UNIVERSITI MALAYSIA KELANTAN

DETECTION AND RISK FACTOR ANALYSIS OF BLOOD PARASITE IN GOATS AND SHEEP IN SELECTED AREAS IN KELANTAN

By

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DETECTION AND RISK FACTOR ANALYSIS OF BLOOD PARASITE IN GOAT AND SHEEP IN SELECTED AREA IN KELANTAN

ABSTRACT

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

Babesiosis, Anaplasmosis, Theileriosis and *Mycoplasmosis* are major tick-borne diseases caused by blood parasites (*Babesia* spp., *Anaplasma* spp., *Theileria* spp., and *Mycoplasma* spp.), affecting goats and sheep and is mainly transmitted by biting fly, hard-tick and blood contaminated instrument. This study was conducted to determine the detection of blood parasites in goats and sheep in Kelantan, Malaysia and potential risk factors related to its occurrence, microscopically.

A total of 40 blood samples from 20 goats and 20 sheep were collected from 4 different small ruminant farms in Kota Bharu and Bachok area. Samples were prepared and stained using thin blood smear and stained using 10% Giemsa stain for detection and identification of blood parasites. Packed cell volume (PCV) was determined by microhematocrit centrifugation technique and measured to assess the relationship between parasitic infection and severity of anaemia. Data was analysed using Pearson's Chi Square.

Microscopic examination of blood smears revealed an overall blood parasite prevalence of (n = 28; $\chi^2 = 0.476$) 70%. The highest infection rate was recorded in Farm A (**80.0%**), followed by Farm C and D (**70.0%**) and B (**60.0%**) ($p = 0.014$). Infection was slightly higher in sheep (**75.0%**) than goats (**65.0%**) and in females (**75.0%**) than males (**62.5%**), though not significant ($p > 0.05$). A significant association was observed between infection and age ($\chi^2 = 4.977$; $p = 0.026$), with all animals aged below one year (< 1 years) high likely to be infected. Most infections were mild, and PCV values decreased with infection severity, indicating anaemia associated with parasitaemia.

In conclusion, blood parasites are highly prevalent among the goat and sheep population in Kelantan, Malaysia, suggesting these tick-borne disease is still circulating within small ruminant farms in Kelantan and potentially contributing to massive economic impact due to health deterioration.

Keywords: *Prevalence, small ruminants, haemoparasites, pack cell volume (PCV), Giemsa stain*

PREVALENSI DAN ANALISIS FAKTOR RISIKO PARASIT DARAH DALAM KAMBING DAN BIRI-BIRI DI KAWASAN TERPILIH DI KELANTAN

ABSTRAK

Kertas penyelidikan ini dibentangkan kepada Fakulti Perubatan Veterinar, Universiti Malaysia Kelantan sebagai sebahagian daripada keperluan kursus DVT 55204 – Projek Penyelidikan.

Babesiosis, Anaplasmosis, Theileriosis dan Mycoplasmosis merupakan penyakit bawaan kutu utama yang disebabkan oleh parasit darah (*Babesia* spp., *Anaplasma* spp., *Theileria* spp. dan *Mycoplasma* spp.), yang menjangkiti kambing dan biri-biri melalui gigitan lalat penghisap darah, kutu keras serta peralatan yang tercemar dengan darah. Kajian ini dijalankan untuk menentukan kehadiran parasit darah dalam kambing dan biri-biri di Kelantan, Malaysia serta mengenal pasti faktor risiko yang berpotensi mempengaruhi kejadian jangkitan tersebut melalui kaedah mikroskopik.

Sebanyak 40 sampel darah, terdiri daripada 20 kambing dan 20 biri-biri, telah dikumpul dari empat ladang ruminan kecil di kawasan Kota Bharu dan Bachok. Sampel darah telah disediakan dan diwarnakan menggunakan kaedah 'smear' darah nipis dengan pewarnaan Giemsa 10% bagi pengesanan dan pengecaman parasit darah. Nilai pek sel darah (PCV) ditentukan menggunakan teknik sentrifug mikrohematokrit untuk menilai hubungan antara jangkitan parasit dengan tahap keterukan anemia. Data dianalisis menggunakan ujian Khi Kuasa Dua (Chi-Square) Pearson.

Pemeriksaan mikroskopik terhadap smear darah menunjukkan kadar prevalens keseluruhan parasit darah sebanyak 70% ($n = 28$; $\chi^2 = 0.476$). Kadar jangkitan tertinggi direkodkan di Ladang A (**80.0%**), diikuti oleh Ladang C dan D (**70.0%**), manakala Ladang B (**60.0%**) mencatat kadar terendah ($p = 0.014$). Kadar jangkitan juga lebih tinggi pada biri-biri (**75.0%**) berbanding kambing (**65.0%**), serta pada haiwan betina (**75.0%**) berbanding jantan (**62.5%**), namun perbezaan tersebut tidak signifikan ($p > 0.05$). Terdapat hubungan signifikan antara umur dan jangkitan ($\chi^2 = 4.977$; $p = 0.026$), di mana semua haiwan berumur kurang daripada satu tahun dijangkiti. Kebanyakan jangkitan adalah ringan, dan nilai PCV menurun seiring peningkatan keterukan jangkitan, menunjukkan hubungan antara anemia dan parasitemia.

Kesimpulannya, parasit darah masih menunjukkan kadar prevalens yang tinggi dalam populasi kambing dan biri-biri di Kelantan, Malaysia, menandakan penyakit bawaan kutu ini masih beredar dalam ladang ruminan kecil dan berpotensi memberi kesan ekonomi yang besar akibat kemerosotan kesihatan haiwan.

Kata kunci: *Prevalensi, ruminan kecil, hemoparasit, pek sel darah (PCV), pewarnaan Giemsa*

CERTIFICATION

This is to certify that we have read this research paper entitled **'Detection and Risk Factor Analysis of Blood Parasite in Goat and Sheep in Selected Area in Kelantan'** by **Muhammad Azim Syahmi Bin Md Nawi**, and in our opinion, it is satisfactory in term of scope, quality, and presentation as partial fulfilment of requirements for the course DVT – Research Project.

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Lunar, Lola, Luna, and Leo

Thank you so much

DEDICATION

In the Name of Allah, the Most Beneficent, the Most Merciful. We would like to thank the Almighty for giving me the chance to complete the Final Year Project, without His consent I will not be here to finish it. Furthermore, I salute my highest gratitude to the dean of Faculty of Veterinary Medicine, Associate Professor Dr. Mohd Farhan Hanif Bin Reduan for giving the opportunity for my Degree of Doctor of Veterinary Medicine (DVM) year 5 to undergo this project thus allowing me to achieve valuable and irreplaceable practical knowledge that cannot be taught in any class nor books.

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LIST OF ABBREVIATIONS

spp.	Species (plural)
BCS	Body Condition Score
CI	Confidence Interval
DVS	Department of Veterinary Services
EDTA	Ethylenediaminetetraacetic Acid
FAMACHA	Faffa Malan chart
FPV	Fakulti Perubatan Veterinar
PCR	Polymerase Chain Reaction
PCV	Packed Cell Volume
SE	Standard Error
SPSS	Statistical Package for the Social Sciences
UMK	Universiti Malaysia Kelantan
WOAH	World Organisation of Animals Health

LIST OF SYMBOLS

%	Percent
°C	Degrees Celsius
χ^2	Chi-square
$<$	Less than
$>$	Greater than
$\pm$	Plus–minus

CHAPTER 1

1.0 INTRODUCTION

It is expected that by mid-2080 the global population will strike 10.3 billion people, increasing the demand for food and animal products (United Nations, 2024). This suggests a promising bright future for the livestock industry, especially the ruminant sector. However, the sector continues to face challenges such as rising feed cost, diseases, low-quality output, and inefficient production system. Parasitic diseases are one of the major diseases causing significantly poor effects on the growth and production in goats and sheep (Stuen, 2020). Statistics from Department of Veterinary Services (Fig. 1) based on the goat and sheep population in Livestock Statistics 2023/2024 stated that Kelantan has the fifth largest goat population with 30,411 heads after Kedah which ranked first in the list while Kelantan has second largest sheep population with 21,566 head of sheep after Johor. Therefore, any disease outbreak or inefficient management system that has an impact on the livestock population in Kelantan will give a great impact on the national livestock industry in Malaysia.

