UNIVERSITI MALAYSIA KELANTAN

SEROPREVALENCE OF *TOXOPLASMA GONDII* AND ASSOCIATED RISK FACTORS AMONG LOCAL GOATS IN KELANTAN, MALAYSIA

By

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SEROPREVALENCE OF *TOXOPLASMA GONDII* AND ASSOCIATED RISK FACTORS AMONG LOCAL GOATS IN KELANTAN, MALAYSIA

ABSTRACT

Toxoplasma gondii is an intracellular protozoan parasite capable of infecting humans and many warm-blooded animals. Goats are highly susceptible to *T. gondii* infection via ingestion of food or water contaminated with oocysts shed by feline definitive hosts or via transplacental transmission of tachyzoites. Most infections in goats are asymptomatic; however, in pregnant females, toxoplasmosis can cause reproductive failures. Humans can acquire toxoplasmosis by ingesting raw or undercooked meat, including meat-based products. There have been a limited number of recent studies investigating *T. gondii* prevalence in goats in Malaysia, with the studies focusing only on Selangor state. The objective of this study is to assess the present seroprevalence of *T. gondii* among local goats in selected farms in Kelantan, Malaysia, and to identify the associated risk factors. Eighty serum samples collected from goats across four ruminant farms in Kota Bharu and Bachok districts were examined using an indirect Enzyme Linked Immunosorbent Assay (indirect ELISA) to identify the presence of *T. gondii* antibodies. The findings indicated that five of the goat serum samples (6.25%) tested positive for *T. gondii*, with water source identified as a significant associated risk factor. Overall, even though the seroprevalence of *T. gondii* among goats in Kelantan was relatively low, the positive detection of infection indicates ongoing environmental exposure. These findings highlight the need for improved farm hygiene, water management, and biosecurity to further reduce infection in goats and minimise the potential zoonotic risk to farmers and consumers.

Keywords: *T. gondii*, seroprevalence, risk factors, goat, indirect ELISA, Kelantan

SEROPREVALENSI DAN ANALISIS FAKTOR RISIKO JANGKITAN *TOXOPLASMA GONDII* DI ANTARA KAMBING TEMPATAN DI KELANTAN, MALAYSIA

ABSTRAK

Toxoplasma gondii ialah protozoa parasit intrasel yang mampu menjangkiti manusia dan kebanyakan haiwan berdarah panas. Kambing sangat mudah dijangkiti oleh *T. gondii* melalui pengambilan makanan atau air yang tercemar dengan oosista yang disebarkan oleh perumah definitif daripada keluarga kucing, atau melalui penularan transplasenta oleh takizoit. Kebanyakan jangkitan pada kambing adalah tanpa gejala, namun bagi kambing betina bunting, toxoplasmosis boleh menyebabkan kegagalan sistem pembiakan. Manusia boleh dijangkiti toxoplasmosis melalui pemakanan daging atau produk berasaskan daging yang mentah atau tidak dimasak sepenuhnya. Hanya terdapat sejumlah kecil kajian terkini yang menyiasat prevalensi *T. gondii* dalam kalangan kambing di Malaysia, dan kajian tersebut hanya tertumpu di negeri Selangor. Objektif kajian ini adalah untuk menilai kadar seroprevalensi semasa *T. gondii* dalam kalangan kambing tempatan di ladang-ladang terpilih di Kelantan, Malaysia serta mengenal pasti faktor risiko yang berkaitan. Sebanyak 80 sampel serum yang dikumpulkan daripada kambing di empat ladang ruminan di daerah Kota Bharu dan Bachok telah dianalisis, dengan menggunakan ujian ELISA tidak langsung untuk mengesan kehadiran antibodi *T. gondii*. Hasil kajian menunjukkan bahawa lima sampel serum kambing (6.25%) adalah positif terhadap *T. gondii*, dan sumber air dikenal pasti sebagai faktor risiko yang signifikan. Secara keseluruhannya, walaupun seroprevalensi *T. gondii* dalam kalangan kambing di Kelantan adalah sangat rendah, pengesanan positif jangkitan menunjukkan bahawa pendedahan kepada persekitaran masih berlaku. Hasil kajian ini menekankan keperluan untuk penambahbaikan dalam kebersihan ladang, pengurusan sumber air dan biosekuriti bagi mengurangkan lagi jangkitan dalam kambing serta meminimumkan risiko zoonotik kepada para penternak dan pengguna.

Kata kunci: *T. gondii*, seroprevalensi, faktor risiko, kambing, ELISA tidak langsung, Kelantan

CERTIFICATION

It is hereby to certify that we have read this research paper entitled "**Seroprevalence of *Toxoplasma gondii* and Associated Risk Factors Among Local Goats In Kelantan, Malaysia**" by Lee Yi Heng, and in our opinion, it is satisfactory in terms of scope, quality, and presentation as partial fulfillment of the requirements for the course of DVT 55204 – Research Project.

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DVM 5 Lycan Class of 2025

Thank you so much.

DEDICATIONS

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LIST OF ABBREVIATIONS

AIDS	Acquired Immunodeficiency Syndrome
CI	Confidence Intervals
DNA	Deoxyribonucleic Acid
DVS	Department of Veterinary Services
GRA	Dense Granule Antigens
ELISA	Enzyme-Linked Immunosorbent Assay
IFAT	Indirect Fluorescent Antibody Test
IgG	Immunoglobulin G
LAT	Latex Agglutination Test
LD	Lethal Dose
MAT	Modified Agglutination Test
MIC	Microneme Antigens
NC	Negative Control
O.D.	Optical Density
OR	Odds Ratios
P	P-values
PC	Positive Control
PCR	Polymerase Chain Reaction
PV	Parasitophorous Vacuole

ROP	Rhoptry antigens
S/P	Sample/Positive ratio
T. gondii	*Toxoplasma gondii*

LIST OF SYMBOLS

%	Percentage
℃	Degree Celsius
µL	Microlitre
M	Molarity
nm	Nanometre
rpm	Revolutions per minute

CHAPTER 1

INTRODUCTION

1.1 Research Background

Toxoplasma gondii is an obligate intracellular protozoan from the phylum Apicomplexa, infecting both humans and various species of warm-blooded animals (Sadiq *et al*., 2023). The parasite was discovered in 1908 in a species of North African rodents, and it was proven to infect humans and animals in the late 1930s (Tenter *et al*., 2000). This protozoa is genetically diverse with three main genotype lineages, namely type I, II, and III, and some minor and mixed genotypes (Norouzi *et al*., 2016; Hosseini *et al*., 2018).

