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# SEROLOGICAL DETECTION AND RISK FACTORS OF AKABANE VIRUS IN GOATS FROM SELECTED FARMS IN KELANTAN, MALAYSIA

By

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

FACULTY OF VETERINARY MEDICINE
UNIVERSITI MALAYSIA KELANTAN

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**SEROLOGICAL DETECTION AND RISK FACTORS OF AKABANE VIRUS IN GOATS FROM SELECTED FARMS IN KELANTAN, MALAYSIA**

## ABSTRACT

The Akabane virus, a member of the *Orthobunyavirus* genus within the *Bunyaviridae* family, belongs to the Simbu serogroup and is classified as an arbovirus. It primarily replicates in blood-feeding arthropods and is transmitted through the bites of biting midges. The virus is known to infect a diverse range of domestic and wild ruminants, with symptomatic infections observed in cattle, goats, sheep and swine. This thesis presents the serological detection of Akabane virus (AKAV) antibodies among goats in Kelantan, Malaysia, to evaluate the occurrence and associated risk factors of AKAV infection within the goat population. Some studies on the AKAV, including its detection, genetic analysis and case reports, have been conducted across Asia, particularly in countries like China, Japan and Indonesia, indicating that the virus has spread into Southeast Asia. However, no prevalence studies have been conducted on this virus in livestock animals in Malaysia. Therefore, this study aimed to examine the presence of AKAV infection in serum samples from goats in selected districts of Kelantan, Malaysia and to determine the seroprevalence of AKAV antibody titers. Forty (n=40) goats were randomly selected from farms in Kota Bharu and Bachok districts and screened for AKAV antibodies using a competitive ELISA assay. Blood samples were collected via the jugular vein, processed and analyzed for antibody detection. The results showed that only two goats from Bachok tested positive for AKAV antibodies, indicating a district-specific prevalence of 8.7 %, while no positive cases were identified in Kota Bharu. The overall prevalence across both districts was 5.0 %, suggesting a low-level presence of the virus in Kelantan's goat population. Risk factors including sex, age, farm management practices and vector control were hypothesized to influence AKAV infection rates, highlighting the need for further research to understand these associations better. This study underscores the potential risk of AKAV introduction and circulation within Malaysian goat populations due to regional livestock importation and vector species in tropical climates.

Keywords: Akabane virus, Akabane disease, District-specific prevalence, competitive ELISA.



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**PENGESANAN SEROLOGI DAN FAKTOR RISIKO VIRUS AKABANE PADA KAMBING DARIPADA LADANG TERPILIH DI KELANTAN, MALAYSIA**


## ABSTRAK

Virus Akabane di bawah genus *Orthobunyavirus* dalam keluarga *Bunyaviridae*, tergolong dalam serogrup Simbu dan diklasifikasikan sebagai arbovirus. Virus ini terutamanya mereplikasi dalam serangga penghisap darah dan disebarkan melalui gigitan midges. Virus ini diketahui menjangkiti pelbagai jenis domestik ruminan dan ruminan liar, dengan jangkitan simptomatik dilaporkan pada lembu, kambing, biri-biri dan khinzir. Tesis ini membentangkan pengesanan serologi antibodi virus Akabane (AKAV) pada kambing di Kelantan, Malaysia, dengan tujuan menilai kejadian dan faktor risiko yang berkaitan dengan jangkitan AKAV dalam populasi kambing. Pelbagai kajian mengenai AKAV, termasuk pengesanan, analisis genetik dan laporan kes, telah dijalankan di Asia, terutamanya di negara seperti China, Jepun, dan Indonesia, yang menunjukkan penyebaran virus ini ke Asia Tenggara. Namun, tiada kajian ke atas virus ini dijalankan di Malaysia. Oleh itu, kajian ini dijalankan untuk menyiasat kehadiran jangkitan AKAV dalam sampel serum kambing di daerah terpilih di Kelantan, Malaysia, serta menentukan seroprevalen titer antibodi AKAV. Sebanyak empat puluh (n=40) kambing telah dipilih secara rawak dari ladang di daerah Kota Bharu dan Bachok dan disaring untuk antibodi AKAV menggunakan ujian ELISA kompetitif. Sampel darah diambil melalui vena jugular, diproses dan dianalisis untuk pengesanan antibodi. Hasil kajian menunjukkan dua kambing dari Bachok positif untuk antibodi AKAV, menunjukkan prevalen spesifik daerah sebanyak 8.7 %, manakala tiada kes positif yang dikenal pasti di Kota Bharu. Prevalen keseluruhan di kedua-dua daerah adalah 5.0 %, yang mencadangkan kewujudan virus ini pada tahap rendah dalam populasi kambing di Kelantan. Faktor risiko termasuk jantina, umur, amalan pengurusan ladang dan kawalan vektor dihipotesiskan mempengaruhi kadar jangkitan AKAV, menekankan keperluan untuk penyelidikan lanjut bagi memahami hubungan ini dengan lebih mendalam. Kajian ini menekankan potensi risiko penyebaran AKAV dalam populasi kambing di Malaysia disebabkan oleh importasi ternakan dari rantau lain serta kehadiran spesies vektor di iklim tropika.