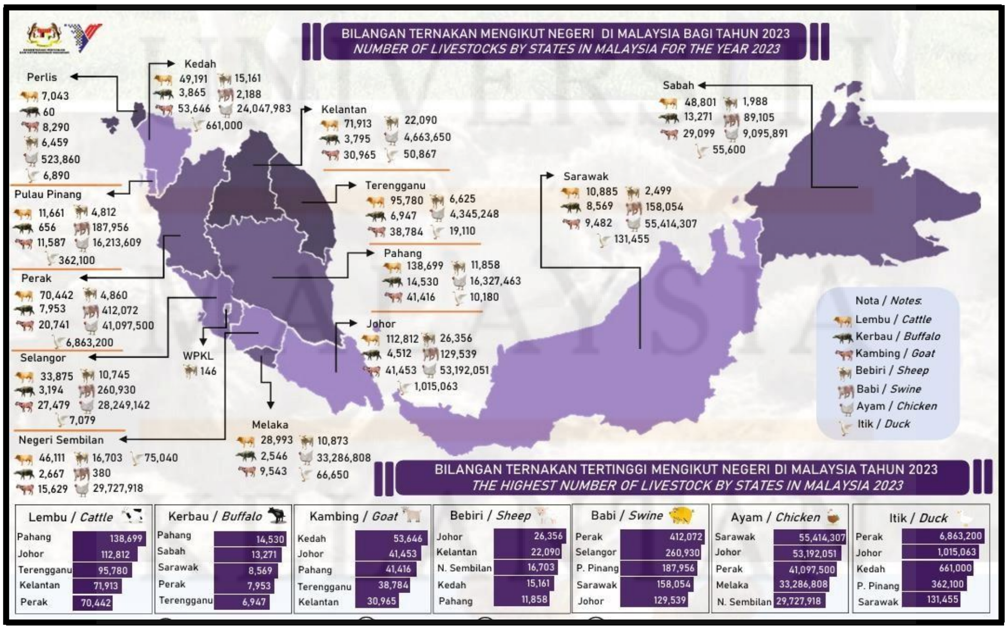

Figure 1 **:** Number of livestock by states in Malaysia for the years 2023/2024

Tick-borne diseases in small ruminants is a worldwide concern, particularly in tropical and subtropical countries like Malaysia that provide favourable climatic conditions for the tick vector to breed. There are several tick-borne diseases that commonly affect small ruminants, primarily caused by blood parasites such as *Babesia, Anaplasma, Theileria,* and *Mycoplasma* species. These infections can lead to a range of clinical signs including high fever, anaemia, jaundice, swollen lymph nodes, poor weight gain, and even sudden death, especially in animals under stress or with weakened immunity. In many cases, the diseases remain subclinical, making early detection difficult and only becoming apparent when significant damage has already occurred. The destruction of red blood cells caused by these parasites lead to reduced oxygen-carrying capacity, resulting in lethargy, decreased feed intake, and stunted growth. Collectively, these effects contribute to serious economic losses through reduced productivity, lower meat and milk yield, increased veterinary costs, and in severe outbreaks, high mortality rates. If not properly managed, these infections can severely undermine the health and profitability of small ruminant farms, especially in high-risk regions like Kelantan.

In Malaysia, tick-borne infections continue to impact small ruminant production, particularly in goat herds, with notable consequences for animal welfare and the overall farm income. Since different small ruminant breeds have varying levels of resistance to disease, blood-borne parasite infections in livestock can often remain undetectable and are usually only identified if active surveillance is done carefully. The frequent detection of haemoparasites in various regions emphasizes the need for early diagnostic efforts and effective control measures. Since goat and sheep farming represents a vital component of the country's agricultural economy, investing in early intervention strategies could play a key role in reducing mortality, enhancing productivity, and supporting long-term farm sustainability (Guswanto *et al.,* 2020).

Goats are widely valued as a source of high-quality meat, especially due to their rapid reproductive cycles and the general absence of cultural or religious restrictions on goat meat consumption. As a rich source of protein, goat meat plays a crucial role in improving dietary protein intake, especially in resource-limited settings. However, blood-borne parasitism remains a major challenge in goat farming, contributing to considerable economic losses through reduced growth rates, poor feed efficiency, and, in severe cases, mortality. Among the most commonly encountered haemoparasites in goats and sheep are *Anaplasma* spp. and *Babesia* spp., which can severely affect animal health performance and farm productivity (Ros-García *et al.,* 2021).

Hence, the primary objective of this study is to determine the detection of blood parasite infections among goats and sheep in Kelantan. Specifically, it focuses on detecting the presence of *Anaplasma* spp., *Babesia* spp., *Theileria* spp. and *Mycoplasma* spp., using microscopic diagnostic techniques. Additionally, this research aims to analyze the potential risk factors contributing to the transmission of these haemoparasites within the small ruminant population. By enhancing the understanding of disease occurrence and associated risk factors will help farmers and veterinarians better prevent and control these diseases, leading to healthier animals and better farm production.

1.1 RESEARCH PROBLEM STATEMENT

Tick-borne diseases continue to represent a major challenge to livestock health and productivity across the globe. Among these, infections caused by *Anaplasma* sp., *Babesia* sp., *Theileria* sp. and *Mycoplasma* sp., are particularly concerning for small ruminants such as goats and sheep, especially in tropical regions. In Kelantan, a study published in 2023 by Malaysia's Department of Veterinary Services (DVS) documented the occurrence of these pathogens, along with *Mycoplasma* spp., in local small ruminant populations. Malaysia is endemic for Babesiosis, Anaplasmosis, and Theileriosis but not Mycoplasmosis. However, there are a few studies on goat and sheep Mycoplasmosis in Malaysia.

Addressing these infections is critical, as they can lead to serious clinical outcomes including decrease growth rate, anaemia, reduced milk and meat production and increase in cost of treatments (Bock *et al.,* 2004). These health issues not only reduce productivity but also strain local farming communities, which rely heavily on livestock for income and food security. Gaining insight into the presence and distribution of these pathogens in Kelantan is essential for designing effective disease management programs. Furthermore, the risk factors associated with blood parasite infections in goats and sheep in Kelantan have not been well studied.

This study specifically focuses on goats and sheep within Kelantan's farming systems, a state known to contribute substantially to Malaysia's small ruminant population. Due to high livestock density, any disease outbreak in herd health management could lead to a significant impact on both local producers and the national livestock sector. Therefore, understanding the prevalence and risk factor of blood parasite infections in this region is crucial, offering data that could support general disease control policies throughout similar agro-ecological zones in Malaysia.

1.2 RESEARCH QUESTION

1. Is there any detection of blood parasites infection among goats and sheep in selected areas (Kota Bharu and Bachok) in Kelantan?
2. What are the potential risk factors associated with blood parasite infection among goats and sheep in selected areas in Kelantan?