T. gondii has a complex life cycle, involving a sexual stage in intestinal epithelial cells of the definitive hosts and an asexual stage in different tissues in a broad range of intermediate hosts (Norouzi *et al*., 2016; Amouei *et al*., 2019). During the life cycle of *T. gondii*, three developmental stages can infect cells, which are tachyzoite (rapid multiplies and is found in the acute infections), bradyzoite (slow multiplies and is found in the chronic infections as tissue cysts), and sporozoite (produced only during sexual stage in the feline definitive host and released in the oocysts with cat feces) (Attias *et al*., 2020).

Small ruminants, including goats and sheep, are particularly vulnerable to *T. gondii* infection via the consumption of food or water contaminated with oocysts shed by feline definitive hosts, or through the transplacental transmission of tachyzoites. Most infections in small ruminants are asymptomatic; however, in pregnant females, toxoplasmosis can cause reproductive failures, such as abortions, stillbirths, fetal mummification, and the birth of weak kids/lambs (Holec-Gasior & Sołowinska, 2023). As few as 10 sporulated oocysts are adequate to cause abortion in experimentally infected goats (Parigi, 2014).

Since 1969, global seroprevalence surveys have reported that approximately one-third of the human population worldwide has been infected with *T. gondii* (Kean, 1972; Jackson & Hutchison, 1989; Saadatnia & Golkar, 2012; Wana *et al*., 2020). Toxoplasmosis in humans is significantly associated with the consumption of raw or undercooked meat or meat products (Bahreh *et al*., 2021). Contaminated cooking surfaces and utensils during meat processing can serve as a transmission route to humans (Rahman *et al*., 2018). A study conducted by Rani *et al.* in 2020 showed the presence of viable *T. gondii* tissue cysts in as small as 5 grams of goat and sheep meat (Rani *et al*., 2020). It may cause toxoplasmic encephalitis or retinochoroiditis in immunodeficient individuals, such as those with AIDS, cancer, or who have undergone organ transplants. In pregnant women, *T. gondii* may cross the placenta and infect fetuses, causing retinochoroiditis, mental retardation, or even fetal death (Rahman *et al*., 2018).

In Malaysia, toxoplasmosis is included as one of the reportable diseases in the Manual of Listed Notifiable Diseases 2020 (*Buku Panduan Senarai Penyakit Wajib Lapor 2020*) by the Department of Veterinary Services Malaysia (DVS) owing to its economic implications for the small ruminant sector and public health concerns. Hence, this study aims to assess the seroprevalence of toxoplasmosis among local goats in Kelantan and to identify the associated risk factors, enabling the organization of effective preventive and control measures based on these risk factors.

1.2 Research Problem

Studies related to the prevalence of *T. gondii* have been conducted globally in humans and other animals, including goats. Nonetheless, to date, only a few studies regarding the prevalence of *T. gondii* in goats have been conducted in Malaysia recently, with the studies only focusing on Selangor state. There remains a deficiency of studies to indicate the prevalence of *T. gondii* in goats in other states, such as Kelantan.

1.3 Research Questions

1. Is *T. gondii* serologically detectable among local goats in Kelantan?

2. What are the risk factors associated with *T. gondii* infection among local goats in Kelantan?

1.4 Research Hypothesis

1. The prevalence of *T. gondii* infection based on antibody detection among local goats across various farms in Bachok and Kota Bharu, Kelantan, ranges from moderate (30 - 50%) to high (> 50%).
2. The associated risk factors of *T. gondii* infection among local goats in Kelantan are age, sex, rearing system, source of animals, water source, herd size, and presence of small mammals.

1.5 Research Objectives

1. To assess the presence of antibodies against *T. gondii* among local goats in selected farms in Bachok and Kota Bharu, Kelantan.
2. To identify the associated risk factors of *T. gondii* infection among local goats in the targeted farms.

CHAPTER 2

LITERATURE REVIEW

2.1 Classification and Morphology of Protozoa

T. gondii is the only species of the *Toxoplasma* genus and is a member of the Apicomplex phylum, Conoidasida class, Eucoccidiorida order, and Sarcocystidae family (Masombuka *et al*., 2024). There are a variety of *T. gondii* genotypes across the globe, which are mainly classified into lineages I, II, and III, along with atypical or exotic variants. Type I lineages are highly lethal with a lethal dose (LD100) of 1 in all species of laboratory mice; meanwhile, type II and III lineages are less virulent with LD100⩾103 (Hosseini *et al*., 2018). Common genotypes in Asia include ToxoDB#9 (Chinese1), types I and II, the II variant, and type III. The most common genotype in Malaysia is type II, which has been documented in free-range ducks and wild boars (Chaichan *et al*., 2017). The genetic diversity of *T. gondii* can lead to genetic recombination events among strains, causing the generation of new strains with potentially increased virulence to people and animals (Amouei *et al*., 2019).

The antigens of *T. gondii* are found throughout the outer membrane, cytosol, and various secretory organelles. During host cell invasion, the microneme antigens (MIC), the rhoptry antigens (ROP), and the dense granule antigens (GRA) are among the secretory organelles that are discharged into the newly formed parasitophorous vacuole (PV) or into the tissue cyst (Carruthers *et al*., 1999; Holec-Gasior & Sołowinska, 2022). The contents of the three secretory organelles will be released in sequence according to a cascade mode (Joiner & Roos, 2002). MICs are the first components released upon encountering host cells, facilitating their recognition and attachment (Kotresha & Noordin, 2010). This is followed by the release of ROPs, which help in the formation of the PV and promote its association with host cell organelles (Sam-Yellowe, 1996). Subsequently, GRAs are released sub-apically both during and after the parasite invades the PV (Mercier *et al*., 2005). After secretion, they are believed to alter the conditions inside the PV to support the parasite's survival and replication within host cells (Holec-Gasior & Sołowinska, 2022). The parasite tachyzoites and bradyzoites possess surface coatings composed of glycosylphosphatidylinositol (GPI)-anchored antigens, largely belonging to the surface antigen 1 (SAG1) and surface antigen 2 (SAG2) families. These antigenic proteins are thought to be involved in host cell invasion, modulation of the host immune response, and regulation of virulence, while also potentially providing environmental protection that aids the parasite's survival outside the host (Lekutis *et al.*, 2001). P30, which is a member of SAG1, is the most immunogenic component of tachyzoites (Rodriguez *et al.*, 1985). Thus, the P30 antigen is widely employed in ELISA tests to identify antibodies against *T. gondii*.