Kata Kunci: Virus Akabane, penyakit Akabane, prevalen spesifik daerah, ELISA kompetitif.



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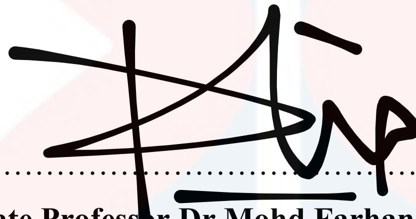

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## LIST OF ABBREVIATIONS

| | |
|---|---|
| **AKAV** | Akabane Virus |
| **Ods** | Optical Densities |
| **ssRNA** | Single Stranded Ribonucleic Acid |
| **NS** | Non-Structural |
| **c-ELISA** | Competitive Enzyme-Linked Immunosorbent Assay |
| **Mab** | Monoclonal antibody |
| **HRP** | Horseradish Peroxidase |
| **TMB** | Tetramethylbenzidine |
| **Cpos** | Positive Control |
| **Cneg** | Negative Control |

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# CHAPTER 1

## INTRODUCTION

The Akabane virus (AKAV) is classified within the *Orthobunyavirus* genus in *Bunyaviridae* family, specifically falling under the Simbu serogroup among the genus's 18 serogroups (Mohamed *et al.,* 2009). The virus is identified as an arbovirus, primarily replicating in blood-feeding arthropods and transmitted through bites from midges of the *Culicoides* genus. Most viruses within the Simbu serogroup have been discovered from arthropod vectors (Saeed *et al.,* 2001). AKAV infection can infect a diverse array of wild ruminants and wildlife animals. Symptomatic infections of AKAV have been observed in goats, cattle, swine and sheep. Conversely, deer, camel, buffalo, horse and wild boar are examples of species that are asymptomatically but tested positive serologically for antibodies against the Akabane virus (Kirkland, 2015).

AKAV is the agent that causes Akabane disease, which is also known as enzootic arthrogryposis-hydranencephaly, affecting cattle, sheep and goats (Yanase *et al.,* 2020). The term 'Akabane Disease' has been employed to describe the clinical condition resulting from an *in utero* infection by the AKAV (Kirkland, 2015). This nomenclature has been used since the virus was first isolated from *Aedes vexans* and identified in Akabane, Japan in 1959 (Oya *et al.,* 1961).

Akabane disease manifests through two primary modes of transmission, where vertical transmission plays a crucial role in its epidemiology distribution (Spicler, 2017). This mode is particularly noteworthy due to the ability of the viruses from a non-immunized dam to infect the placenta, affecting the developing fetus directly and resulting in congenital abnormalities.

Simultaneously, horizontal transmission depends on *Culicoides spp.* biting midges, primarily of the *Culicoides* genus, act as the AKAV's primary vectors. Geographically, the distribution of these vectors varies, with *Culicoides oxystoma* being prevalent in Japan, *C. brevitarsis* in Australia and *C. imicola* predominantly observed in Africa (Özsoy & Yildirim, 2021). Even though reports suggest that *Aedes spp.* and *Culex spp.* can carry the Akabane virus,

Culicoides biting midges are currently acknowledged as the primary vectors for the virus (Kirkland, 2015). The interplay between vertical and horizontal transmission underscores the complex epidemiology of AKAV.

Akabane disease is classified as a teratogenic pathogen (Aiello *et al.,* 2016). It has the potential to impact newborn animals relying on the gestational stage at which the dam is infected, resulting in outcomes like abortions, stillbirths and a range of congenital deformities in neonates (Alsaad *et al.,* 2017). Blindness, congenital arthrogryposis and hydranencephaly are prevalent clinical manifestations documented in offspring affected by AKAV infection. The majority of afflicted neonates either succumb to the disease or necessitate euthanasia. These factors significantly contribute to the global recognition of Akabane disease, given its severe impact on economic livestock losses.

The AKAV survives in tropical and subtropical regions and has been documented in various locations, including Australia, Southeast Asia, Africa and the Middle East (Spicler, 2017). Malaysia, situated in Southeast Asia, experiences a consistent temperature range of 21°C to 35°C, high humidity and 80% average annual rainfall (Malaysian Meteorological Department, 2024). Given that the Akabane virus is known to be transmitted by biological vectors, the warm and humid conditions during late spring and early summer create a favourable environment for an increased presence of vectors (Kirkland, 2015). This implies a higher probability of heightened Akabane virus vector abundance, aligning with Malaysia's tropical climate.

