness, particularly in resource-limited settings (Noaman & Shayan, 2010). Furthermore, blood smears serve as a permanent record that can be revisited for reanalysis or further study (Hammed & Albadrani, 2021).

Despite these benefits, microscopy is not without its limitations. The method is labor-intensive, time-consuming, and highly dependent on the expertise of the operator, especially when differentiating parasites from artifacts like Howell-Jolly bodies (Noaman & Shayan, 2010). Its sensitivity decreases significantly in cases of low parasitemia, such as subclinical infections or chronic carriers, which may result in false negatives (Sawitri et al., 2022). Additionally, the morphological similarity among some parasites can make species identification challenging, further limiting its reliability in epidemiological studies (Hammed & Albadrani, 2021).

2.5 ADVANCEMENTS IN DIAGNOSTIC METHODS

In response to the limitations of microscopy, diagnostic techniques have evolved significantly over time. Modified staining methods, like rapid Giemsa, Leishman-Giemsa or Giemsa-toluidine blue have been developed to improve the speed and clarity of parasite detection. These advancements reduce the time required for staining and enhance the visibility of parasitic structures, making the process more efficient and accurate (Hammed & Albadrani, 2021).

The advent of molecular diagnostics, particularly PCR, has revolutionized the field by offering higher sensitivity and specificity, helping in better detection of low parasitemia and the differentiation of closely related species. These molecular methods have become invaluable in identifying carriers and conducting large-scale epidemiological studies where traditional microscopy falls short (Sawitri et al., 2022).

While these modern techniques address many of the challenges associated with microscopy, they often require specialized equipment and trained personnel, making them less accessible in certain regions. As a result, microscopy remains a cornerstone of blood parasite diagnostics, particularly when integrated with molecular methods to achieve a comprehensive and balanced diagnostic approach (Noaman & Shayan, 2010).

Each diagnostic method has its advantages and limitations. PCV determination is quick and provides valuable information about the animal's haematological status but lacks specificity for the type of infection. Thin blood smear examination allows for direct visualization of parasites but requires significant expertise and may not detect low parasitaemia levels. PCR offers high sensitivity and specificity, making it ideal for early detection and confirmation of infections, even when parasitaemia is low. Integrating these diagnostic methods can provide a comprehensive approach to diagnosing hemoparasitic infections. For instance, initial screening with PCV determination can identify animals with potential anemia. These cases can then be further investigated using thin blood smear examination for preliminary identification of parasites. Finally, PCR can be employed to confirm the diagnosis and differentiate between species, especially in cases with low parasitaemia or ambiguous smear results (Desta et al., 2020).

CHAPTER 3

3.0 MATERIALS AND METHODS

3.1 ETHICAL CONSIDERATIONS

Animal ethics approval was obtained from the Fakulti Perubatan Veterinar (FPV) Animal Ethics Committee (UMK/FPV/ACUE/FYP/012/2024) before commencement of the study.

3.2 SAMPLE COLLECTION AND PREPARATION

A total of 17 animals consisting of 16 cattle and a buffalo were sampled in Veterinary Teaching Farm, UMK Bachok. The animals were guided into the crush to restrict their movement and secure their heads for restraining purposes. Gloves and face masks were worn before proceeding with blood collection.

Blood was collected from the coccygeal vein of each animal. The tail was lifted, and the site was disinfected with cotton balls soaked in alcohol. The vacutainer needle was inserted perpendicularly to withdraw the blood. Once the blood was collected into an EDTA tube, the needle was removed, and pressure was applied to stop any bleeding. The blood tubes were labelled, stored in an icebox and transported to the Parasitology Laboratory, FPV UMK on the same day of sampling.

3.3 PCV MEASUREMENT

For the PCV measurement, the glass capillary tubes were filled with blood and sealed using Cristaseal (Hawskley and Sons Ltd, United Kingdom). The sealed tubes were then centrifuged at 220 rcf for 5 minutes, and the volume was measured using a rotoreader (Hawskley and Sons Ltd, United Kingdom) (Nur-Sabrina et al., 2024). The results for each sample were then recorded.

3.4 MICROSCOPIC EXAMINATION

Thin blood smears were prepared adhering to the standard protocol by the Veterinary Research Institute, Department of Veterinary Services (DVS) Malaysia (Omar et al., 2016) . The smears were air-dried and then fixed with methanol. They were subsequently stained with eosinophilic and basophilic stains from the Diff-Quick solution, following the manufacturer's guidelines (Labchem Sdn. Bhd, Malaysia). The prepared smears were examined at 100x magnification with a light microscope (Olympus CX21, Japan) to identify the presence of blood parasites according to Koonyosying et al. (2022). The results were then interpreted and documented in the form of digital images.

3.5 DATA MANAGEMENT

All data were collected and recorded using Microsoft Excel and analyzed using the software Statistical Package for the Social Sciences (SPSS) Version 27.0. Spearman's Rank Correlation Coefficient was used to assess the relationships between the presence of blood parasites (*Anaplasma* spp., *Babesia* spp., and *Theileria* spp.) and PCV. This non-parametric test was chosen as the data were not normally distributed. It also allows for the evaluation of monotonic relationships between ordinal or continuous variables. The significance level was set at $p < 0.05$. Correlation coefficients were interpreted as weak, moderate, or strong based on the guidelines according to Kuckartz et al. (2013). The results were considered statistically significant when p-values were less than 0.05.