2.2 Life Cycle

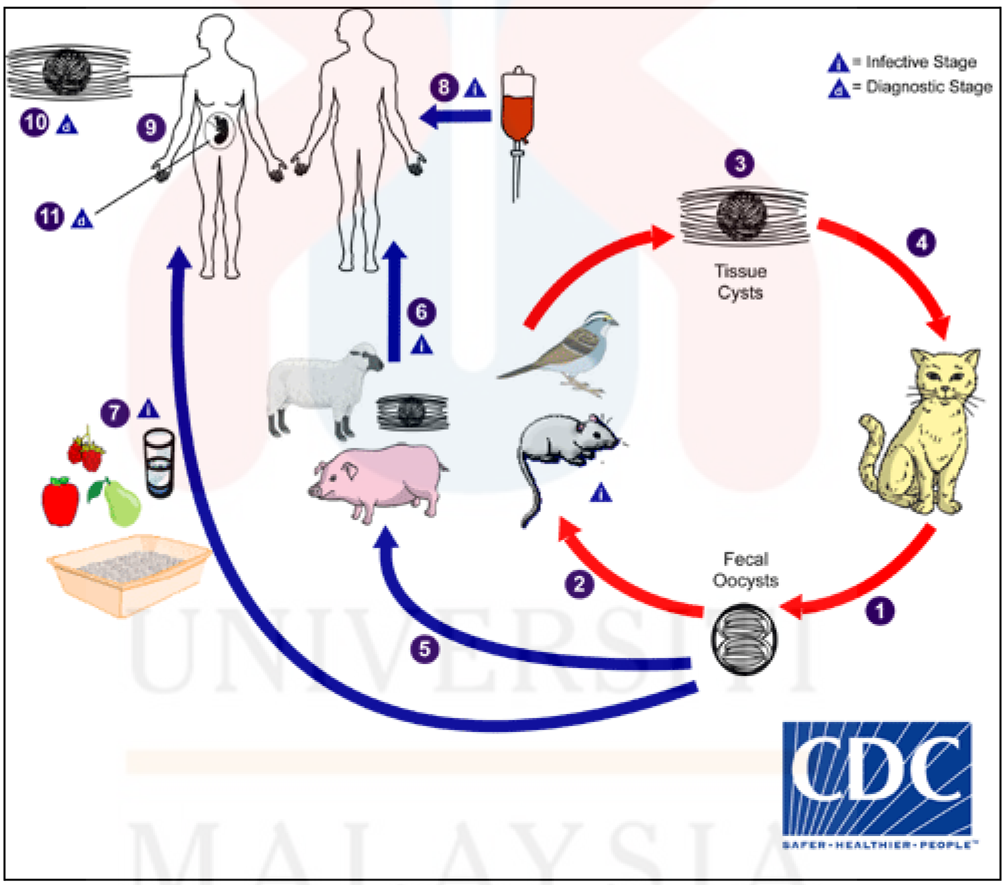

Figure 2.1: Life cycle of *T. gondii.* Adapted from Centers for Disease Control and Prevention (n.d.)

T. gondii has two life cycles: a sexual cycle that occurs in the small intestine of definitive hosts (feline species) and an asexual cycle in intermediate hosts (infected animals and humans) (Saadatnia & Golkar, 2012; Rahman *et al.*, 2018). Cats can be infected with any of the three forms of the parasite (tachyzoites, bradyzoites, or sporozoites) and release the infectious oocysts into the environment (Saadatnia & Golkar, 2012). Predation of prey (e.g., birds and rodents) that harbor tissue cysts will establish the parasitic transmission route (Figure 2.1) (Holec-Gasior & Sołowinska, 2023). Alternatively, ingestion of oocysts containing sporozoites excreted by another cat can also facilitate transmission among felines. Digestion of the cyst wall or oocyst will release bradyzoites or sporozoites, respectively. They will invade intestinal epithelial cells and form schizonts. The process of schizogony produces many daughter cells known as merozoites within feline enterocytes. When these enterocytes rupture, they release numerous merozoites that can then invade other enterocytes and undergo further multiplication through schizogony. Some merozoites begin to differentiate into gametes (microgamonts or macrogametes) within a period of three to fifteen days following the primary infection in feline species. Fertilization of male and female gametes will give rise to unsporulated oocysts, which are shed with the feline feces. Sporulation occurs in the environment and produces two sporocysts, each containing four sporozoites (Attias *et al*., 2020).

Meanwhile, humans and other warm-blooded mammals can acquire the infection by ingestion of tissue cysts (in undercooked meat) or sporulated oocysts (in soil or vegetables contaminated with cat feces) (Figure 2.1) (Saadatnia & Golkar, 2012). Then, the external walls of the cysts or oocysts rupture, leading to the discharge of bradyzoites and sporozoites into the intestinal lumen. After that, these organisms penetrate the enterocytes and proliferate, transforming into tachyzoites. Subsequently, the tachyzoites circulate through the blood and/or lymphatic vessels to other body systems (acute phase). The body's immune response will cause the conversion of rapidly dividing tachyzoites into quiescent bradyzoites within tissue cysts (chronic phase) (Saadatnia & Golkar, 2012; Wannapong *et al*., 2024). However, the bradyzoites can differentiate back to tachyzoites if the immune system of an infected host deteriorates. This will trigger a recurrence of the acute phase, leading to tachyzoite dissemination throughout the body. In pregnant hosts, fetuses can acquire infection via congenital transmission of tachyzoites (Wannapong *et al*., 2024).

2.3 Host and Clinical Manifestation

The feline species, including the domestic cat, is the only definitive host for *T. gondii*; meanwhile, most species of warm-blooded animals, including ruminants and humans, are intermediate hosts in which the parasite can survive and multiply (Centers for Disease Control and Prevention, n.d.). As compared to large ruminants, small ruminants are predisposed to *T. gondii* infection. Small ruminants can graze at lower heights compared to cattle. Thus, they may have a higher chance of ingesting soil contaminated with *T. gondii* oocysts (Rahman *et al*., 2011). Besides that, cattle are not suitable intermediate hosts for *T. gondii* because they are able to reduce or eradicate the amounts of tissue cysts to undetectable levels several weeks post-infection, which may be due to their innate resistance (Hamid *et al*., 2020).

The stage of infection, the parasite strain, and the animals' immune status determine the severity of clinical signs in small ruminants (Daro *et al*., 2024). Toxoplasmosis may cause reproductive failures in small ruminants, including fetal mummification, abortion, stillbirths, and the delivery of weak offspring or the occurrence of congenital diseases. Surviving lambs and kids usually grow normally, but can serve as a source of *T. gondii* infection in humans. Consumption of the infected meat, cured meat products, or unpasteurized milk and dairy products can cause zoonotic transmission (Holec-Gasior & Sołowinska, 2023). In the USA, *T. gondii* is one of three pathogens (together with *Salmonella* and *Listeria*) that account for three-quarters of all deaths from foodborne diseases (Dubey & Jones, 2008). For immunocompetent persons, toxoplasmosis is often characterized by mild, transient symptoms including fever, general malaise, and lymph node enlargement. In contrast, clinical signs are usually more severe in immunocompromised people and pregnant women, causing more complications such as encephalitis, retinochoroiditis, abortion, splenomegaly, and pneumonia (Hosseini *et al*., 2018).