The disease investigation of Akabane disease can be achieved using diagnostic tools such as viral isolation and identification via RT-PCR, immunohistochemistry, or genetic analysis. However, serological techniques are the most practical approach for confirming this disease (Oluwayelu *et al.,* 2016). In Malaysia, detecting Akabane Disease in goat species using any diagnostic workup is scarce. Therefore, this study will be conducted to detect the serological detection of the AKAV using goat blood samples and serve as a reference for understanding the status of Akabane disease within caprine species in Kelantan, Malaysia.



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## 1.1 Research Problem

In the context of Akabane Disease in Malaysia, specifically within goat species, the current diagnostic work up for investigating this disease has never been established. Our country still lacks seroprevalence studies to understand the extent of Akabane virus exposure in goat populations. Even though the most practical diagnostic workup (ELISA) can be done in a short period, our country has not performed it due to Malaysia being the non-endemic Akabane region status. This issue poses a significant constraint in surveillance and diagnostic efforts for Akabane Disease in Malaysia. This scarcity impedes the early detection and understanding of the prevalence of the Akabane virus in the local goat population.

The absence of active surveillance and diagnostic protocols, specifically for the Akabane virus, not only constrains diagnostic capabilities but also poses a potential risk to the health of livestock. This is because Akabane Disease has the potential to lead to severe economic losses due to its negative impact on livestock's reproductive system.

In the absence of precise data on AKAV prevalence in goat populations, the development and implementation of effective control strategies become challenging. Thus, the study aim to serologically determine the presence of AKAV infection and establish information regarding the occurrence of the virus in the goat population in Kelantan, Malaysia. These data findings can serve as the initial alert for the potential occurrence of this disease in our country.

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## 1.2 Research Questions

I. What is the occurrence of Akabane virus infection in goat populations in Kelantan, Malaysia?
II. What are the contributing factors to the risk of the Akabane virus infection in Kelantan, Malaysia?

## 1.3 Research Hypothesis

I. There is a low to moderate serological occurrence of the Akabane virus in goat species in Kelantan, Malaysia.
II. Sex, age, farm management type and vector control management contribute to the risk factors for Akabane virus infection.

## 1.4 Research Objectives

I. To assess the occurrence of antibody titers against the Akabane virus within the goat population on selected farms in Kelantan, Malaysia.
II. To determine the association between risk factors and the occurrence of the Akabane virus among the goat population in Kelantan, Malaysia.

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# CHAPTER 2

# LITERATURE REVIEW

## 2.1 Classification and Morphology of AKAV

The taxonomy of the AKAV is grouped under the genus *Orthobunyavirus* in the family *Bunyaviridae*. This family holds the distinction of being the largest among mammalian viruses (Soldan & González-Scarano, 2014). Regarding serology, the Akabane virus is categorized within the Simbu serogroup of the *Orthobunyavirus* genus.

This virus is a unique creature characterized by a spherical-shape, lipid icosahedral enveloped containing tripartite and negative-sense RNA genome (Elliott, 2014). The tripartite region is encoded with 3 different protein genomes which are small (S), medium (M) and large (L) (Elliott, 2014). These viral genomes are enveloped by prominent glycoprotein peplomers responsible for integral functions in viral attachment, penetration and replication within host cells.

In the context of viral replication, the AKAV undergoes replication within the cytoplasm of host cells (Ogawa *et al.,* 2007). There, the transcription and translation processes of virion RNA, result in the generation of new virions. These new virions are subsequently released from host cells through either lysis or exocytosis, allowing them to infect new host cells.

## 2.2 Vector and Geographic Distribution of AKAV

The AKAV has a global distribution, especially in countries with tropical and subtropical climates (Aiello *et al.,* 2016). This arbovirus versatile utilization of the arthropod vector, *Culicoides spp.* enhances the likelihood of the virus expanding its prevalence and geographic reach under suitable environmental conditions. The commonly acknowledged belief identifies Culicoides biting midges as the primary carriers of the virus, despite reports suggesting the potential involvement of *Aedes spp.* and *Culex spp.* in transmitting the Akabane virus (Kirkland, 2015). In tropical regions, biological vectors are consistently present throughout the year, while in most temperate regions, the adult Culicoides population significantly declines or becomes nearly

nonexistent during the dry season (Gao *et al.,* 2022). However, Culicoides and mosquito species exhibit a broad distribution from low-latitude valleys to high mountain plains (Duan *et al.,* 2019).