CHAPTER 4

4.0 RESULTS

A total of 17 samples were analyzed for the presence of blood parasites namely *Anaplasma* spp. (Figure 1 & 2), *Babesia* spp. (Figure 3), and *Theileria* spp. (Figure 4), with two samples deemed invalid due to red blood cells coagulation, resulting in 15 valid samples. Among these, *Anaplasma* spp. had the highest prevalence, with 13 samples testing positive, accounting for 86.7% of the valid samples, and two samples testing negative, representing 13.3%. For *Babesia* spp., three samples were positive, making up 20% of the valid samples, while 12 samples were negative, comprising 80%. The prevalence of *Theileria* spp. was the lowest, with only one sample testing positive (6.7%) and 14 samples testing negative (93.3%). The percentage of invalid samples due to coagulation was consistent across all three parasites, with two invalid samples (11.8%) in total. In conclusion, the overall data shows that *Anaplasma* spp. was the most prevalent parasite, while *Theileria* spp. was the least prevalent among the sampled animals. The data collected is represented in Table 1.

Table 1. The overall prevalence of blood parasites among cattle and buffalo in the Veterinary Teaching Farm, UMK Bachok

Blood Parasites	Prevalence (%)	Negative samples (%)
Anaplasma spp.	86.7	13.3
Babesia spp.	20	80
Theileria spp.	6.7	93.3

The prevalence of co-infections between *Anaplasma* spp. and *Babesia* spp. were observed in 20% of the samples. Co-infections between *Anaplasma* spp. and *Theileria* spp. were less frequent, with a prevalence of 6.7%. Additionally, no co-infections between *Babesia* spp. and *Theileria* spp. were detected. The data is documented in Table 2.

Table 2. The prevalence of co-infections between blood parasites in cattle and buffalo in the Veterinary Teaching Farm, UMK Bachok

Blood Parasites	Prevalence (%)
Anaplasma spp. & *Babesia* spp.	20.0
Anaplasma spp. & *Theileria* spp.	6.7
Theileria spp. & *Babesia* spp.	0.0