2.4 Diagnosis of Toxoplasmosis

Several laboratory techniques are employed for the diagnosis of toxoplasmosis in animals, including ELISA, indirect fluorescent antibody tests (IFAT), microscopic examination, mouse bioassays, and PCR. The most commonly used diagnostic methods are serological tests, with ELISA having higher sensitivity and specificity than other serological assays (Rahman *et al*., 2011; Tan *et al*., 2020). Bioassays using mice or cats provide the most reliable detection of viable *T. gondii*, but their laborious, time-intensive, and expensive nature restricts their use in large-scale studies. Thus, ELISA has been widely documented in epidemiological studies for the detection of *T. gondii* antibodies in live ruminants (Hamid *et al*., 2020). Conventional serological methods, such as ELISA, have limitations in determining the duration of *T. gondii* infection. In other words, they cannot differentiate between acute and chronic infections. At present, distinguishing between recent and past infections primarily relies on assessing the IgG avidity of antibodies specific to *T. gondii*. Low-avidity IgG antibodies are typically produced during the early stages of infection, whereas high-avidity IgG antibodies are characteristic of chronic infection (Holec-Gasior & Sołowinska, 2022).

2.5 Epidemiology of Toxoplasmosis in Small Ruminants

The prevalence of *T. gondii* in small ruminants is higher in countries with a humid climate than in those with a hot, dry climate (Rahman *et al*., 2011). The reported seroprevalence of *T. gondii* infection in goats varies widely, ranging from 0% to 77% across different countries and regions (Jung *et al*., 2014). A comprehensive literature review and meta-analysis by Rodrigues *et al*. on the prevalence of *T. gondii* antibodies in goats from 2000 to 2020 found a global seroprevalence of 27.49% with 20.74% in Asia. Possible risk factors include sex, age, rearing system, breed, presence of cats, and presence of other species (Rodrigues *et al*., 2022).

Several studies have also been conducted in Malaysia to assess the prevalence of *T. gondii* in goats. One quarter of ruminant meat samples from wet markets in Klang Valley and abattoirs in Selangor, Malaysia, were seropositive for *T. gondii* IgG antibodies, with the highest documented in goat meat samples (55%), and females were more likely to be seropositive than males (Hamid *et al*., 2020). Sadiq *et al*. indicated that more than half (69.8%) of goats in Selangor, Malaysia, were seropositive for *T. gondii* antibodies in 2022. This study also demonstrated that older animals, semi-intensive rearing systems, the presence of cats on farms, large herd size, and the source of replacement stock increased the exposure risk to toxoplasmosis (Sadiq *et al*., 2023). In 2008, antibodies to *T. gondii* were detected in 35.5% of goats' sera in peninsular Malaysia (Chandrawathani *et al*., 2008). Based on previous studies in Malaysia, the expected prevalence of *T. gondii* in goats in Kelantan is estimated to be 30-70% (moderate to high). To date, no recent study has been carried out in Kelantan to generate data on *T. gondii* infection in local goats. Thus, this will be the first study to assess the seroprevalence of *T. gondii* and identify potential risk factors affecting goats in Kelantan.

2.6 Treatment Available and Prevention Management

Treatment of toxoplasmosis in small ruminants involves sulfonamides, pyrimethamine, clindamycin, spiramycin, and toltrazuril. However, the treatment options are not very effective because most drugs can only kill the active forms of the parasite, not the dormant cysts (Daro *et al*., 2024).

To prevent infection, as the feline is the only definitive host for *T. gondii*, it is important to keep cats out of farm areas, including feed and water troughs. Furthermore, newly purchased animals should be screened for *T. gondii* infection and only sourced from reputable suppliers (Wannapong *et al*., 2024). Toxovax, a vaccine containing live attenuated *T. gondii* S48 strain tachyzoites, is currently used in sheep to prevent congenital infection with *T. gondii*, but the vaccine is not registered to be used in humans and other animals (Saadatnia & Golkar, 2012; Holec-Gasior & Sołowinska, 2023). To prevent food-borne disease in humans, some literature suggests that livestock meat for human consumption should be tested thoroughly and labeled as '*Toxoplasma*-free meat'. As recommended, meat that tests seropositive should undergo thermal processing or freezing before it can be considered safe for sale (Holec-Gasior & Sołowinska, 2023).

CHAPTER 3

MATERIAL AND METHODOLOGY

This chapter covers the ethical considerations, study area, study design, study population, selection criteria, sampling method and procedures, data collection, and data analysis for this study. Below is the summary of the methodology employed in this research (Figure 3.1).

Animal Ethics Approval

Study area:

- UMK Teaching Farm in Bachok, Kelantan.
- 1 private farm in Bachok, Kelantan ((UMK/FPV/ACUE/FYP/011/2025)
- 2 private goat farms located in Kota Bharu and Bachok, Kelantan (archived samples from previous study - UMK/FPV/ACVE/FYP/009/2024).

Study design:

Cross-sectional study

Study population:

- Serum samples of 51 goats randomly selected from the UMK Teaching Farm and private farm in Bachok.
- 29 archived samples from previous study.

Selection criteria:

- **Inclusion criteria**: Serum samples of goats regardless of their ages, sexes, breeds, health status, and body condition scores.
- **Exclusion criteria**: None.

Sampling method and procedure:

1. Collection of information about goats' signalment and farm management from farm workers in UMK Teaching Farm.
2. Physical examination of the study objects.
3. Collection of 51 blood samples via jugular venipuncture by using plain tube.
4. Sample processing and storage.
5. Another 29 serum samples are archived samples from previous study. Collection of information required from former researcher and related farm workers.
6. Serological detection by using indirect ELISA.

Data collection tools:

- Record goats' signalment & farm management information and tabulate by using Microsoft Excel.
- Read the ELISA results (OD) by using plate reader, then record in Microsoft Excel.

Plan for data analysis:

Data will be analysed with frequency, percentage, odd ratio, 95% CI and P-value by using IBM SPSS Statistics 31 to determine the prevalence and associated risk factors.

Figure 3.1: The flowchart of the methodology conducted in this study

3.1 Ethical Considerations

Approval for animal use in this study was obtained from the Institutional Animal Care and Use Committee (IACUC), Faculty of Veterinary Medicine, Universiti Malaysia Kelantan (UMK/FPV/ACUE/FYP/011/2025). Furthermore, animal ethics approval for routine blood collection from goats at the UMK Teaching Farm had been granted for teaching and learning purposes. This study also used archived sera from previous studies (UMK/FPV/ACVE/FYP/009/2024).