1.3 RESEARCH HYPOTHESIS

1. Detection of blood parasite infection among goats and sheep in selected areas (Kota Bharu and Bachok) in Kelantan is high.
2. The associated risk factors for blood parasite are age, sex, breed, physiological status, vector control and farm management system.

1.4 RESEARCH OBJECTIVE

1. To determine the detection of blood parasite infection in 20 goats and 20 sheep in selected areas (Kota Bharu and Bachok) in Kelantan.
2. To determine the potential risk factor associated with blood parasite infection among goats and sheep in selected areas (Kota Bharu and Bachok) in Kelantan.

CHAPTER 2

2.0 LITERATURE REVIEW

2.1 BLOOD-BORNE PARASITE IN SMALL RUMINANT

Blood parasites are organisms that tend to inhabit erythrocytes or white blood cells and are most likely to be detected during the acute phase of infection. Parasitic organisms survive by attaching themselves to a host and using the host's body to thrive, generally at the host's expense (Dean *et al.,* 2024). Parasites find themselves protected from the harsh temperature and moisture changes of the external world when they live within the rich, warm body of their host (Ismail *et al.,* 2023). The method of transmission varies depending on the parasite, but often they are transmitted through the bites of ticks or flies (Sébastien Grech-Angelini *et al.,* 2015). There are several small ruminant diseases that are caused by blood parasites that can be found in goats and sheep including Babesiosis, Anaplasmosis, Theileriosis and Mycoplasmosis (Galon *et al.,* 2022). These parasites can be transmitted by ticks and possibly blood-contaminated instruments (Gondard *et al.,* 2017). Therefore, the combination of another possible risk factor may promote the transmission of arthropod-borne organisms in animals (F. Jongejan, 2004).

2.2 PREVALENCE

Babesiosis is caused by intraerythrocytic protozoa of the genus Babesia, with *Babesia ovis* and *Babesia motasi* being the principal species affecting small ruminants (Bozan *et al.,* 2024). *B. ovis* is the most pathogenic and commonly affects sheep, whereas *B. motasi* primarily affects goats and is generally less virulent, although severe cases have been documented (Gerrit Uilenberg, 2006). These parasites are transmitted by ticks, particularly species of the

Rhipicephalus and *Haemaphysalis* genera (Sezayi Ozubek *et al.,* 2024). As of 2022, the World Organisation for Animal Health (WOAH) recognizes several Babesia species as significant pathogens in small ruminants, particularly sheep and goats. The primary species of *Babesia* spp. including *Babesia ovis, Babesia motasi,* and *Babesia crassa*. *B. crassa* have been identified in small ruminants but are considered to have low or negligible pathogenicity. *B. ovis* and *B. motasi* are more clinically significant with the former posing a higher risk, especially in sheep (Ulucesme *et al.,* 2024). In Malaysia, there is a notable lack of published molecular studies reporting the detection of *Babesia* species in small ruminants. While *Babesia* infections have been documented in cattle within the country, data pertaining to sheep and goats remain undocumented (Galon *et al.,* 2022).

Anaplasma ovis is an obligate intracellular rickettsial organism that infects red blood cells of sheep and goats (Tabor, 2022). It is transmitted predominantly by ticks of the *Rhipicephalus* and *Dermacentor* genera (T.L., 2024). A study conducted in Peninsular Malaysia detected *Anaplasma bovis* in a goat, highlighting the potential for *Anaplasma* infections in small ruminants within the region, although the study mainly focused on cattle and deer (Koh *et al.,* 2018). The simplified pathogenesis involves invasion of erythrocytes followed by immune-mediated destruction, leading to haemolytic anaemia (Zadeh *et al.,* 2024). Although infections are often subclinical, they can lead to significant production losses when associated with secondary infections or poor management (Ostrovskii *et al.,* 2025). Prevalence is high in regions such as the Iran and Pakistan, and sporadic outbreaks have been reported worldwide (Zadeh *et al.,* 2024).

Theileriosis in small ruminants is caused by protozoa of the genus *Theileria*, with *Theileria lestoquardi* (formerly *T. hirci*) being the most pathogenic species (Morrison, 2022). Other species include *T. ovis, T. uilenbergi*, and *T. luwenshuni* (Riaz *et al.,* 2024). These parasites are primarily transmitted by ticks from the *Hyalomma, Rhipicephalus,* and *Haemaphysalis* genera (Ashraf *et al.,* 2024). Theilerial pathogenesis involves the infection of both lymphocytes and erythrocytes, which lead to immunopathological damage and anaemia. A molecular survey in Malaysia detected theilerial DNA in 90% of sheep samples, indicating a high prevalence (Kho *et al.,* 2017).

Mycoplasma ovis, previously known as *Eperythrozoon ovis*, is a haemotropic bacterium that parasitizes erythrocytes in small ruminants, particularly sheep and to a lesser extent goats (Neimark *et al.,* 2004). It is transmitted mechanically via biting insects such as S*tomoxys calcitrans* and *Melophagus ovinus,* as well as through contaminated instruments used during routine management procedures, including vaccination or ear-tagging (Paul *et al.,* 2023). The pathogenesis involves the adhesion of the organism to red blood cells, prompting to their destruction and resulting in anaemia. In Malaysia, a recent study reported an overall prevalence of 50.7%, with higher rates in lactating, pregnant, and certain breeds such as Boer goats (Thlama *et al.,* 2021).

2.3 CLINICAL SIGN

Clinical signs of tick-borne blood parasite infections in small ruminants can vary in severity but often share common features (Snorre Stuen, 2020). Affected animals may show high pyrexia, anaemia, jaundice, lethargy, and weight loss (Adejinmi *et al.,* 2010). In more severe cases, haemoglobinuria, swollen lymph nodes, and abortion may occur. Infections like *Babesia* *ovis* and *Theileria lestoquardi* can lead to acute illness with rapid deterioration and even death, particularly in stressed or immunocompromised animals (Bijan *et al.,* 2025). Chronic or subclinical cases are also common, often resulting in poor weight gain, pale mucous membranes, ill-thrift, and persistent anaemia (Abdelsalam *et al.,* 2023). Lambs, lactating or pregnant animals, and certain susceptible breeds tend to be more severely affected, especially under poor management or in the presence of concurrent infections (Khan *et al.,* 2021).

2.4 RISK FACTOR

Risk factors associated with blood parasites in animals can be classified into three categories which are related to the characteristics of the animal population, the farm management and the biology of the disease (Franklin, 2020). In detail, the incidence of tick-borne infection is influenced by management factor, herd size, population density, type of animal breed and biological features such as herd immunity (Alvi *et al.,* 2024). Young animals are more susceptible to the infection but latent infection may occur (Munir Aktas *et al.,* 2006). Mixed farming especially sheep in addition to goats increase the risk of transmission by up to 6 time compared to keeping only goat (Batool *et al.,* 2022).

2.5 DIAGNOSIS

Microscopic examination of blood smears stained with 10% Giemsa was the earliest method used for the detection of haemoplasmas (Gulland *et al.,* 1987) and is still the first line in current laboratory diagnosis of *M. ovis* because it is fast, cheap and easy to perform (Alsaad, 2019). Under the light microscope, haemoplasma may be bound to the surface of mammalian erythrocytes or found lying loosely in the plasma due to detachment from the cell, especially after prolonged storage of blood samples (Biondo *et al.,* 2009). Thus, the method of staining the blood smear is considered suggestive in diagnosing blood parasites in small ruminants.