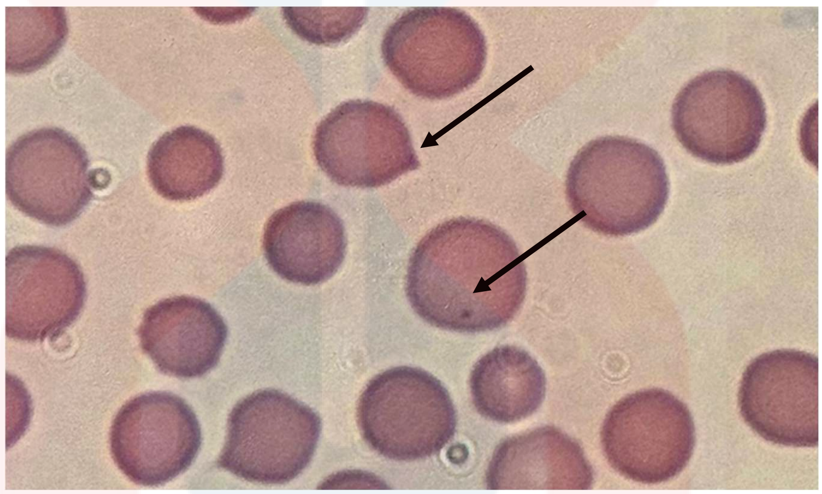


Figure 1: Presence of *Anaplasma* spp. in red blood cells

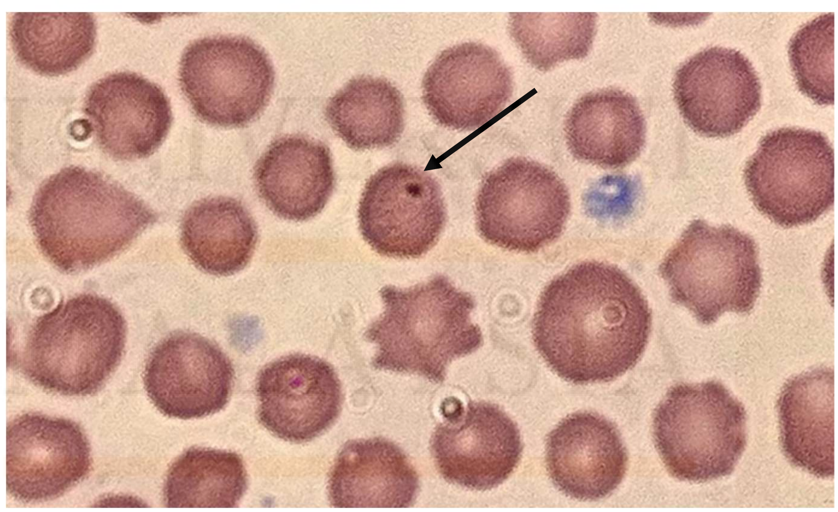


Figure 2: Presence of *Anaplasma* spp. in red blood cell

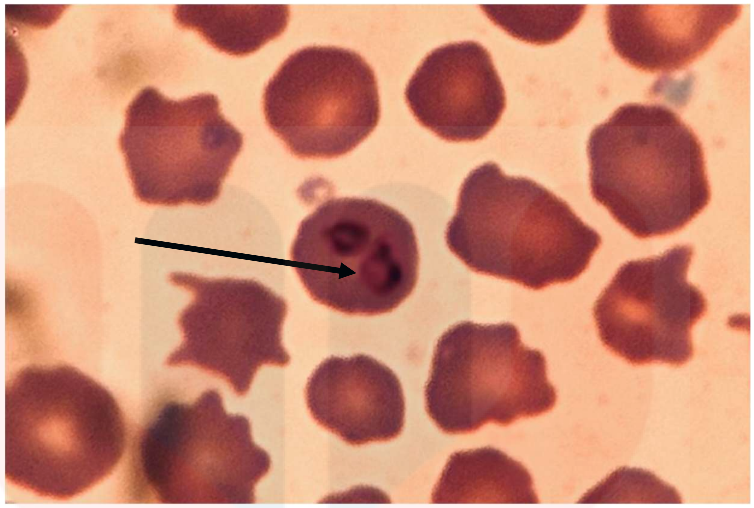


Figure 3: Presence of *Babesia* spp. in red blood cell

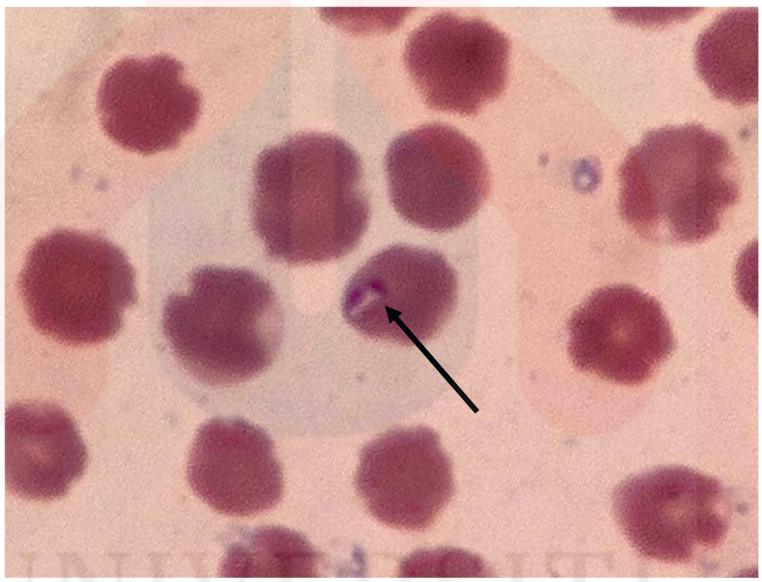


Figure 4: Presence of *Theileria* spp. in red blood cell

Out of the 17 samples analyzed, the PCV values were classified into three categories: low, normal, and high, based on the reference range of normal PCV for cattle between 24-46% and buffaloes' which ranged between 25.3-38.5% (Dhillon et al., 2020) as shown in Table 3. A sample had a PCV value that fell below the normal range, which suggests the presence of anemia. This occurred on a cattle that was detected with co-infection of *Anaplasma* spp. and *Babesia* spp. On the other hand, 14 samples showed normal PCV values and only two samples had high PCV.

Table 3. Distribution of PCV Categories in the Sampled Population

PCV Category	Normal Range (%)	Number of Samples
Low	Below	1
Normal	Within	14
High	Above	2

A Spearman's Rank Correlation Coefficient was conducted to evaluate the relationships among the presence of different blood parasites (*Anaplasma* spp., *Babesia* spp., and *Theileria* spp.) and PCV values. The correlation between the presence of *Anaplasma* spp. and *Babesia* spp. was weak and positive (r (15) = 0.196, p = 0.484), indicating no statistically significant relationship. Similarly, the correlation between the presence of *Anaplasma* spp. and *Theileria* spp. was weak and positive (r (15) = 0.105, p = 0.710), suggesting no significant association between these variables. A weak negative correlation was observed between *Anaplasma* spp. and PCV (r (15) = -0.296, p = 0.284), which was also not statistically significant.

The relationship between *Babesia* spp. and *Theileria* spp. was weak and negative (r (15) = -0.134, p = 0.635), indicating no significant association. Additionally, the correlation between *Babesia* spp. and PCV was very weak and negative (r (15) = -0.019, p = 0.945), which was not statistically significant. Finally, a moderate positive correlation was found between *Theileria* spp. and PCV (r (15) = 0.372, p = 0.172), but this relationship was also not statistically significant. The correlation coefficients among the blood parasites and the PCV is visualized in Figure 5.

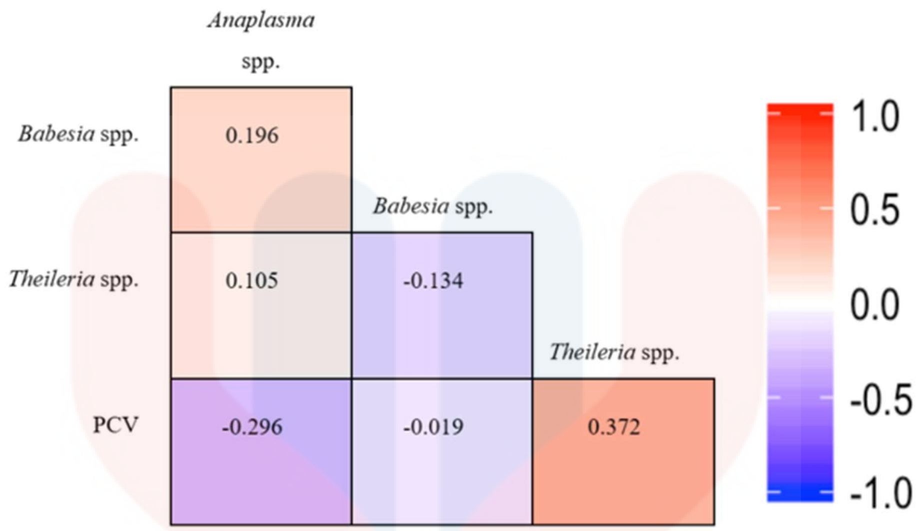


Figure 5: Spearman's Rank Correlations Coefficient among *Anaplasma* spp., *Babesia* spp., *Theileria* spp. and PCV. **=P< 0.05; **=P<0.01, ***P=<0.001*