3.2 Study Area

The study focused on four local goat farms in Kelantan, Malaysia. The farms were selected from two of the eleven districts in Kelantan: Bachok and Kota Bharu. These study areas experience consistently warm temperatures year-round, with a three-month rainy season from October to December. The selected farms included UMK Teaching Farm in Bachok, two private farms in Bachok, and one private farm in Kota Bharu.

3.3 Study Design

A cross-sectional study design was employed, whereby the serum samples were collected, processed, and tested for *T. gondii* antibodies, and the data were analyzed to identify the association between the independent variables and *T. gondii* seropositivity.

3.4 Study Population

Fifty-one (51) goats were randomly selected from Bachok district in Kelantan, Malaysia, comprising thirty-six (36) goats from Farm A and fifteen (15) goats from Farm B. Twenty-nine archived samples from the previous study were also selected, which included fourteen (14) goats from Farm C, and fifteen (15) goats from Farm D, to get a total sample of eighty (n=80) in this study.

3.5 Selection Criteria

3.5.1 Inclusion Criteria

Convenience sampling was applied to select two goat farms in Bachok, Kelantan. The goats on these farms were subsequently selected by simple random sampling, irrespective of sex, age, breed, body condition score, or health status.

3.5.2 Exclusion Criteria

No exclusion criteria were defined for this study.

3.6 Sampling Method and Procedures

3.6.1 Animal Identification

Before blood sampling, a physical examination was conducted on each selected goat, and its signalment was documented in the animal information form (Table 3.1).

Table 3.1: Animal identification form

Goat ID	Sex	Age	Remark

3.6.2 Sample Collection

A total of fifty-one (51) blood samples were obtained through jugular venipuncture using 1-inch, 21-gauge vacutainer needles and plain tubes. Firstly, the jugular vein was located by drawing an imaginary line from the lateral canthus of the goat's eye ventrally to the neck region. Secondly, the jugular vein was occluded at the jugular groove by using the thumb of the non-dominant hand. Thirdly, the bulging vein was swabbed with alcohol. Fourthly, the needle, which was connected to the vacutainer holder, was inserted at a 45° angle into the vein. Fifthly, the vacutainer tube with the holder was stabilized using the middle and index fingers, and the palm of the dominant hand. Sixthly, by applying pressure with the palm, the vacutainer tube was inserted into the needle located in the holder. Seventhly, once the vacutainer was two-thirds full of blood, it was detached from the holder to prevent blood from bursting out due to the vacutainer's pressure. Eighthly, the needle was withdrawn from the vein, and the venipuncture site was occluded by the thumb of the free hand to stop bleeding. Ninthly, each plain tube was labelled with the animal identification number using a permanent marker. Tenthly, the blood samples were stored in an ice box during transit from the farm to the laboratory. Upon arrival at the laboratory, the samples were promptly transferred to a chiller maintained at 2–8 °C.

3.6.3 Sample Processing

Blood samples in plain tubes were centrifuged at 2500 rpm for 15 minutes to facilitate the separation of blood clots. After that, the serum samples were collected using Pasteur pipettes and placed into microcentrifuge tubes, each labelled with the appropriate animal ID for precise documentation. The serum samples were subsequently stored at −20 °C in a freezer. Prior to conducting the ELISA test, the samples were allowed to thaw at room temperature.

3.6.4 Serological Detection - Indirect ELISA

Serological detection of *T. gondii* was performed utilizing an Indirect ELISA kit (IDVet, France). The kit components included microplates coated with P30 antigen, a 10× Concentrated Conjugate, the Positive and Negative Controls, the Dilution Buffers 2 and 3, a 20× Concentrated Wash Solution, a Substrate Solution, and a 0.5 M Stop Solution.

Firstly, all materials required but not provided, including multichannel pipettes, disposable tips, a 96-well microplate, distilled water, a wash system, and a 96-well microplate reader, were prepared. All reagents were brought to room temperature (21 °C ± 5 °C) before use. All reagents were homogenized by inversion or vortexing.

Secondly, the 1× Wash Solution was prepared by diluting the 20× Wash Concentrate with distilled water at a 1:20 ratio. The calculation is shown below (3.1).

200µL x 2 x 3 x 96 = 115200 µL = 115.2 mL

≈ 120 mL of Wash Solution was used for two wash steps (each step was washed 3 times)

$$M1V1 = M2V2 \tag{3.1}$$

$$(20)\ V = (1)\ (120)$$

$$V1 = 6\ \text{mL}$$

* 6 mL of 20x Wash Concentrate was mixed with 114 mL of distilled water to produce a total volume of 120 mL of 1x Wash Solution.

Thirdly, an indirect ELISA was performed according to the manufacturer's guidelines. Serum samples were diluted at a final ratio of 1:10 in this serological test. 90 µL of Dilution Buffer 2 was added to each microwell. Then, 10 µL of the Negative Control was added to wells A1, A2, B1, and B2; meanwhile, 10 µL of the Positive Control was added to wells C1, C2, D1, and D2. Following this, 10 µL of each test sample was added to the remaining wells. Fourthly, the plate was covered with aluminium foil and incubated for 45 minutes ± 4 minutes at 21 °C ± 5 °C.

Fifthly, while the 96-well microplate was incubating, the 1× Conjugate was prepared by diluting the 10× Concentrated Conjugate 1:10 with Dilution Buffer 3. The calculation is shown below (3.2).

100µL x 96 wells x 1 = 9.6 ml

≈ 10 ml

$$M1V1 = M2V2 \tag{3.2}$$

$$(10)\ V = (1)\ (10)$$

$$V1 = 1\ \text{mL}$$

*1 mL of 10x Concentrated Conjugate was mixed with 9 mL of Dilution Buffer to produce a total volume of 10mL of 1x Conjugate.

Sixthly, after incubation, the wells were emptied. Each well was washed three times with at least 200 µL of 1x Wash Solution. Seventhly, 100 µL of 1x Conjugate was added to each well. Eighthly, the plate was covered with aluminium foil and incubated for 30 minutes ± 3 minutes at 21 °C ± 5 °C.

Ninthly, after incubation, the wells were emptied. Each well was washed three times with at least 200 µL of 1x Wash Solution. Tenthly, 100 µL of Substrate Solution was added to each well. Eleventhly, the plate was covered with aluminium foil and incubated for 15 minutes ± 2 minutes at 21 °C ± 5 °C in a dark place. Twelfthly, after incubation, 100 µL of Stop Solution was added to each well in the same order as in the tenth step to stop the reaction. Lastly, the optical density (O.D.) was measured at 450 nm and recorded.