The AKAV was initially identified in Japan and it was subsequently detected in Australia, Kenya and South Africa. AKAV infections have been extensively documented in both Australian and Asian nations, ranging from tropical Indonesia to temperate regions like Japan and Korea (Gao *et al.,* 2022). The existing endemic countries with the presence of AKAV such as Australia, could lead to significant economic losses in livestock and forecast the likelihood of future outbreaks in Egypt (Samy Metwally *et al.,* 2023). This mirrors occurrences in the Republic of Korea (Oem *et al.,* 2012) and Iraq (Alsaad *et al.,* 2017).

Furthermore, there are documented cases of the AKAV being isolated in West Java, Indonesia (Edi *et al.,* 2017). Thus, there is a considerable likelihood that the Akabane virus is actively circulating within the tropical zones of Malaysia, despite the absence of reported cases or studies related to the isolation and characterization of the Akabane virus within the caprine species. Besides, commencing in 2016, Malaysia's sustained reliance on importing live goat meat from diverse sources, including Australia, New Zealand, Africa and India (Mat Amin *et al.,* 2022). This reveals the potential introduction of Akabane virus-infected goats to Malaysia, particularly given Australia's endemic status for the Akabane virus.

**2.3 Host Range and Clinical Manifestation of AKAV**

AKAV exhibits infection in a diverse range of domesticated ruminants and wildlife species, with high antibody prevalence reported in endemic areas among cattle, buffalo, sheep, goats and horses. While the AKAV can infect a broad spectrum of ruminant species, notable disease outbreaks primarily occur in cattle (Aiello *et al.,* 2016). The reproductive and musculoskeletal systems are commonly affected in susceptible hosts during AKAV infection.

In infected cows, the clinical signs are revealed depending on the gestational stage (Spicler, 2017). Initially, infections occurring during the third month of pregnancy lead to a relatively low disease incidence. Newborn calves from these pregnancies are often late in gestation and exhibit signs of acute encephalitis, such as flaccid paralysis of the legs, blindness and difficulty suckling.



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Secondly, calves infected in the fifth or sixth month of gestation may be born with arthrogryposis, displaying deformities on limbs and the spinal cord, including multiple lesions on joints in all limbs and lordosis. In the final stage, during the last trimester of pregnancy, infected cows may experience a catastrophic outcome, resulting in abortion, dystocia and stillbirths due to the severity of cerebral lesions of newborns either hydranencephaly or cerebellar hypoplasia (Kirkland, 2015).

For goats, recent study reported that doe who are pregnant during vector (*Culicoides spp.*) season would be prone to be infected by the AKAV between days 28 to 56 of gestation (Aiello *et al.,* 2016). However, the sequential progression of newborn defects in AKAV-infected cattle could not occur in the goat species due to goats having a shorter period. The common likely clinical signs observed in infected goats include a spectrum of musculoskeletal and central nervous system defects. Affected kids may exhibit severe hydranencephaly and arthrogryposis, with the same possibility of conditions affecting the infected AKAV calves (Kirkland, 2015).

**2.4 Pathogenesis of AKAV**

The Akabane virus is the causative agent for Akabane disease, commonly called the enzootic arthrogryposis-hydranencephaly syndrome. The susceptible hosts for this virus are mainly livestock animals such as cattle, sheep and goats (Yanase *et al.,* 2020). Two modes of transmission are implicated in eliciting clinical signs in the host animal during AKAV infection: the horizontal route and the vertical route.

Horizontal transmission of the AKAV involves transmitting between infected and healthy hosts of the same species through the intermediary of Culicoides midges. An infected animal can introduce the virus into the environment in tropical and subtropical areas, where Akabane is prevalent (Aiello *et al.,* 2016). When Culicoides midges bite the infected host, they can acquire the virus. The Akabane virus then multiplies and persists within the Culicoides. When these infected Culicoides bite a healthy host of the same species, they can transfer the virus to the susceptible host, facilitating its horizontal transmission. This cycle sustains the dissemination of the Akabane virus within the host population.

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Vertical transmission is also a critical factor in the transmission of the AKAV, especially when the dam lacks immunity to the Akabane virus (Spicler, 2017). When the dam lacks immunity, the virus is vertically transmitted from the non-immune dam to the fetus through the placenta. This process leads to viremia in the newborn and culminates in the development of congenital anomalies. This specific mode of AKAV transmission has significantly contributed to the prevalence and impact of Akabane disease within susceptible animal populations.

Viremia of AKAV within infected host circulation typically initiates within one to six days of AKAV infection, lasting for four to six days before the detection of antibodies that indicate viral clearance (Kirkland, 2015). Serological tests can identify these antibodies approximately 14 days post-infection. Notably, the virus may persist for an extended period in the developing fetus. Clinical manifestations are typically not evident for several months, only becoming apparent when an affected fetus is either aborted or reaches full term.