CHAPTER 5

5.0 DISCUSSION

This study hypothesized that blood parasites, specifically *Anaplasma* spp., *Babesia* spp., and *Theileria* spp., were present among cattle and buffaloes at the Veterinary Teaching Farm, UMK Bachok. The primary objectives were to detect these parasites using microscopic methods and to determine their genus from blood samples. The findings (Table 1) revealed a high prevalence of *Anaplasma* spp. at 86.7%, exceeding the seroprevalence reported in Perak and Kelantan at an average seroprevalence of approximately 45% (Rahman et al., 2010) which range from 30% to 70%. The observed prevalence of *Babesia* spp. was 20%, which is consistent with the 17% prevalence for *B. bovis* and 16% for *B. bigemina* reported by Rahman et al. (2010) in Selangor and Pahang. Meanwhile, *Theileria* spp. showed a prevalence of 6.7%, significantly lower than the rates reported in endemic regions, where prevalence can reach up to 50%, such as in Turkey and Egypt (Aktas, 2014; El-Ashker et al., 2017)

Co-infections were observed, with *Anaplasma* spp. and *Babesia* spp. co-infections recorded at 20%, as well as *Anaplasma* spp. and *Theileria* spp. co-infection at 6.7%. There was no co-infection observed between *Theileria* spp. and *Babesia* spp. in the farm (Table 2). These results highlight notable differences in the prevalence of these parasites at the study site compared to findings from other regions.

The findings of *Anaplasma* spp., *Babesia* spp. and *Theileria* spp. among cattle and buffalo in the Veterinary Teaching Farm, UMK Bachok can be attributed to a combination of environmental, management, and sample-related factors. The tropical climate in Bachok, with its average annual temperature of 26.9°C and significant rainfall, supports the survival and activity of tick vectors, which are known to transmit *Anaplasma* spp., *Babesia* spp., and *Theileria* spp. However, while the climate is generally favorable for tick survival, the specific environmental conditions for peak tick activity such as relative humidity (75.91–78.24%) and temperature (28.81–30.64°C) during the months of August and September mentioned by Patel et al. (2012) may not have aligned with the sampling period, potentially limiting parasite transmission. Presumably, the farm practice of outdoor grazing in the farm increases exposure to ticks, but this exposure may not be constant or uniform, depending on the tick population at the time of sampling.

Host factors such as age, breed, sex and immunity were not included in this study because the primary objective was to screen for the presence of blood parasites and identify which genus were present in the cattle and buffalo population on the farm. The study was designed with a focus on detecting and identifying the parasites, not on analyzing how host-specific factors might influence infection rates. As such, this study prioritized understanding the general prevalence of blood parasites in the population without considering host-related variations. Thus, the parasite prevalence reported here reflects the general exposure and environmental conditions rather than host-specific factors.

Next, the findings of this study also reveal variations in mono- and co-species infections among *Anaplasma* spp., *Babesia* spp., and *Theileria* spp. Co-infections between *Anaplasma* spp. and *Babesia* spp. were the most frequent (20.0%), whereas co-infections involving *Theileria* spp. were rare, and no co-infections between *Babesia* spp. and *Theileria* spp. were observed. This pattern may be contributed by the mixture of immune system interactions, competitive dynamics between parasites, and vector feeding behaviors.

Blood parasites could exploit the host's immune system, potentially increasing susceptibility to co-infections. *Theileria* spp. manipulate host macrophages and lymphocytes, causing systemic immunosuppression that later compromises the host's ability to eliminate the concurrent infections (Bursakov et al., 2019). Similarly, *Anaplasma* spp. use antigenic variation to establish chronic infections, opening the window for secondary infections (Ola-Fadunsin et al., 2017). These immune-modulating mechanisms could facilitate co-infections involving *Anaplasma* spp., which aligns with the relatively higher prevalence of *Anaplasma* spp. and its co-infection with *Babesia* spp. in this study.

Competitive interactions among parasites also play a role in the infection patterns. While co-infections of *Anaplasma* spp. and *Theileria* spp. were observed, the absence of *Babesia* spp. and *Theileria* spp. co-infections suggests potential competition or ecological separation. This finding aligns with the studies that show *Babesia* spp. and *Theileria* spp. may occupy distinct ecological niches or have competitive dynamics that limit simultaneous infections within the same host (Bursakov et al., 2019).

Vector feeding behaviors further influence co-infection prevalence. Ticks, particularly ixodid species, can transmit multiple pathogens during a single feeding cycle. Microbial interactions within ticks may promote or inhibit the transmission of multiple parasites simultaneously (Bursakov et al., 2019). Additionally, seasonal variations in vector activity influence parasite transmission dynamics. The summer months, which correlate with peak tick feeding activity typically see higher transmission rates for *Anaplasma* spp. and *Theileria* spp. (Bursakov et al., 2019). However, if sampling occurs outside these peak periods, the prevalence of co-infections could be lower.