Prior to result interpretation, the ELISA assay was validated. Validation criteria were met if the mean O.D. of the Positive Control exceeded 0.350 and the ratio of the mean O.D. values of the Positive to Negative Control was greater than 3. After validation, S/P (%) was calculated using the formula below (3.3) to determine the status of each sampled goat for *T. gondii* antibodies as positive, doubtful, or negative (Table 3.2).

$$\text{Formula to calculate S/P \%} = (\text{OD sample} - \text{OD}_{NC}) / (\text{OD}_{PC} - \text{OD}_{NC}) \times 100 \qquad (3.3)$$

Table 3.2: Interpretation of *T. gondii* status by S/P %

Result	Status
S/P % ≤ 40%	Negative
40% < S/P % < 50%	Doubtful
S/P % ≥ 50%	Positive

3.7 Data Collection

Data on potential risk factors were collected through physical examination (age and sex) and an interviewer-administered questionnaire given to former researchers and related farm workers. The questionnaire covered herd management practices, including rearing system, animal source, water source, herd size, and presence of small mammals (Figure 3.2). The results of the serological detection of *T. gondii* were analyzed with a plate reader. All information was recorded manually and organized in Microsoft Excel.

QUESTIONNAIRE

1. **Types of rearing system in the goat farm?**

   ☐ Intensive ☐ Semi-intensive

2. **Source of animals in the farm?**

   ☐ Local ☐ Import

3. **Types of water source used for farm animals?**

   ☐ Underground water (*air boring*)

   ☐ River water

   ☐ Commercial water (*Air Kelantan Sdn. Bhd*)

   ☐ Others

4. **Size of goat's herd in the farm?**

   ☐ <100 ☐ >100

5. **Presence of small mammals like cats and rodents in the farm?**

   ☐ Yes ☐ No

Figure 3.2: A questionnaire conducted in this study

3.8 Data Analysis

The categorical data will be summarized using the frequency table and bar chart, presenting both frequencies and percentages, as part of the descriptive analysis. Subsequently, as part of the inferential analysis, the data will be analyzed using odds ratios, 95% confidence intervals, and P-values in IBM SPSS Statistics 31 to assess the prevalence and associated risk factors.

CHAPTER 4

RESULT AND DISCUSSION

4.1 Results

In this research, 80 serum samples from goats were obtained across four farms in Kelantan: 36 from Farm A, 15 from Farm B, 14 from Farm C, and 15 from Farm D. The Indirect ELISA results showed that 6.25% (5/80) of the goat samples were seropositive, indicating a low seroprevalence of *T. gondii* in the local goat population of Kelantan (Table 4.1). The breakdown of the results is demonstrated in Figure 4.1.

Univariate analysis using Fisher's Exact test showed no significant correlations between age ($p>0.05$), sex ($p>0.05$), rearing system ($p>0.05$), source of animals ($p>0.05$), or herd size ($p>0.05$) and *T. gondii* seropositivity, indicating that the observed differences were likely due to random fluctuations instead of being a genuine effect of these factors. Among the factors analyzed, only the farm's water source ($p<0.05$) showed a statistically significant association (Table 4.2). Small mammals, including cats and rodents, were present on all selected farms. Therefore, the presence of small mammals was excluded as a risk factor in this study.

Table 4.1: Seroprevalence of *T. gondii* among local goats in Kelantan in this study

Farm	Total number of samples (n)	Positive (n)	Negative (n)
A	36	1	35
B	15	0	15
C	14	4	10
D	15	0	15
Total (n)	**80**	**5**	**75**
Total Percentage	**100 %**	**6.25 %**	**93.75 %**

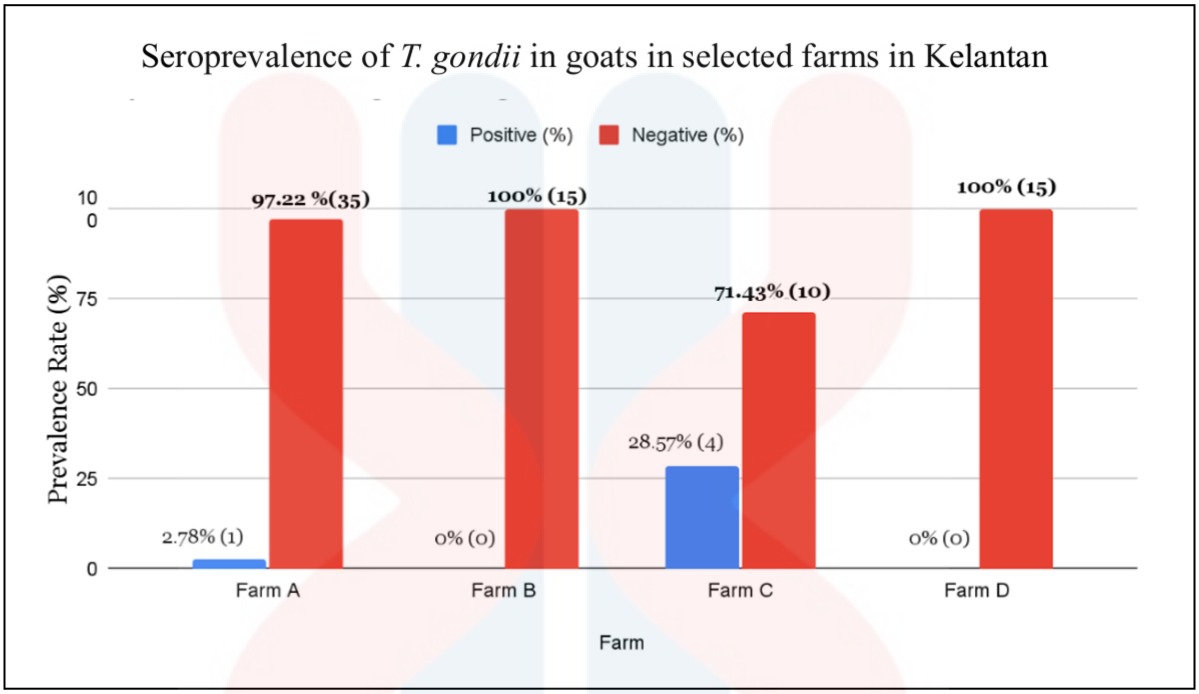

Figure 4.1: Bar chart of seroprevalence of *T. gondii* among local goats in Kelantan in this study

Table 4.2: Relationship between *T. gondii* seropositivity and the potential risk factors

Variables	**Tested (n)**	**Positive (n)**	**Total Seropositive Level (%)**	**p**	**OR**	**95% CI**
Age (Years)				0.076		
<1	8	2	25.000		7.667	1.065 - 55.202
1-6	72	3	4.200			
Sex				1.000		
Male	27	2	7.400		1.333	0.209 - 8.501
Female	53	3	5.700			
Rearing System				0.055		
Intensive	51	1	2.000			
Semi-intensive	29	4	13.800		8.000	0.849 - 75.394
Source of Animals				0.151		
Local	50	5	10.000		-	-
Import	30	0	0		-	-
Water Source				0.006[a]		
Air Kelantan	36	1	2.800			
Air Kelantan + Underground Water	15	0	0		-	-
Underground Water	14	4	28.600	14.000[b]	1.402	139.812
River Water	15	0	0		-	-
Herd Size				0.055		
<100	51	1	2.000			
>100	29	4	13.800		8.000	0.849 - 75.394