Other diagnostic tools that are available to detect presence of blood parasite including the usage of advanced molecular approach which is polymerase chain reaction (PCR) and sequencing of the 16S rRNA gene are now widely used to detect and characterise haemoparasite in animal and human infections (Wang *et al.,* 2017). PCR is one of the tools offering high sensitivity and specificity with an aid to detect blood parasites with low or acute parasitism and also able to differentiate between species invasion (Flaherty *et al.,* 2018).

2.6 TREATMENT

Animals found positive for Anaplasmosis were treated with tetracycline antibiotics like oxytetracycline intravenously (10 mg/kg) daily for 3 days (Huda *et al.,* 2023). This antibiotic is commonly used to treat haemoparasite and provide long lasting immunity up to a month. Imidocarb dipropionate was used successfully and efficiently in the management of heavy tick infestation in animals (Curtis *et al.,* 2021). The most common method of tick control in Malaysia is by chemical dipping or spraying. Pour-on formulations are also available in the market, especially for dairy cattle farmers. (Rahman *et al.,* 2010). Treatment may diminish clinical symptoms but may not clear the organisms from the body (Thomas *et al.,* 2009). Controls methods involve good preventive measures such as enough nourishment of good quality, minimizing the risk of biting flies and avoiding the spread of the organism by needles and surgical instruments (Snorre Stuen, 2020).

CHAPTER 3

3.0 MATERIALS AND METHODS

3.1 STUDY AREA

The blood samples were collected from 20 goats and 20 sheep, in 2 districts in Kelantan namely Kota Bharu and Bachok (Fig. 2) based on convenience sampling. Before collecting blood samples, general and physical examinations were performed on the elementary units.

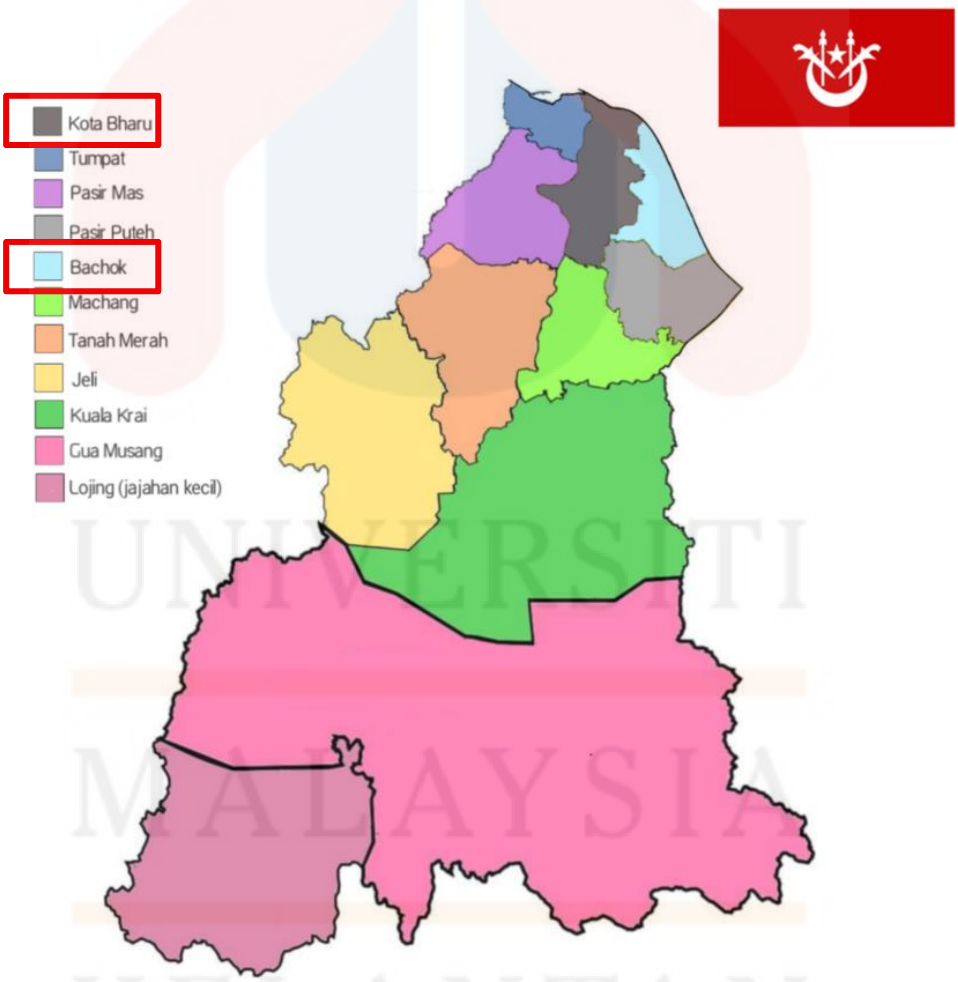

Figure 2 **:** Study area for sample collection

3.2 STUDY DESIGN

This was a cross-sectional study design which the selected blood samples were processed and observed for presence of haemoparasite from the sample population at the point of sampling.

3.3 STUDY POPULATION

A total of forty (n = 40) small ruminants from two districts in Kelantan, comprising twenty (n = 20) from Kota Bharu and twenty (n = 20) from Bachok were randomly sampled. The sampling consisted of ten (10) sheep from Farm A, ten (10) sheep from Farm B, ten (10) goats from Farm C, and ten (10) goats from Farm D.

3.4 SELECTION CRITERIA

3.4.1 INCLUSION CRITERIA

Goat and sheep farms in Kota Bharu and Bachok were chosen by convenience sampling. Goat and sheep on selected farm were chosen by simple random sampling, including breed, age, sex, physiological status, vector control and body condition score.

3.4.2 EXCLUSION CRITERIA

Severely ill animals were excluded from the sampling.

3.5 SAMPLING METHOD AND PROCEDURE

Thorough physical examination was performed on individual animals during each farm visit to determine their age, sex, breed, reproductive status, Faffa Malan chart (FAMACHA), presence of ectoparasite and body condition score (BCS). Animals were sorted by age using dentition and stratified into young (less than one year) and adult (one year and above) categories. Identification of individual sex and breed of small ruminant using physical characteristics. The colour of ocular mucous membrane of each animal was examined once and were classified according to FAMACHA as 1 = red (non-anaemic), 2= red-pink (non-anaemic), 3 = pink (mildly-anaemic), 4 = pink-white (anaemic), 5 = white (severely anaemic). The determination of BCS of goats and sheep by palpation to estimate the muscle and fat cover in lumbar and sternum region and were classified as emaciated (1), thin (2), average (3), fat (4) and obese (5). History taking from the farmer were recorded to furnish data on the farm management, biosecurity and herd health practice for risk analysis.

Five to ten ml blood from each goat and sheep were collected via jugular vein using 21G vacutainer needle and placed in ethylene diamine tetra-acetic (EDTA) tube for anticoagulant. All the relevant information such as breed, age, sex, and presence of ectoparasite were recorded for further analysis. These samples were placed in an ice box and transported to the Parasitology Laboratory, FPV UMK for analysis on the same day.

3.6 BLOOD SMEAR AND PACKED CELL VOLUME

A single drop of blood from a sample container (EDTA) tube was taken and placed on one end of a labelled clean glass slide. The slides labels were checked with the sample bottle to make sure the label was correct. Then, the drop was spread by using another slide (spreader). The slide was moved in a forward direction allowing the blood to spread as thin layer on the surface of the slide. The smear will be allowed to air dry. Then cytological examination of blood smear for the presence of organisms was carried out using Giemsa stain.