The sampling for this study was conducted in early October in Bachok, Kelantan, when the weather was recorded between 32°C and 33°C with a relative humidity of 86%. This period follows the months of August and September, during which temperatures were slightly lower but still high, with humidity levels ranging from 82% to 83%. Even though Malaysia does not experience four seasons like temperate climates, the environmental conditions in October which are usually warm and humid are still favourable for tick activity and parasite transmission just like the conditions observed in the previous months.

Therefore, while October may be slightly outside the peak months observed in temperate regions, it still falls within a period conducive to tick and parasite prevalence in tropical climates, as noted by Patel et al. (2012). In fact, tropical regions like Malaysia experience relatively consistent conditions throughout the year, meaning that the sampling in October can still be considered within a peak period for tick activity, albeit slightly reduced compared to the preceding months. This aligns with findings from studies in tropical environments, where the focus is on consistent humidity and temperature levels rather than the four distinct seasons seen in temperate regions like Russia, as mentioned by Bursakov et al. (2019).

The study also explored the relationship between the presence of blood parasites and PCV levels in the cattle and buffalo at the Veterinary Teaching Farm, UMK Bachok (Table 3). One sample had a low PCV value, falling below the normal range, which suggests the presence of anemia. This was observed in a cattle sample that was diagnosed with a mixed infection of *Anaplasma* spp. and *Babesia* spp., both of which are known to cause haemolytic anemia by destroying red blood cells hence reducing the PCV level (Aiello et al., 2016). Other than that, 14 samples exhibited normal PCV values, indicating no significant issues with red blood cell count and suggesting that the animals were generally healthy in terms of oxygen-carrying capacity. Two samples had higher PCV values than the normal range, which could indicate dehydration or polycythaemia, conditions in which the blood becomes more concentrated, often due to fluid loss or an increased production of red blood cells. High PCV values are usually associated with these conditions and should be investigated more to rule out other potential causes such as underlying chronic diseases (Bell-Sakyi et al., 2004). These findings highlight the importance of monitoring PCV levels in cattle and buffaloes since abnormal values can provide valuable insights into the health status of the animals and also possible presence of parasitic infections of the animals.

While weak correlations were observed between some of the parasites and PCV, none of the correlations were statistically significant, as all p-values exceeded the threshold of 0.05. Weak trends were observed but these correlations were not strong enough to draw definitive conclusions. The small sample size ($n = 17$) was most likely the reason to the lack of statistical significance, and future studies with larger sample sizes or alternative methods may be needed for a better understanding of the relationships among the blood parasites and PCV level.

This lack of correlation may be caused by several factors. First, the parasitaemia levels in the animals could have been low, which would not have resulted in significant changes in PCV levels. Parasitic infections, particularly in the early stages, may not always produce enough of an effect on red blood cell destruction to cause noticeable drops in PCV (Zweygarth & Josemans, 2003). Additionally, the small sample size ($n = 17$) may have limited the power of the statistical analysis, making it difficult to detect a significant correlation even if one exists.

Other than that, the blood parasites control at Veterinary Teaching Farm, UMK Bachok may require several strategies to address the challenges posed by the tropical climate and outdoor grazing practices. The farm's location in Bachok, with its high temperatures and significant rainfall, creates an ideal environment for tick vectors. In addition, the farm practicing outdoor grazing also increases cattle exposure to these vectors. An integrated approach that includes tick control, vaccination, and treatment strategies would be most effective to manage these blood parasites' infections.

Tick control is a primary measure for reducing the transmission of diseases like anaplasmosis, babesiosis, and theileriosis. While acaricides are commonly used, resistance in tick populations is becoming a significant issue. Nickel (2024) points out that while acaricide use is essential for controlling tick populations, resistance is rising, and their effectiveness may decrease over time. This underscores the importance of integrating other control measures, such as rotational grazing and maintaining pasture health, to reduce tick exposure. Additionally, Carter and Rolls (2022) suggest that chemical tick control alone cannot fully prevent the transmission of *Babesia* spp., and integrating multiple strategies, could be more effective. Given the close proximity of cattle grazing areas to deers' grazing areas, as well as the presence of sheep, chickens, and horses within approximately 1 km, Veterinary Teaching Farm, UMK Bachok could benefit from a regional approach to tick control. This could involve coordinated efforts in managing tick populations across different animal species and their shared grazing and living areas. Synchronizing acaricide treatments, implementing effective pasture management strategies, and controlling the movement of animals between areas could help prevent tick infestations and reduce the risk of blood parasites transmission. By combining these methods, the farm can better manage tick populations within the farm and minimize the spread of diseases.

Vaccination is also a critical component of blood parasites control. Nickel (2024) discusses the potential for vaccines as a key element in controlling tick-borne diseases on farms with high exposure to vectors. Vaccination with live *Anaplasma centrale*, a less pathogenic strain of *Anaplasma*, has been successfully used in regions such as South Africa and Australia to provide partial protection against *A. marginale* (Tabor, 2022). Similarly, live attenuated vaccines for *Babesia* and *Theileria* have been used in countries like Argentina and Brazil to control these diseases (Carter and Rolls, 2022; Morrison, 2022). Introducing these vaccines to Veterinary Teaching Farm, UMK Bachok could significantly reduce the likelihood of infection, particularly when combined with tick control.

In cases where infections do occur, prompt treatment is essential to prevent severe disease. According to Tabor (2022), tetracycline antibiotics such as oxytetracycline are commonly used to treat *Anaplasma* spp. infections and can provide immunity for several months. Imidocarb, while effective against *Babesia* spp. and *Theileria* spp., has longer withholding periods and requires careful management (Carter and Rolls, 2022; Morrison, 2022). Additionally, supportive treatments such as blood transfusions and anti-inflammatory drugs can improve the survival rate of affected animals, especially in severe cases (Carter and Rolls, 2022).