[a]*p value < 0.05 is considered significant.*

[b]*Odds ratio of water source (underground water as compared to Air Kelantan).*

4.2 Discussion

The study findings indicated a low seroprevalence of *T. gondii* among the goats sampled in Kelantan, with 5 out of 80 samples (6.25%) showing seropositivity for *T. gondii* antibodies. The results of this study were lower than the recent reports of caprine toxoplasmosis in Selangor, Malaysia, in 2022 (69.8% by ELISA; Sadiq *et al*., 2023) and in 2020 (55% by ELISA; Hamid *et al*., 2020). The seroprevalence rates reported in the neighboring countries like Thailand (27.9% by LAT, and 28.5% by ELISA), and Indonesia (93.9% by MAT) were found to be higher than those of our current study (Jittapalapong *et al*., 2004; Udonsom *et al*., 2021; Fatmawati *et al*., 2023). Our result was also below the estimated global average prevalence of 27.49% (Rodrigues *et al*., 2022). However, a lower seroprevalence than that reported in our current study, based on ELISA, was reported in Nigeria (4.6%) and Korea (5.1%) (Kamani *et al*., 2010; Jung *et al*., 2014). Variations between countries and regions may be influenced by factors such as ecological conditions, climate, diagnostic techniques employed, and the population density of small mammals (including cats and rodents) in the study areas (Jung *et al*., 2014). Moisture is important for *T. gondii* sporulation and survival. Previously reported seroprevalence of *T. gondii* is higher than that of our present study, which may be attributed to the longer rainy season, for instance, in Selangor (9 months) as compared to Kelantan (3 months). A similar observation was reported in Thailand (Jittapalapong *et al*., 2004), Indonesia (Fatmawati *et al*., 2023), and Nigeria (Kamani *et al*., 2010), where higher prevalence was attributed to humid areas compared to dry regions.

Furthermore, our results, which indicated a prevalence lower than the global average, may reflect a comparatively smaller population of feral and free-roaming owned cats in the study areas. Besides that, the restricted movement and limited grazing practices in the farm management system likely helped reduce goats' exposure to environments contaminated with oocysts shed by cats, thereby preventing *T. gondii* infection (Jung *et al*., 2014). However, small mammals (cats and rodents) were present in all four selected farms in this study area. Thus, there remains a potential risk of *T. gondii* transmission among goats in Kelantan since a single cat can excrete up to 10 million resistant oocysts daily, which may contaminate the environment (Lee *et al*., 2011).

The only factor significantly linked to *T. gondii* seropositivity in goats in this study was the water source ($p<0.05$). This was aligned with several studies that identified water source as a significant factor that influences the detection of *T. gondii* IgG in goats (Fatmawati *et al*., 2023; Masombuka *et al*., 2024). Oocysts present in feces act as intermediates in the transmission of *T. gondii*, and water is a suitable medium for their survival. The oocysts capable of persisting in water are the sporulated forms (Mufa *et al*., 2025). Soil contaminated with cat feces serves as the main reservoir for *T. gondii* oocysts before they spread into the environment (Shapiro *et al*., 2019). The natural behavior of felids to bury their feces causes the oocysts to become embedded in the upper soil layers (Frenkel *et al*., 1975; Aramini *et al*., 1999). This habit may help prevent oocysts from being inactivated by solar UV exposure, extreme temperature changes, and drying (Kinsey *et al*., 2023). Additionally, oocysts can be passively carried by soil-dwelling organisms, such as coprophagous insects and earthworms, either on their surfaces or within their guts, thereby aiding in the distribution of oocysts in the soil (Frenkel *et al*., 1975; Ruiz & Frenkel, 1980). Besides biotic influences, factors such as soil composition and moisture significantly affect the survival of infectious oocysts in the soil. Together with rainfall intensity, these factors also influence the horizontal and vertical movement of oocysts within the soil and their eventual transfer to surface and groundwater (Shapiro *et al*., 2019). Even though the movement of oocysts from soil into water bodies dilutes their concentration, the low infectious dose required (as few as 1–10 oocysts) still poses a potential infection risk to animals and humans who consume contaminated water (Kinsey *et al*., 2023).

In this study, goats supplied with underground water had 1.4 times the likelihood of being seropositive with *T. gondii* in comparison to goats supplied with treated water (Air Kelantan). It is essential to implement preventive measures by ensuring that water is treated before being provided to livestock (Mufa *et al*., 2025). Preventing the cat from entering the farm area and regular cleaning of the water trough are equally important. Regardless of the water source, practices such as leaving water in troughs for several days and merely refilling them when they are empty, along with allowing cats access to the water, can increase the risk of *T. gondii* contamination (Hotea *et al*., 2021).

No significant relationship was observed between goat age and *T. gondii* seroprevalence (p>0.05). This finding contrasted with previous studies, which indicated that prolonged exposure to various risk factors may contribute to a higher prevalence of toxoplasmosis among adult goats (Jittapalapong *et al*., 2004; Sadiq *et al*., 2023). On the other hand, some studies also showed no significant differences in the seroprevalence of these parasites across age groups (Jung *et al*., 2014; Udonsom *et al*., 2021). Even though the age of goats did not show a significant correlation with *T. gondii* seropositivity in this research, the odds ratio for age was 7.7 when comparing goats below one year old with those aged between one and six years. This suggests that the age group may become a significant risk factor as the sample size increases.

The seroprevalence of *T. gondii* was similar in both male and female goats (p>0.05). Our result was contrary to findings reported in previous studies, which suggested that females are more prone to *T. gondii* infection than males because of hormonal variations, immunosuppression during pregnancy, stress from lactation, and other physiological changes (Jittapalapong *et al*., 2004; Rodrigues *et al*., 2022; Masombuka *et al*., 2024). However, one study by Kamani *et al.* found that sex did not significantly influence the seroprevalence of *T. gondii* in goats (Kamani *et al*., 2010), indicating that both sexes of goats have a similar risk of *T. gondii* infection.