The slides were air dried and fixed in 100% methanol for 5 minutes. Then, the slides were immersed in 10 % Giemsa stain for 30 minutes, carefully washed under tap water to get rid of excess stain, blotted and air dried. Slides were examined under light microscope (40x) to observe erythrocyte morphology and (100x) using immersion oil for the detection and identification of blood parasites (*Babesia* spp., *Anaplasma* spp., *Theileria* spp., and *Mycoplasma* spp.) detection of one blood parasite was considered positive.

For PCV measurement, the glass capillary tubes were filled with blood and sealed using Cristaseal. The sealed tubes were then centrifuged at 220 rcf for 5 minutes and the volume was measured using an EZ Reader. The results for each sample were then recorded.

3.7 DATA COLLECTION TOOLS

Data resulting from the microscopic examination and haematocrit evaluation from blood samples were manually recorded and tabulated using Microsoft Excel.

3.8 DATA ANALYSIS

The collected data was analysed using IBM Statistical Package for the Social Science (SPSS) Version 27.0. Pearson Chi-square test was used to assess association between two categorical variables. The significant level was set at $p < 0.05$. The result was considered statistically significant when p-value were less than 0.05.

3.9 ETHICAL CONSIDERATION

Animal ethics approval was obtained from the Fakulti Perubatan Veterinar (FPV) Animal Ethics Committee *(UMK/FPV/ACUE/FYP/017/2025)* before commencement of the study.

CHAPTER 4

4.0 RESULT

A total of 80 blood smears (including duplicate smears from all animals) were prepared on the same day as blood collection by using a 10% Giemsa stain for cytologic evaluation. Blood smears were prepared from a total of 20 goats and 20 sheep. Slides were evaluated by using compound microscopy under oil with a 100× objective (model CX22LED, Olympus, Tokyo, Japan). A minimum of 10 high-powered fields were evaluated per smear. Organisms were identified as basophilic pleomorphic coccoid or ring-form bacteria or double pear-shape, appearing as single organisms within or on the surface of erythrocytes. However, some basophilic material consistent with organisms, Howell–Jolly bodies, stain precipitate, or other debris may be seen. Even a single haemoparasite detected indicated a suspected positive result, for which basophilic bodies consistent with hemoplasma were found in small numbers in all fields evaluated.

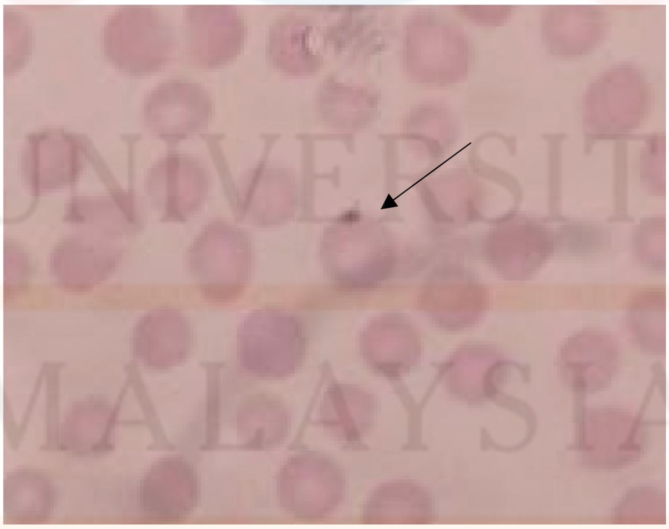

Figure 3 : Presence of *Mycoplasma* spp. in red blood cell (arrow).

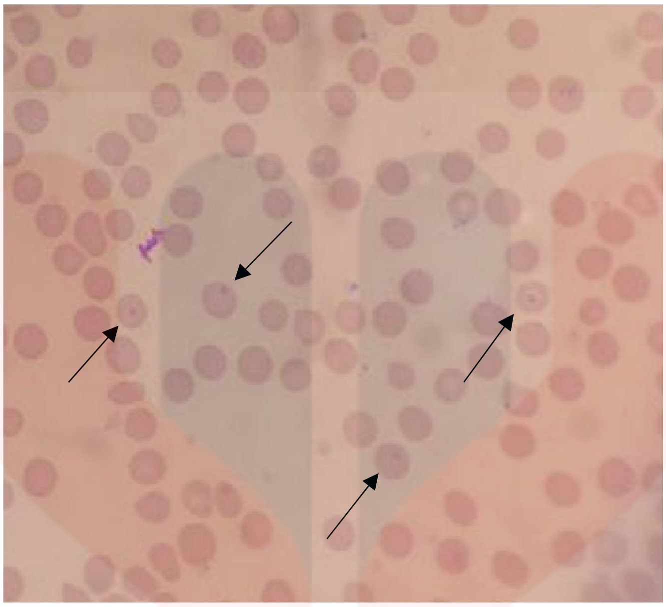

Figure 4 : Presence of *Anaplasma* spp. in red blood cells (arrows).

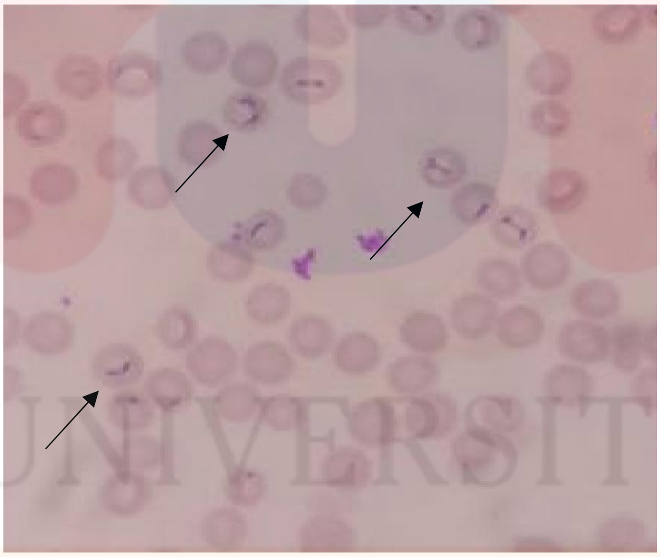

Figure 5 : Presence of *Babesia* spp. in red blood cells (arrows).

A total of 40 small ruminants comprising 20 goats and 20 sheep from four selected farms in Kelantan. Thin blood smear examination revealed that **28** (**70%**) animals were positive for blood parasites (Table 1). The infection rate varied slightly between farms, ranging from 60.0% to 80.0%. The highest prevalence was recorded in Farm A (n =10) with 80.0% positive animals, followed by Farm C (n = 10) and Farm D (n =10) (70.0% each), while Farm B (n = 10) recorded the lowest prevalence at 60.0%. Statistical analysis revealed a significant difference ($p = 0.014$) in infection prevalence among the study farms.

Table 1 : Overall prevalence of blood parasite in different small ruminant farms.

Farm location	No. of examined	Prevalence (%)	95% CI	*P*-value
A	10	8 (80.0)	*Ref*	0.014*
B	10	6 (60.0)	0.05-2.77	
C	10	7(70.0)	0.08-4.56	
D	10	7 (70.0)	0.08-4.56	

P-value with asterisk (*) is significant ($p<0.05$)

Analysis was restricted to positive cases, *Anaplasma* spp. was the predominant blood parasite detected, accounting for (67.9%; 19/28) of infections. This was followed by *Mycoplasma* spp. (14.3%; 4/28) and *Babesia* spp. (3.6%; 1/28). Mixed infections were observed in 14.3% (4/28) of the positive samples, comprising of two samples co-infected with *Mycoplasma* spp. and *Anaplasma* spp., and two samples co-infected with *Babesia* spp. and *Anaplasma* spp. The distribution pattern indicates that *Anaplasma* spp. is the most prevalent blood parasite among the infected goats and sheep, suggesting its higher endemicity or vector transmission efficiency compared to *Mycoplasma* and *Babesia* species. A pie chart representation (Fig. 3) illustrates the proportional distribution of these infections, emphasizing the dominance of *Anaplasma* spp. infections within the studied population.