During the clinical period, the Akabane virus displays clinical signs in the newborn fetus, highlighting its potent teratogenic effects primarily on the developing fetus (Kirkland, 2015). When a dam is infected with the virus during pregnancy, it can lead to severe fetal defects, particularly affecting the limbs, often resulting in arthrogryposis and the central nervous system. Brain defects include small cystic issues like porencephaly and hydranencephaly (George & Standfast, 2019). Newborns infected around the time of birth or shortly after may experience encephalitis due to Akabane virus infection. These manifestations highlight the significant impact of the virus on the fetal clinical period.

**2.5 Diagnosis of AKAV**

Detecting Akabane disease necessitates a comprehensive approach, beginning with a thorough history from the owner, especially when congenital anomalies in newborn livestock arise on farms in late spring and early summer (Kirkland, 2015). A suspected case of Akabane disease needs a thorough physical examination of the infected dam. The examination of gross pathology, particularly abnormalities within the central nervous system (CNS), is carefully investigated. The common findings of post-mortem examinations of infected fetuses are hydranencephaly and arthrogryposis providing evidence of Akabane disease (Aiello *et al.,* 2016). Regarding AKAV-



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infected dams, they exhibit tendencies characterized by abortion and retained fetal membranes, contributing to the AKAV infection diagnostic process.

The diagnostic workup encompasses several key components to rule in the Akabane disease. Serological detection involves identifying specific antibodies in the blood or fluids of affected neonates. Fetuses and neonates deprived of colostrum are tested for elevated IgG levels, indicating the presence of an infectious agent (Wanner & Husband, 1974). The common Akabane viral detection for serological methods include virus neutralization tests and enzyme-linked immunosorbent assays (ELISA). Besides, the alternative tests reported to be utilized include agar gel immunodiffusion, hemagglutination inhibition and hemolysis inhibition assays (Spicler, 2017).

Lastly, confirmatory testing for the Akabane virus involves virus isolation and identification through Polymerase Chain Reaction (PCR). Real-time PCR is particularly useful for detecting residual AKAV Ribonucleic acid (RNA) in the affected tissues of neonates (Stram *et al.,* 2004). Swabs taken from the surface of cotyledons of the placenta may provide positive results (Kirkland, 2015). Thus, the diagnostic process for Akabane disease involves a comprehensive evaluation, combining historical information, physical examinations, post-mortem assessments and a range of diagnostic tests adapted to different stages of pregnancy and clinical symptoms.

### 2.6 Treatment and Prevention Management of Akabane Virus

There is no specific treatment available for animals affected by Akabane disease (Aiello *et al.,* 2016). Consequently, veterinarians must educate farmers on effective control management for livestock animals at risk of Akabane virus infection.

Education becomes the first line of defense, with farmers needing to recognize symptoms, particularly dystocia in animals. A quick response involves immediate consultation with veterinarians for diagnosis. Suspected cases should be reported promptly to state or federal veterinary authorities, facilitating timely control measures and preventing further spread.

Another crucial aspect is the implementation of vector control measures. This is critical to limiting the population of Culicoides, the primary vectors of the Akabane virus. A practical



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example of managing herds is through fogging, which involves dispersing pyrethroids and pyrethrin as the active ingredients of fogging. Farmers can choose substances targeting Culicoides to prevent their growth.

Furthermore, importing animals from AKAV Endemic-Free Areas can be a prudent strategy (Kirkland, 2015). Farmers may choose to import breeds of animals from regions free of endemic Akabane virus. This precautionary measure aims to prevent the introduction of the virus to the farm through the acquisition of new livestock.

Moreover, the introduction of animals from non-endemic to endemic areas is essential. Controlled introduction involves moving animals from non-endemic to endemic areas before their first breeding, allowing them to develop immunity to the virus before potential exposure (Kirkland, 2015). This emphasizes the need for careful timing.

Lastly, vaccine production is a crucial component of Akabane virus control. While some countries, such as Japan, have utilized live attenuated vaccines (Kurogi *et al.,* 1979), ongoing research is essential. This is due to reported antigenic differences between vaccine strains and viruses causing postnatal encephalomyelitis in newborns (Kirkland, 2015). These collective measures contribute to a comprehensive and effective livestock control strategy against the Akabane virus.

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# CHAPTER 3

# MATERIALS AND METHODS

## 3.1 Ethical Considerations

This study obtained ethical approval from the Institutional Animal Care and Use Committee (IACUC), Faculty of Veterinary Medicine, Universiti Malaysia Kelantan (Appendix A) under approval code (UMK/FPV/ACUE/FYP/009/2024).

## 3.2 Study Area and Target Population

The target population for this study was goats in Kelantan, Malaysia. Forty goats (n=40) were randomly selected from the Bachok and Kota Bharu districts, as shown in Table 3.1.