An integrated control approach that combines tick management, vaccination, and prompt treatment is recommended for effective blood parasites control at Veterinary Teaching Farm, UMK Bachok.

CHAPTER 6

6.0 CONCLUSION AND RECOMMENDATION

In summary, the genus of blood parasites identified among the cattle and buffalo at the Veterinary Teaching Farm, UMK Bachok, consisted of *Anaplasma* spp., *Babesia* spp., and *Theileria* spp. The prevalence of *Anaplasma* spp. was high at 86.7%, while *Babesia* spp. and *Theileria* spp. were less prevalent at 20% and 6.7%, respectively. Co-infections occurred between *Anaplasma* spp. and *Babesia* spp. were observed at 20%.

In conclusion, the study successfully detected and identified blood parasites in the farm's cattle and buffalo population. Future studies could improve by increasing the sample size and evaluating the risk factors on hosts such as age, breed, and immunity. The integration of molecular techniques such as PCR alongside microscopic detection, could enhance the detection of low parasitaemia cases, improving both sensitivity and accuracy (Shkap et al., 2008). Regular screening programs and monitoring of health parameters like PCV should also be conducted to enable timely control measures. Exploring additional variables such as nutritional status and immune responses will provide a more comprehensive understanding of parasitic infections.

7.0 APPENDICES

Figure 6: Sample Collection at Veterinary Teaching Farm, UMK Bachok

Figure 7: Sample Processing at Parasitology Laboratory, FPV UMK

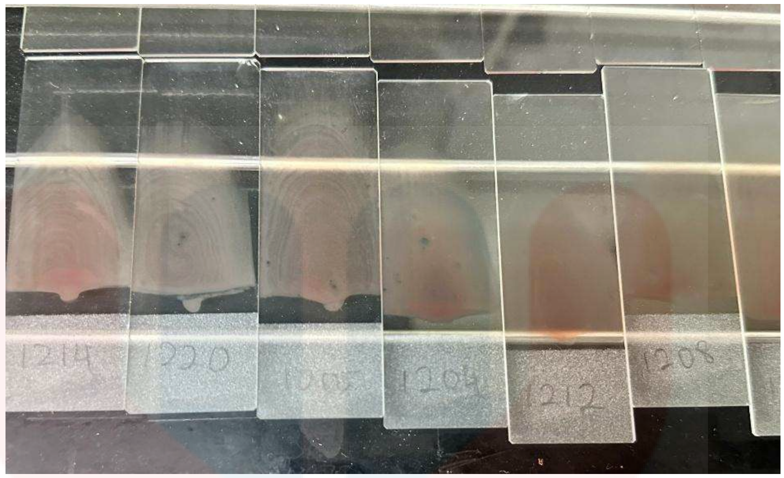


Figure 8: Thin Blood Smears

8.0 REFERENCES

1. A.M Nur-Sabrina, H.A.M Muhamad-Ali, N Nur-Amalina, H Hasimah, W.A Wan-Ladiana, Salma, C. W., Wan, K. L., & Hayyan, B. N. (2024). Relationship between parasitism with milk yield and body weight of Mafriwal cattle. *Veterinary Integrative Sciences*, *22*(1), 141–159. https://doi.org/10.12982/vis.2024.012
2. Adjou Moumouni, P.F., Aboge, G.O., Terkawi, M.A., Masatani, T., Cao, S., Kamyingkird, K., Jirapattharasate, C., Zhou, M., Wang, G., Liu, M., Iguchi. A., Vudriko. P., Ybanez. A.P., Inokuma. H., Umemiya. R.S., Suzuki. H., Xuan. X., (2015). Molecular detection and characterization of *Babesia bovis*, *Babesia bigemina*, *Theileria* species and *Anaplasma marginale* isolated from cattle in Kenya. Parasit. Vectors. 8(1), 1-14.
3. Agina, O. A., Shaari, M. R., Isa, Ajat, M., Zamri-Saad, M., Mazlan, M., Muhamad, A. S., Afrah Alhana Kassim, A. A., Lee, C. H., Rusli, F. H., Masaud, D., & Hamzah, H. (2021). Molecular detection of Theileria species, Anaplasma species, Candidatus Mycoplasma haemobos, Trypanosoma evansi and first evidence of Theileria sinensis-associated bovine anaemia in crossbred Kedah-Kelantan x Brahman cattle. *BMC Veterinary Research*, *17*(1). https://doi.org/10.1186/s12917-021-02902-0
4. Aiello, S. E., Moses, M. A., & Allen, D. G. (2016). *The Merck veterinary manual* (11th ed.). Merck & Co., Inc.
5. Aktas, M. (2014). A survey of ixodid tick species and molecular identification of tick-borne pathogens. *Veterinary Parasitology*, *200*(3-4), 276–283. https://doi.org/10.1016/j.vetpar.2013.12.008
6. Bell-Sakyi, L., Koney, E. B. M., Dogbey, O., & Walker, A. R. (2004). Incidence and prevalence of tick-borne haemoparasites in domestic ruminants in Ghana. *Veterinary Parasitology*, *124*(1-2), 25–42. https://doi.org/10.1016/j.vetpar.2004.05.027
7. Bock, R., Jackson, L., De Vos, A., & Jorgensen, W. (2004). Babesiosis of cattle. *Parasitology*, *129*(S1), S247–S269. https://doi.org/10.1017/s0031182004005190
8. Bursakov, S. A., & Kovalchuk, S. N. (2019). Co-infection with tick-borne disease agents in cattle in Russia. *Ticks and Tick-Borne Diseases*, *10*(3), 709–713. https://doi.org/10.1016/j.ttbdis.2019.03.004
9. Carter, P. D., & Rolls, P. (2022, March). *Babesiosis in Animals - Circulatory System*. MSD Veterinary Manual. https://www.msdvetmanual.com/circulatory-system/blood-parasites/babesiosis-in-animals
10. Desta, T., Lobago, F., & Bekele, J. (2020). Hematological and serological detection of bovine babesiosis in cattle of Tigray, Northern Ethiopia. Journal of Veterinary Medicine and Animal Health, 12(4), 93-99. https://doi.org/10.5897/JVMAH2020.0820