Moreover, the statistical analysis conducted in the study revealed no significant correlation between the rearing system, source of animals, and herd size with the seroprevalence of *T. gondii* in goats (p>0.05). This was inconsistent with the findings of a recent study by Sadiq *et al*., which reported higher odds of seropositivity in goats raised under semi-intensive systems compared to intensive ones, in farms with large herd sizes (over 100 animals) compared to smaller farms, and in farms that used their own animals for replacement compared to those with multiple or external sources (Sadiq *et al*., 2023). However, in this study, goats reared in semi-intensive systems and from large herd sizes were 8.0 times more likely to be seropositive compared to goats reared in intensive systems and from small herd sizes, respectively. In this context, the rearing system and herd size are potential risk factors for toxoplasmosis in goats. The lack of a significant association between these two variables and seropositivity in the study may be due to the relatively small sample size. A systematic review and meta-analysis by Rodrigues *et al*. revealed that goats raised under extensive and semi-intensive management systems exhibited a higher seroprevalence of *T. gondii* compared to those in intensive systems, likely due to greater environmental exposure to oocysts (Rodrigues *et al*., 2022).

No clinical signs of toxoplasmosis were observed in the *T. gondii*–positive goats, which remained healthy throughout the entire sampling period. Studies have shown that *T. gondii* typically infects hosts without causing clinical signs and only rarely leads to severe illness in non-pregnant goats. It usually results in abortion and stillbirth in pregnant goats (Pavone *et al*., 2020). However, occasional cases of acquired toxoplasmosis in adult goats have been documented in the literature. The common clinical sign is a brief episode of fever and anorexia lasting three to ten days after infection (Stelzer *et al*., 2019). Although death in adult goats is uncommon, cases of disseminated acute toxoplasmosis have been reported, with the most frequent clinical signs including encephalitis, fever, diarrhea, dyspnea, and lethargy (de Moura *et al*., 2016). Therefore, the manifestation of clinical signs of toxoplasmosis in animals largely depends on the host's immune response and the pathogenicity of *T. gondii* (da Silva *et al*., 2013).

Furthermore, a positive ELISA result does not necessarily indicate active infection with *T. gondii*. It merely indicates exposure to this parasite. A definitive diagnosis of the infection requires direct detection of the parasite in blood or other clinical specimens. Molecular techniques are valuable for diagnosing primary, reactivated, or chronic cases of toxoplasmosis, as they can identify circulating parasites. Currently, DNA amplification–based molecular methods are the standard diagnostic tools for detecting potential *T. gondii* infections in small ruminants (Koonyosying *et al*., 2025). Measurement of IgG avidity of *T. gondii*-specific antibodies can also be applied to distinguish between recent and past infections (Holec-Gasior & Sołowinska, 2022).

Toxoplasmosis in goat populations significantly affects public health and the advancement of goat farming. An outbreak of caprine toxoplasmosis had been reported in Brazil in 2018, which caused reproductive losses of 60% of the affected females (Neto *et al*., 2018). The protozoa is among the three major pathogens—along with *Salmonella* and *Listeria*—responsible for about 70% of foodborne illness-related deaths in the United States, primarily resulting from the consumption of raw or undercooked meat contaminated with *T. gondii* (Guo *et al*., 2015). Although the goat industry in Malaysia remains relatively small, it maintains a steady demand, particularly among Muslim and Hindu communities. Muslims commonly slaughter goats and distribute the meat (mutton) during significant religious occasions such as Aqiqah ceremonies to commemorate births. Meanwhile, Hindus, who abstain from consuming beef, regard mutton to be their primary source of red meat. In 2023, mutton consumption, encompassing both goat and sheep meat, rose to 1.4 kg per capita, up from 1.0 kg in 2022 (Malaymail, 2025). This will indirectly increase the risk of foodborne transmission of toxoplasmosis from infected goat meat, which is particularly significant in Kelantan, where the population consists of about 95.5% Muslims and 0.2% Hindus (Department of Statistics Malaysia, 2025). Thus, as a notifiable disease in Malaysia, DVS should be informed of the positive toxoplasmosis cases, even though this study recorded a low seroprevalence of *T. gondii* in goats. To our knowledge, the DVS has not developed any active surveillance programs for toxoplasmosis in goats. Therefore, notification of this finding can assist DVS in formulating future plans to screen goat serum samples and meat products in Kelantan, as well as in developing strategies aimed at achieving zero *T. gondii* prevalence in goats to ensure food safety.

Limitations such as a small sample size, limited variability among sampled farms, and the inclusion of only a single species restrict the generalizability of the findings to the broader goat population in Kelantan. The limited size of samples in this study may reduce statistical power, restricting the ability to detect trends and associations between *T. gondii* seroprevalence and its potential risk factors. For example, age, rearing system, and herd size may become significant risk factors as the sample size increases. Moreover, the study's geographic limitation, focusing on only two districts in Kelantan, may reduce the potential to extrapolate the findings to the broader agricultural sector across Malaysia.

CHAPTER 5

CONCLUSION AND RECOMMENDATION

5.1 Conclusion

Serological testing using indirect ELISA confirmed the presence of *T. gondii* antibodies in goat serum samples from selected farms in Kelantan, Malaysia. This study revealed a low seroprevalence of *T. gondii* among local goats from the selected farms in Kelantan, with water source being the sole significant risk factor. This emphasizes the importance of using treated water in goat farming. No significant relationships were observed between goats' sex, age, rearing system, source, or herd size and *T. gondii* seroprevalence in this study.

5.2 Recommendation for Future Studies

These findings suggest that future studies should include a larger sample, a broader range of animal species, and ruminant farms from multiple districts in Kelantan to achieve a more thorough understanding of *T. gondii* epidemiology in livestock in Kelantan. Additionally, indirect ELISA should be used as the principal serological method in future studies, given its high accuracy in detecting *T. gondii* antibodies and its simplicity for mass-screening surveys. DVS Malaysia should conduct active surveillance of toxoplasmosis in livestock to evaluate the potential risk of disease outbreaks and public health issues. Since cats were present on all sampled farms in this study, samples (e.g., feces and serum) should be collected from the farm cats to assess their potential association with *T. gondii* infection in ruminant livestock.

APPENDICES

Figure 6.1: Group photo after discussion of FYP proposal with supervisor and co-supervisor

Figure 6.2 The team performing blood sampling at UMK Teaching Farm

Figure 6.3: Jugular venipuncture of a goat

Figure 6.4: ID Screen Toxoplasmosis Multi-species Indirect ELISA

Figure 6.5: Addition of Dilution Buffer 2 into each microwell of the test kit

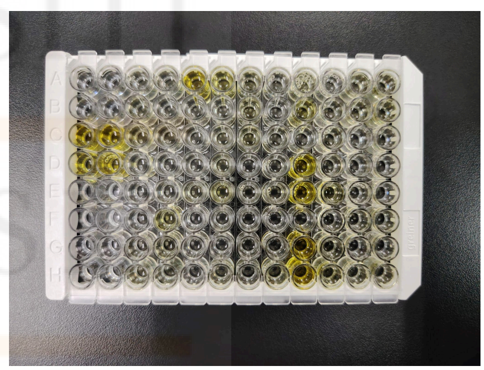

Figure 6.6 Toxoplasmosis Indirect ELISA result

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