Table 3.1: Samples Collected According to Districts in Kelantan

| No. | Districts | Number of samples collected |
|---|---|---|
| **1.** | Kota Bharu | 23 |
| **2**. | Bachok | 17 |
| | **TOTAL** | **40** |

## 3.3 Factors Determination for Each Sampled Goat

The factors determined for each sampled goat were recorded, including sex (categorized as male or female) and age (classified as either below 1 year or above 1 year). Farm management practices were assessed and categorized into intensive or semi-intensive systems. Vector control management was evaluated by documenting the implementation of preventive measures, such as fogging activities and the use of mosquito killer lamps, to mitigate vector-borne risks. These parameters were meticulously recorded to facilitate a comprehensive analysis of their potential impact on the study outcomes.

### 3.4 Sample Collection and Procedure

Each goat was examined for its vital signs, including body temperature, respiratory and heart rates. Their Identification Number (ID) were determined before blood sampling. The blood samples were collected using a ¾-inch 21-gauge vacutainer needle via the jugular vein method. A total of 5 ml of blood was collected from each goat. The blood samples were transferred into plain red tubes to allow serum separation. Each tube was labelled with an ID number using a marker pen. After that, the samples were kept chilled in a cold box packed with ice packs during transportation. Upon arrival at the laboratory, the samples were stored in a chiller at 2 to 8 °C.

### 3.5 Sample Processing

The next step involved the removal of blood clots through centrifugation at a speed ranging from 2500 to 3000 rpm using a centrifuge machine. Following centrifugation, the serum was carefully extracted using a Pasteur pipette. The serum was then transferred into microcentrifuge tubes labeled with the corresponding identification number for accurate record-keeping. The microcentrifuge tubes containing the serum were sealed with wax tape to prevent evaporation during storage. Afterward, the processed samples were slated to be stored in a refrigerated environment at temperatures between 2 to 8 °C for up to 5 days.

### 3.6 Serological Detection by ELISA

A commercial ELISA kit (ID vet, Grabels, France) was used to perform a competitive ELISA assay to detect antibody titers against Akabane virus (AKAV) in serum samples. The instructions indicated that the ELISA kit is highly sensitive to AKAV antibodies. It demonstrated a strong correlation (96.52%) with the Virus Neutralisation Test (VNT), as validated by (Li *et al.,* 2019). In that study, a 93.5 % correlation was observed and no cross-reactivity with other viruses in the Bunyaviridae family was detected.

The ELISA kit targets anti-g1 antibodies directed against the Akabane virus. The microwells were pre-coated with purified AKAV. After adding the test samples and controls to the 96-well microplates, any anti-Akabane antibodies would form an antigen-antibody complex,



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masking the viral epitopes on the wells. An anti-g1 monoclonal antibody (Mab) conjugated to Horseradish Peroxidase (HRP) was added, binding to any remaining free AKAV epitopes, creating an antigen-Mab-HRP complex.

Tetramethylbenzidine (TMB) substrate was added to the wells after washing to eliminate excess conjugate. The degree of color change was directly related to the number of specific antibodies in the tested sample. If the sample lacked antibodies, the solution would turn yellow, indicating a negative result. A clear solution signified a positive result. Optical density (OD) was measured at 450 nm using an ELISA plate reader and the competition percentage was calculated using a specific formula.

### 3.7 Sample Preparation and Procedure

All apparatus and equipment, including multichannel pipettes, disposable tips, 96-well microplates and the distilled water system, were prepared and autoclaved prior to the procedure. The wash concentrate (20X) was diluted with distilled water to prepare the diluted wash solution (1X) as specified in Table 3.2.

Table 3.2: Wash Solution Dilution Preparation and Calculation

300µ x 5 times wash x 96-well microplate x 2 = 288,000 µl

Conversion from µl to ml = 288,000 µl ÷ 1000
= 288 ml of distilled water required to dilute the wash solution

Assume 500 ml of distilled water is needed to dilute the wash solution, so use the dilution formula M1V1 = M2V2 to obtain the volume of wash concentrate needed to mix in distilled water.

$$M1V1 = M2V2$$
$$20(V1) = 1(500)$$
$$V1 = 25 \text{ ml}$$

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Next, all reagents were homogenized using a vortex or inversion and maintained at room temperature (21 ℃ ± 5 ℃). The test and control samples were initially prepared in a 96-well microplate, which was then transferred into the ELISA microplate using a multichannel pipette to minimize variations in incubation times across samples. The competitive ELISA steps for detecting anti-Akabane antibodies were conducted according to the manufacturer's instructions.

Pre-dilution microplates were prepared and 50 µl of positive control (Cpos) was added to wells A11, A12, B11 and B12. Simultaneously, 50 µl of negative controls (Cneg) was added to wells C11, C12, D11 and D12. Then, 75 µl of dilution buffer 19 was added to each well, followed by 25 µl of the serum samples to the remaining wells.