11. Dhillon, K. S., Randhawa, C. S., Gupta, K., Singh, R. S., & Chhabra, S. (2020). Reference values for haematological and biochemical profile in adult Indian buffaloes. *Buffalo Bulletin*, *39*(2), 145–154.
12. El-Ashker, M., Hotzel, H., Gwida, M., El-Beskawy, M., Silaghi, C., Tomaso, H., & Morsy, T. A. (2017). Molecular biological identification of *Theileria* and *Babesia* in ticks collected from grazing cattle in Egypt. Parasitology Research, 116(11), 3083-3090. https://doi.org/10.1007/s00436-017-5610-1
13. Hadi, N., Wong, M. L., & Abdullah, F. F. (2012). Prevalence of *Theileria orientalis* in Malaysian cattle. Tropical Animal Health and Production, 44(3), 701-705. https://doi.org/10.1007/s11250-011-9956-7
14. Hammed, S. M., & Albadrani, B. A. (2021, September 1). *New and ModifiedStaining TechniquesforRapid Diagnosis of Hemoparasites in Blood Smears of Cows*. https://doi.org/10.9790/2380-1409021120
15. Hammer, J.F., Emery, D., Bogema, D.R., Jenkins, C., (2015). Detection of *Theileria orientalis* genotypes in Haemaphysalis longicornis ticks from southern Australia. Parasit. Vectors. 8, 229–336. https://doi.org/10.1186/s13071-015-0839-9
16. Hunfeld, K.P., Hildebrandt, A., Gray, J.S., (2008). Babesiosis: recent insights into an ancient disease. Int. J. Parasitol. 38, 1219–1237
17. Jenkins, C., Micallef, M., Alex, S., Collins, D., Djordjevic, S., & Bogema, D. (2015). Identification of a novel *Theileria orientalis* type in Australian cattle. Veterinary Parasitology, 210(3-4), 324-330. https://doi.org/10.1016/j.vetpar.2015.04.017
18. Kaba, J., Mwacharo, J., Mwaura, S., & Githaka, N. (2014). Progress towards the control of East Coast fever in Kenya. Parasit Vectors, 7, 472. https://doi.org/10.1186/s13071-014-0472-3
19. Kocan, K. M., de la Fuente, J., Blouin, E. F., & Garcia-Garcia, J. C. (2004). *Anaplasma marginale* (Rickettsiales: *Anaplasmataceae*): recent advances in defining host-pathogen adaptations of a tick-borne rickettsia. Parasitology, 129(Suppl), S285-S300. https://doi.org/10.1017/S0031182003004685
20. Koonyosying, P., Rittipornlertrak, A., Chomjit, P., Sangkakam, K., Muenthaisong, A., Nambooppha, B., Srisawat, W., Apinda, N., Singhla, T., & Sthitmatee, N. (2022). Incidence of hemoparasitic infections in cattle from central and northern Thailand. PeerJ, 10, e13835. https://doi.org/10.7717/peerj.13835
21. Kuckartz, U., RädikerS., & Ebert, T. (2013). *Statistik : Eine verständliche Einführung* (p. 213). Vs Verlag Für Sozialwissenschaften.
22. Martins, T.M., Pedro, O.C., Caldeira, R.A., Do Rosario, ´ V.E., Neves, L., Domingos, A., (2008). Detection of bovine babesiosis in Mozambique by a novel seminested hot-start PCR method. Vet. Parasitol. 153, 225–230.

23. Morrison, W. I. (2015). The aetiology, pathogenesis and control of theileriosis in domestic animals. Revue Scientifique et Technique (International Office of Epizootics), 34(2), 599-611. https://doi.org/10.20506/rst.34.2.2373

24. Nickel, T. (2024). *Effective management practices for parasite control in cattle*. Boehringer-Ingelheim.com. https://www.boehringer-ingelheim.com/ca/animal-health/livestock/bovine/effective-management-practices-parasite-control-cattle

25. Nik Him, N. A. I. I., Pong, S. C. Y., Zary, S. Y., Premaalatha, B., Zaini, C. M., Chandrawathani, P., ... & Ramlan, M. (2013). Seroprevalence of anaplasmosis (*Anaplasma marginale*) in local cattle in Malaysia. Malaysian Journal of Veterinary Research, 4(2), 27-31.

26. Noaman, & Shayan, P. (2010). Comparison of Microscopy and PCR-RFLP for detection of Anaplasma marginale in carrier cattle. *PubMed*, *2*.