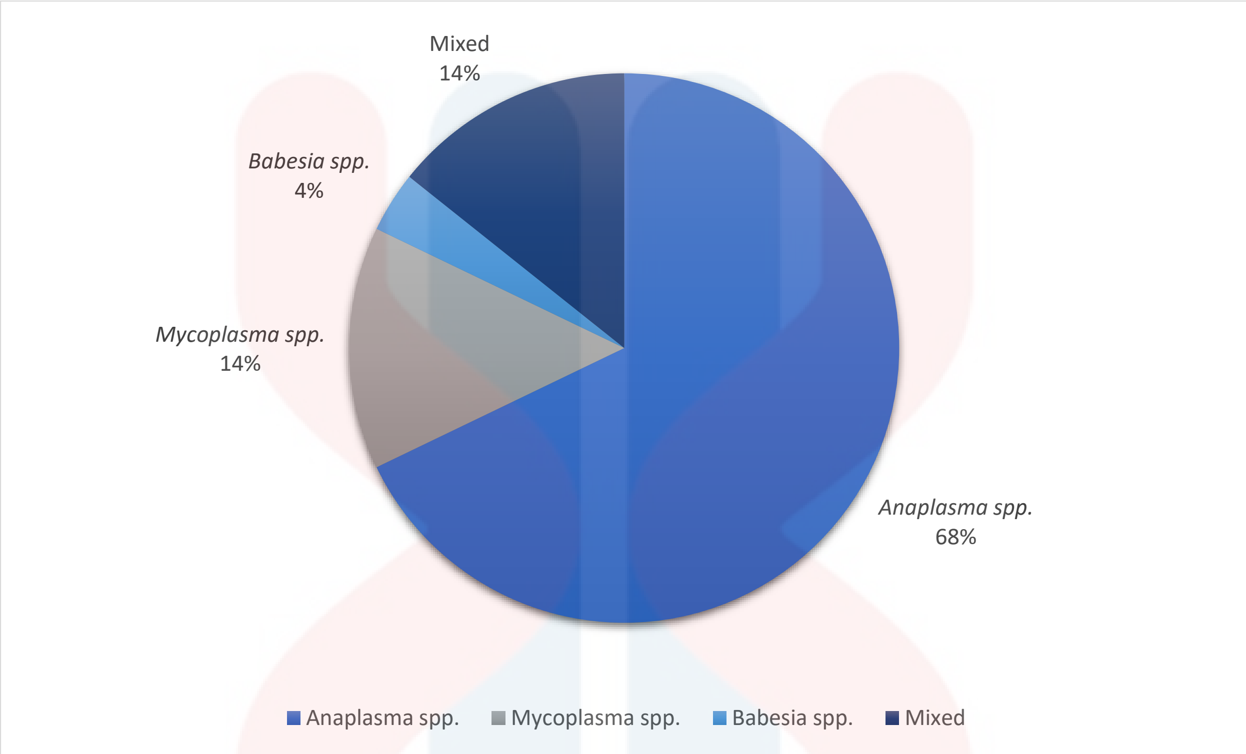

Figure 6 : Distribution of blood parasite infections detected in positive goat and sheep blood smears.

Out of 12 infected goats, 66.67% (n = 8) exhibited mild infection, 25.0% (n = 3) showed moderate infection, while only one goat (8.33%) was severely affected. The mean PCV was inversely proportional to the severity of infection, from 20.25 ± 0.41% in mild, 15.67 ± 0.33% in moderate, to 8.00% in severe cases represented in Table 2.

Table 2 : Distribution of blood parasite infection severity and mean PCV in goat

Severity	**N (%)**	**PCV**	
		Mean ± SE	**Range**
Mild	8 (66.67)	20.25 ± 0.41	18-21
Moderate	3 (25.0)	15.67 ± 0.33	15-16
Severe	1 (8.33)	8.00 ± —	0-8

In sheep, 4 (66.6%) showed mild infection and 2 (33.3%) moderate infection, as shown in Table 3. With mean PCV of 24.75 ± 0.75% and 20.50 ± 0.50%, respectively.

Table 3 : Distribution of blood parasite infection severity and mean PCV in sheep

Severity	**N (%)**	**PCV**	
		Mean ± SE	**Range**
Mild	4 (66.6)	24.75 ± 0.75	23-26
Moderate	2 (33.3)	20.50 ± 0.50	20-21
Severe	0 (0.0)	0.0 ± 0.0	0-0

Univariable analysis of risk factors revealed that infection was slightly higher in sheep (75.0%) than in goats (65.0%), but the difference was not significant ($\chi^2 = 0.476$; $p = 0.490$). Similarly, infection was higher in females (75.0%) than in males (62.5%) ($\chi^2 = 0.714$; $p = 0.398$). A significant association was found between age and infection, where animals aged <1 year (100.0%) were more infected than those aged >1 year (61.3%) ($\chi^2 = 4.977$; $p = 0.026$). Infection rate was higher in animals with low body condition scores (100.0%) than those with high body condition (64.7%), but this was not significant ($\chi^2 = 3.025$; $p = 0.082$).

No significant association was observed between infection and mucous membrane colour ($\chi^2 = 1.726$; $p = 0.189$) or PCV category ($\chi^2 = 1.759$; $p = 0.185$). All animals examined were free from visible tick infestation, and the presence of ticks was therefore not analysed statistically. The frequency of vector control showed no significant difference ($\chi^2 = 0.476$; $p = 0.490$), with infection rates of 65.0% and 75.0% among farms practicing control every six months and occasionally, respectively. The data documented in Table 4.

Table 4 : Result of association between blood parasite infection and risk factor

Variable	Category	No. examine (%)	No. Positive (%)	Chi-square (χ^2)	*P*-value
Species	Goats	20 (50.0)	13 (65.0)	0.476	0.49
	Sheep	20 (50.0)	15 (75.0)		
Sex	Male	16 (40.0)	10 (62.5)	0.714	0.398
	Female	24 (60.0)	18 (75.0)		
Age (years)	< 1	9 (22.5)	9 (100.0)	4.977	0.026*
	> 1	31 (77.5)	19 (61.3)		
General body condition	High score	34 (85.0)	22 (64.7)	3.025	0.082
	Low score	6 (15.0)	6 (100.0)		
Mucous membrane	Normal	29 (72.5)	22 (75.9)	1.726	0.189
	Pale	11 (27.5)	6 (54.5)		
	Congested	0 (0.0)	0 (0.0)		
PCV category	Non-anaemic	23 (57.5)	18 (78.3)	1.759	0.185
	Anaemic	17 (42.5)	10 (58.8)		
Frequency of vector control	Every three month	0 (0.0)	0 (0.0)	0.476	0.490
	Every 6 month	20 (50.0)	13 (65.0)		
	Occasional	20 (50.0)	15 (75.0)		

P-value with asterisk (*) is significant (p<0.05)