After that, the 96-well plate containing the test and control samples was transferred into an ELISA microplate using a multichannel pipette and incubated at (37 ℃ ± 2 ℃) for (45 minutes ± 4 minutes). During the incubation, the 1X conjugate was prepared by diluting the concentrated Anti-Akabane-HRP conjugate (10X) to 1/10 in dilution buffer 5, as shown in Table 3.3.

Table 3.3: Conjugate 1X Preparation and Calculation

| Conjugate 10X → 1X |
| --- |
| 100 µl dilution buffer x 96 x 2 = 19,200 µl |
| Conversion from µl to ml = 19,200 x 0.001 = 19.2 ml (20 ml dilution buffer) |
| Use dilution formula M1V1 = M2V2 to obtain a volume of conjugate 1X needed to mix in a 20 ml dilution buffer. |
| M1V1 = M2V2 |
| 10X(V1) = 1(20) |
| V1 = 2 ml |

After the incubation, the wells were emptied and washed five times with approximately 300 µl of the prepared wash solution per well. Next, 100 µl of the 1X conjugate was added to each well. The plate was then covered and incubated for (60 minutes ± 3 minutes) at (21 ℃ ± 5 ℃). Following the second incubation, the wells were emptied and again washed five times with



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approximately 300 µl of the wash solution per well. Then, 100 µl of the substrate solution was added to each well and the plate was covered and incubated for (30 minutes ± 3 minutes) at (21 ℃ ± 5 ℃) in a dark room. Finally, 100 µl of the stop solution was added to each well to terminate the reaction. The optical density (O.D.) was measured and recorded at 450 nm.

### 3.8 Calculation and Interpretation

The test was considered valid if the mean optical density (O.D.) of the negative control exceeded 0.6 and the ratio of the mean O.D. values of the positive and negative controls were less than 0.5. For the results, the optical densities (ODs) of the test samples were used to calculate the competition percentage by determining the sample (S) to the negative control (N) ratio, using the following formula: S/N (%) = (OD sample / OD negative control) x 100%. Samples with an S/N (%) greater than or equal to 30% were classified as negative, while those with an S/N (%) less than 30% were classified as positive.

### 3.9 Data Analysis

The collected data were summarized using frequencies and presented through frequency tables and comparative bar charts. The percentage distribution of positive samples was displayed in a pie chart. The farm-level prevalence of herd exposure to Akabane virus antibodies was calculated using a contingency table method. The data was analyzed using IBM SPSS Statistics 27 software and Pearson Chi-square was be used to determine the association between risk factors and the occurrence of Akabane virus among goat population in this study.

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# CHAPTER 4

# RESULT

## 4.1 Demographic Data of Selected Goats

Table 4.1 presents the demographic data of the animals enrolled in the study. Of the 40 goats sampled, 55.00% were male and 45.00% were female. The majority were adults, ranging in age from 1 to 6 years (87.50%), while 12.50% were young goats under 1 year old. Additionally, 42.50% of the goats were managed in a semi-intensive system, while the remaining 57.50% were managed intensively. Among the forty goats, only 5.00% exhibited clinical signs of disease, such as diarrhea and rhinitis, while 95.00% were healthy.

Table 4.1: Demographic Data of Goats Sampled in Kelantan (*n* = 40)

| | **No. of goat samples** | **Percentage (%)** |
|---|---|---|
| **Sex** | | |
| Male | 22 | 55.00 |
| Female | 18 | 45.00 |
| **Age** | | |
| Young (< 1 year old) | 5 | 12.50 |
| Adult (1 to < 6 years old) | 35 | 87.50 |
| **Management** | | |
| Semi Intensive | 17 | 42.50 |
| Intensive | 23 | 57.50 |
| **Health Status** | | |
| Healthy | 38 | 95.00 |
| Diseases | 2 | 5.00 |

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### 4.2 Akabane Virus Infection Status from Selected Farms

Based on the results obtained (Appendix B), two out of forty serum samples from the selected goats tested positive for AKAV antibodies, while the remaining thirty-eight samples were negative. Regarding Figure 4.1, the two cases that were serologically positive originated from the Bachok district and none was recorded within Kota Bharu districts. The goats with ID numbers starting with 'N' were from farms in Kota Bharu, while those starting with 'D' were from Bachok (Appendix B), where different farm management practices were observed. The data collected were represented in frequency tables and comparative bar charts (Figure 4.1).