27. Ola-Fadunsin, S. D., Gimba, F. I., Abdullah, D. A., Sharma, R. S. K., Abdullah, F. J. F., & Sani, R. A. (2018). Epidemiology and risk factors associated with Anaplasma marginale infection of cattle in Peninsular Malaysia. *Parasitology International*, *67*(6), 659–665. https://doi.org/10.1016/j.parint.2018.06.013

28. Ola-Fadunsin, S., Maizatul, A., Ibrahim, A., Amlizawathy, A., Chandrawathani, P., Jesse, F., Sani, R., Sharma, R., (2017). Molecular prevalence and species co-infection of bovine haemoparasites in Peninsular Malaysia. Malays. J. Vet. Res. 8(2), 13-22.

29. Omar, J., Chandrawathani Panchadcharam, Premaalatha Bathmanaban, Muizz, A., Tharshini Jegathesuran, & Hassan, M. N. (2016). Blood protozoa findings in pet dogs screened in Ipoh, Malaysia. *Malaysian Journal of Veterinary Research*, *7*.

30. Patel, G., Shanker, D., Jaiswal, A. K., Sudan, V., & Verma, S. K. (2012). Prevalence and seasonal variation in ixodid ticks on cattle of Mathura district, Uttar Pradesh. *Journal of Parasitic Diseases*, *37*(2), 173–176. https://doi.org/10.1007/s12639-012-0154-8

31. Pupin, R.C., Guizelini, C.C., Lemos, R.A.A., Martins, T.B., Borges, F.A., Borges, F.G.L., Gomes, D.C., (2019). Retrospective study of epidemiological, clinical and pathological findings of bovine babesiosis in Mato Grosso do Sul, Brazil (1995–2017). Ticks Tick Borne. Dis. 10, 36–42. https://doi.org/10.1016/j.ttbdis.2018.08.015

32. Radostits, O. M., Gay, C. C., Blood, D. C., & Hinchcliff, K. W. (2007). Veterinary Medicine: A Textbook of the Diseases of Cattle, Horses, Sheep, Pigs and Goats (10th ed.). Saunders Ltd.

33. Rahman, W. A., Lye, Y. P., & P Chandrawathani. (2010). The seroprevalence of bovine babesiosis in Malaysia. *Tropical Biomedicine*, *27*(2), 301–307.

34. Rehman, A., Nijhof, A.M., Sauter-Louis, C., Schauer, B., Staubach, C., Conraths, F.J., (2017). Distribution of ticks infesting ruminants and risk factors associated with high tick prevalence in livestock farms in the semiarid and arid agro-

ecological zones of Pakistan. Parasit. Vectors 10, 190–204. https://doi.org/10.1186/s13071-017-2138-0.

35. Sawitri, D. H., Wardhana, A. H., Ekawasti, F., & Dewi, D. A. (2022, February 24). *Parasitological and Molecular Detection of Babesiosis in Cattle and Buffalo in West and Central Java*. Www.atlantis-Press.com; Atlantis Press. https://doi.org/10.2991/absr.k.220207.028
36. Shahnawaz, S., Ali, M., Aslam, M. A., Fatima, R., Chaudhry, Z. I., Hassan, M. U., Ashraf, K., Khan, M. S., Ullah, R., Ali, M., Iqbal, F., Moutailler, S., & Almeras, L. (2019). A detailed review on the global prevalence, molecular identification and prevention strategies for bovine hemoprotozoan parasites: *Theileria and Babesia* spp. Microbial Pathogenesis, 130, 166-179. https://doi.org/10.1016/j.micpath.2019.03.039
37. Shkap, V., Leibovitz, B., Krigel, Y., Molad, T., Fish, L., Mazuz, M., Fleiderovitz, L., & Savitsky, I. (2008). Concomitant infection of cattle with the vaccine strain Anaplasma marginale ss centrale and field strains of A. marginale. *Veterinary Microbiology*, *130*(3-4), 277–284. https://doi.org/10.1016/j.vetmic.2008.02.013
38. Svensson, J., Hunfeld, K., Persson, K.E.M., (2019). High seroprevalence of *Babesia* antibodies among Borrelia burgdorferi infected humans in Sweden. Ticks Tick Borne. Dis. 10, 186–190. https://doi.org/10.1016/j.ttbdis.2018.10.007
39. Tabor, A. E. (2022, March). *Anaplasmosis in Ruminants - Circulatory System*. MSD Veterinary Manual. https://www.msdvetmanual.com/circulatory-system/blood-parasites/anaplasmosis-in-ruminants
40. Tay, S.T., Koh, F., Kho, K., Ong, B., (2014). Molecular survey and sequence analysis of *Anaplasma* spp. in cattle and ticks in a Malaysian farm. Trop. Biomed. 31, 769-776.
41. Taylor, M. A., Coop, R. L., & Wall, R. (2016). *Veterinary parasitology* (4th ed.). Wiley Blackwell.
42. Tembue, A.A.M., Silva, F.J.M., Silva, J.B., Santos, T.M., Santos, H.A., Soares, C.O., Fonseca, A.H., (2011). Risk factors associated with the frequency of antibodies against *Babesia bovis* and *Babesia bigemina* in cattle in southern Mozambique. Pesqui. Vet. Bras. 31, 663–666.
43. Watts, J. G., Playford, M. C., & Hickey, K. L. (2016). *Theileria orientalis*: A review. New Zealand Veterinary Journal, 64(1), 3-9. https://doi.org/10.1080/00480169.2015.1035761
44. Zweygarth, E., & Josemans, A. I. (2003). *In Vitro* Infection of Nonendothelial Cells by *Ehrlichia ruminantium*. *Annals of the New York Academy of Sciences*, *990*(1), 573–580. https://doi.org/10.1111/j.1749-6632.2003.tb07429.