CHAPTER 5

5.0 DISCUSSION

Many infectious diseases that can affect small ruminants are caused by bacteria, viruses, fungi and parasitic pathogens brought by protozoan parasites. Therefore, the current study involving sheep and goats in the western coastal region of Malaysia, specifically in Negeri Sembilan, had the aim of determining the prevalence of the blood parasite infection and identifying the associated risk factors. This study hypothesized that blood parasites especially *Anaplasma* sp., *Mycoplasma* sp., *Babesia* sp., and *Theileria* sp. were found to be present among the goats and sheep in Kota Bharu and Bachok, Kelantan. The main objectives of the study were to detect occurrence of these blood parasites by using microscopic methods and to determine risk factor analysis from the blood sample. From the microscopic examination, an overall prevalence of 70% was revealed, demonstrating that a substantial burden of haemoparasite infection in the sampled population. The infection rates among the farms varied from 60% to 80%, with Farm A having the highest prevalence of 80%. Microscopic examination of the blood smears revealed an overall prevalence of 70% among individual animals and 100% infection in four small ruminant herds. The observed microscopic prevalence of haemoparasite in this study is similar to the prevalence reported in Turkey (60.2%) (Zhou *et. al.,* 2017), Egypt (73%) (Abdelsalam *et. al.,* 2023) and Iraq (69.2%) (Renneker *et. al.,* 2013).

However, the highest infection rate was recorded at Farm A with the prevalence of 80%, which seemed significantly higher as compared with the rate recorded at Farm B, where the prevalence recorded is 60%. The significant difference in infection prevalence between the farms (p = 0.014) suggests that the environmental management and farm-level practices may influence the disease transmission. This variation is aligned with the findings of Alvi *et. al* (2024), who emphasized that inadequate vector control frequency, poor housing hygiene and management, and mixed grazing can significantly increase tick exposure, therefore elevating the infection risk. Similarly, Tan Li (2024) reported that the farm applying intensive management systems in Malaysia, has higher infection rate in terms of breed and age of the small ruminants. The high prevalence in Kelantan is also consistent with Malaysia studies. Laili *et. al.* (2020) reported the widespread parasitic infections in small ruminants across Perak, while a case study from Paul *et. al.* (2021) documented significant prevalence of *Mycoplasma ovis* in Malaysian smallholder groups. Thus, these results highlight that the haemoparasites infestations continue to be a major constraint to the productivity and health of the small ruminants in tropical regions in Malaysia.

The relationship between the severity of the infection and packed cell volume (PCV) observed in this study, highlighted the pathogenic role of intraerythrocytic blood parasites in causing anaemia. In goats, mild infections corresponded to a mean PCV of 20.25 ± 0.41%, meanwhile moderate and severe infections revealed the mean PCV values of 15.67 ± 0.33% and 8%, respectively. Similarly, infected sheep demonstrated a mean PCV value of 24.75 ± 0.75% in mild infections and 20.50% ± 0.50% in moderate infections. The inverse relationship between the PCV and the severity of infection reflects the well-documented pathophysiological mechanism of haemoparasites diseases. The higher prevalence of mild subclinical *Anaplasma* spp., *Mycoplasma* spp., *Theileria* spp., and *Babesia* spp. infection observed among small ruminants in this study is consistent with Anyanwu *et. al.* (2016), who revealed that there is a high prevalence of the subclinical disease in small ruminants. Frequently, the field infections of small ruminants are chronic and characterised by mild bacteraemia and regenerative anaemia due to low pathogenicity of *Mycoplasma ovis* (Machado *et. al.,* 2017; Stuen *et. al.,* 2020; Villanueva-Saz *et. al.,* 2022; Bijan *et. al.,* 2025). From previous reports, blood parasite severity has been associated with the mean PCV of small ruminants where a lower PCV was documented in mild to severely infected animals. In addition, haemolytic anaemia is recognised as a significant manifestation of haemoparasitism (Paul *et. al.,* 2021; Obeta and Nwaka, 2024; Siddique *et. al.,* 2024) due to the distortion of the erythrocyte membrane (Shibuya *et. al.,* 2018), increased in membrane fragility (Tabor *et. al.,* 2022; Morrison *et. al.,* 2022), erythrophagocytosis (Philips & Henderson, 2018), oxidative injury, enzymatic lysis and disruption of the cell normal functions (Sulaiman *et. al.,* 2025). This is supported by similar findings by Zadeh *et. al.,* (2024) in Iran, where the case report mentioned a significant decline in PCV especially among infected small ruminants, particularly in cases of moderate to severe parasite infestations.

The overall infection rate recorded was 70% (28/40), indicating a high endemicity of haemoparasites infestations among goats and sheep in selected areas, which is in Kota Bharu and Bachok, Kelantan. This discovery demonstrates that blood parasites remain a persistent health challenge among small ruminants in tropical environments, where climate plays a major role in multiplication and survival of tick vectors (Jongejan & Uilenberg, 2004; Stuen, 2020). Tick vectors, which are very well known in spreading haemoparasites, were able to survive and thrive in the tropical climate of Malaysia, specifically in Kelantan which has the average temperature of 26.6°C and heavy rainfall. Regardless of the hot and humid climate, Abdullah *et. al.* (2019) reported that during the months of July and September, the relative humidity (75.9% - 78.2%) and temperature (28.8°C - 30.6°C) that are ideal for tick activity may not have aligned with the sampling period, which could have limited the geographical distribution of parasites.

The sampling for this study was conducted in the early and late of September in Kota Bharu and Bachok, Kelantan. The weather conditions were between 30.0°C - 30.6°C with relative humidity of 88%. This period follows the month of August, where the temperature was slightly colder but still high and humidity level ranging from 82 - 84%. Although Malaysia does not experience four seasons like temperate climate, environmental conditions in September which is usually warm and humid, are still favourable for tick activity and parasite transmission. This aligns with the findings based on a study from Russia (Bursakov & Kovalchuk, 2019), where tropical environments have inconsistent humidity and temperature levels where it is higher than the four distinct seasons seen in temperate climate of Russia.

In this study, age-related factors are one of the most important risk factors in determining the severe infestation of parasites towards small ruminants. The infection rate appears to decrease steadily with increasing age. Animals with young age are reported to have a significant association with higher infection rates of tick-borne blood parasites. Compared to all the risk factors analysed, age has exhibited a statistically significant association (p = 0.026) with infection status. All animals that are less than one year old (< 1 year) showed a 100% infection rate, compared to those that are more than one year old (> 1 year) which is about 61.3%. Similar evidence was reported in another study conducted by Aktas *et. al.,* (2007) and Rahman *et al.,* (2022), are in agreement with the results, where the rate of infection decreases from young, adult to old animals in Turkey and Bangladesh, respectively. These findings reflect the age-related susceptibility, due to the underdevelopment of the immune system of young animals and their limited prior exposure to parasitic agents and other pathogen-borne. This is primarily attributed to the higher likelihood of encountering vector-borne disease agents, which is reflected in higher blood parasite infection rates. While studies in Europe (Cabezas-Cruz *et al.,* 2019; Rubel *et al.,* 2021) indicated that older small ruminants tested positive for haemoparasites of *Anaplasma* sp. more often as compared to the younger groups (Hormok *et al.,* 2007). The exact reasons for these conflicting results require further investigation, as it may be caused by variation in animal husbandry practices, environmental conditions, vector and haemoparasites species.

Even though sex was not significantly associated with the infection rate (p = 0.398), the trends observed were consistent with prior research, with females (75%) demonstrating higher prevalence than males (62.5%). Conversely, the studies in Egypt (Abdelsalam *et al.,* 2023) demonstrated there was significant association between animal sex and the prevalence of the tick-borne blood pathogens. However, previous studies by Benedicto *et al.,* (2020), who reported that females were significantly associated with higher infection rates of haemoparasites protozoa, and emphasized that female animals are often experiencing stress factor-associated immunosuppression, which was primarily related with pregnancy and lactation, which the males did not experience. Hormonal variations and nutritional demands may also impair immune responsiveness, increasing infection risk. However, in large ruminants, such as cattle, no significant association between sex and protozoan blood parasite infection has been reported (Nunez-Regueiro, 2020).

During sampling, no ticks were detected on any of the animals, suggesting that there were certainly other vectors or even potential fomites transmitting these diseases into the selected research farm areas. The high occurrence of blood parasite agent in this study could be associated with nature of environment and management practices which favour mechanical transmission via blood-sucking arthropods such as *Stomoxys* flies, mosquitoes and/or ixodid ticks (Wang *et al.,* 2017; Azizova *et al.,* 2024). Previous studies, have reported a high density of biting dipterid fly vectors in Peninsula Malaysia, which may explain the frequency of haemoparasites even though this study did not discover ticks or lice on sheep and goats throughout investigation (Saad *et al.,* 2014; Syamsul *et al.,* 2020b; Paul *et al.,* 2021). The high rate of diseases transmitted by vectors in the tropics and subtropics is explained by the optimal condition for the spread of haemophagous arthropods (Jongejan and Uilenberg, 2004). In this study, factors such as frequent heavy rainfall, warmth, humidity, farms' close proximity to dense vegetation, and the large number of animals in pens without flyscreen likely contributed to favourable conditions for vectors. Additionally, prior research demonstrated that management procedures that result in bleeding, like flock immunization, ear-tagging, marking, mulesing and wool shearing, promote the iatrogenic propagation of these blood parasites within a flock. (Hope *et al.,* 2018; Alsaad *et al.,* 2019). Thus, the use of shared needles and tag applicators, insufficient herd health programs, the widespread practice of grazing under semi-intensive and intensive management systems, and smallholder farmers' lack of awareness of the disease were identified as an important risk factor for blood parasites spread in the studied area.

While this study successfully determined the prevalence and risk factors of blood parasites in small ruminants using thin blood smear and packed cell volume (PCV) analysis, several improvements could help enhance the accuracy, representativeness, and diagnostic value of future investigations. The statistical power and generalizability of the results would be enhanced by expanding the sample size across more farms. Stratifying samples according to flock size, management system, and ecological zone, as well as extending sampling to more districts in Kelantan, would also allow for stronger epidemiological comparisons. The current sample of 40 animals (10 per farm) offered useful preliminary insight into the infection dynamics within the selected area. Additionally, the study could be significantly enhanced by additional laboratory methods that improve detection sensitivity and species differentiation. Microscopic examination is still an efficient and popular method, but it has limitations especially when it comes to identifying chronic or low-level infections involving low parasitaemia. Therefore, incorporating molecular diagnostic techniques such as polymerase chain reaction (PCR) would allow for more sensitive and specific identification of blood parasites, enabling the detection of mixed infections and latent carriers that might be missed under the microscope. Moreover, tick identification and surveillance during sample collection would significantly improve epidemiological value. Although no ticks were observed during the present study, active tick collection using drag cloths or direct inspection from surrounding vegetation would help determine vector species presence and infestation intensity, (Huda *et al.,* 2023; Alvi *et al.,* 2024).

In the situation where the infection is present, effective control of haemoparasites infections in small ruminants requires an integrated approach that combines immediate therapeutic management with strategic preventive measures addressing both the animal and the vector. Based on the result of the current study, where a high prevalence 70% of blood parasite infection was detected with the absence of visible tick infestation, suggest that subclinical carrier animals and environmental factor play crucial roles in maintaining infection cycles. The choice of drug depends on the suspected or identified causative agent. For infections caused by *Anaplasma* spp., which are commonly found in small ruminants in Malaysia (Huda *et al.,* 2023) the recommended treatment is oxytetracycline administered intramuscularly at a dose of 10 mg/kg body weight for 3 to 5 consecutive days (Tabor, 2022). In suspected cases of *Babesia* spp. infection, the treatment with imidocarb dipropionate or diminazene aceturate are effective in controlling acute babesiosis (Aktas *et al.,* 2006). Additionally, applying acaricide every four to six weeks is recommended (Alvi *et al.,* 2024; Sébastien Grech-Angelini *et al.,* 2015). Field control can also be improved through farmer workshops on topics like safe acaricide use, rotational grazing, tick control, and early disease detection. The Department of Veterinary Services (DVS Malaysia, 2025) supports participatory extension approaches that involve both veterinary professionals and rural farmers to ensure that preventive protocols are practical and sustainable for effective blood parasite control in Malaysia.

CHAPTER 6

6.0 CONCLUSION

In conclusion, the present study successfully addresses the primary objectives by determining the prevalence and analysing the associate risk factors of blood parasite in goats and sheep in selected areas in Kelantan. The finding demonstrated a high prevalence 70% of blood parasite infection, detected through thin blood smear examination and supported by PCV evaluation confirming the initial hypothesis that blood parasite occurrence in small ruminants within study area is high. The occurrence of infection across all farms, despite the absence of visible tick infestation, indicates the possible role of subclinical carriers or alternative mechanical vectors in maintaining transmission within the population. Age was identified as a significant risk factor, with young animals (<1 year) being more susceptible to infection, while species, gender, body condition, and vector control frequency showed no significant associations ($p > 0.05$). The inverse relationship observed between infection severity and PCV confirmed the impact of haemoparasites infection on anaemia development in affected animals. These findings highlight the continuing importance of haemoparasites diseases in small ruminant production systems in Kelantan and underscore the need for strengthened surveillance, regular vector control, improved nutritional and management practices, and farmer awareness programs to reduce disease burden, enhance productivity, and safeguard animal health at both farm and regional levels.

APPENDICES

Figure 7 : Backyard Farm at Kota Bharu

Figure 8 : Sample Collection in Goat

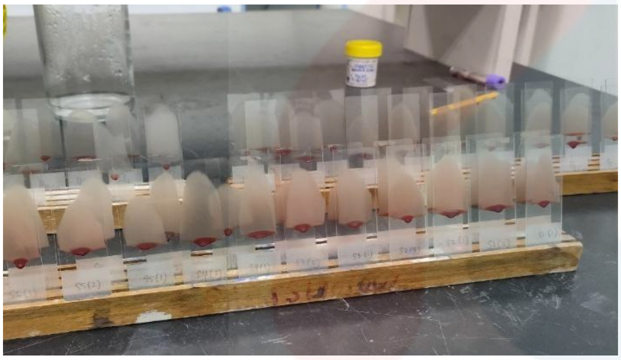

Figure 9 : Thin Bood Smears

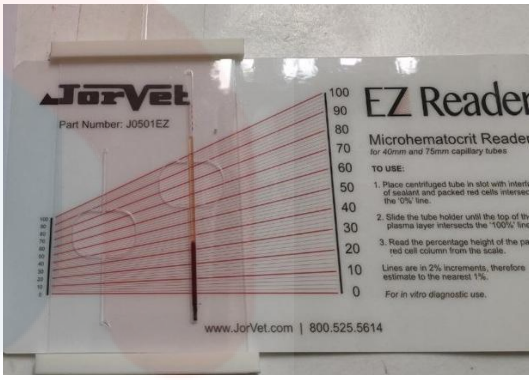

Figure 10 : Packed Cell Volume

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