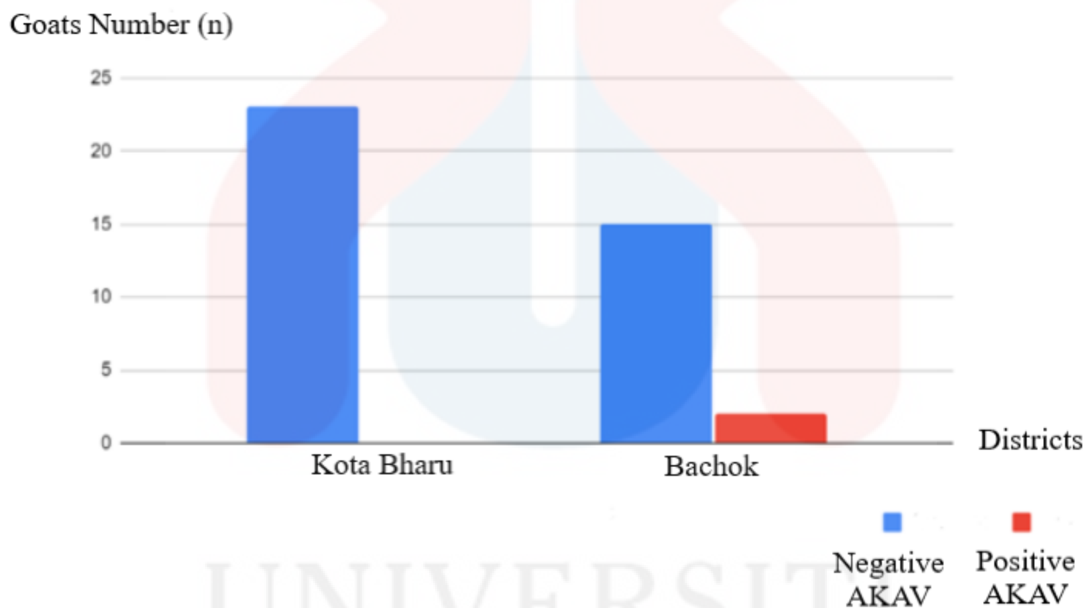


Figure 4.1: Status of AKAV Infection in Goats From Selected Farms in Kelantan, Malaysia

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Based on the data collected, the distribution of positive samples across the selected districts was determined and recorded in Figure 4.2. Goats from Kota Bharu showed a 0% serological detection rate for AKAV infection, indicating no positive cases from this location. In contrast, 100% of the goats from Bachok (n=2) tested positive for AKAV antibodies, reflecting a slight presence of the virus in a particular district in Kelantan.

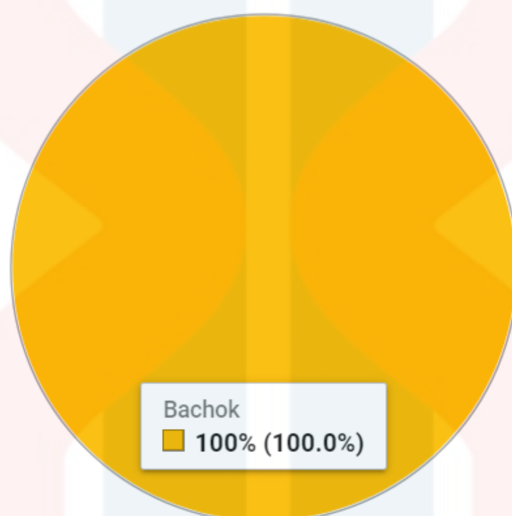


Figure 4.2: Percentage of Positive Sample Distribution Across Districts in Kelantan, Malaysia

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### 4.3 Farm Prevalence Percentage Towards Akabane Virus Antibodies Exposure

According to Table 4.1, all 17 animals tested in Kota Bharu were free from infection, resulting in a 0.00% farm prevalence of Akabane virus antibodies. In contrast, in Bachok, 23 animals were tested, of which 21 were free from infection and 2 were infected, yielding a prevalence rate of 8.70% for this district. Across both districts, 40 animals were tested, of which 38 were free from infection and 2 were infected, resulting in an overall farm prevalence of 5.00%.

Table 4.2: Exposure of Herd with Akabane Virus Antibodies From Selected Districts in Kelantan

| Districts | Animals Free from Infected | Animals Infected | Total Animals | Farm Prevalence (%) |
|---|---|---|---|---|
| **Kota Bharu** | 17 | 0 | 17 | 0.00% |
| **Bachok** | 21 | 2 | 23 | 8.70% |
| **Total** | 38 | 2 | 40 | 5.00% |

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## 4.4 Percentage of Goats Toward Each Identified Risk Factors

### 4.4.1 Sex

Based on the demographic data provided in (Appendix C), there were twenty-two (n=22) male goats (55% of the total sample). Among these, two (n=2) goats tested positive for AKAV, accounting for 9.1% of the male goat population. The remaining twenty (n=20) male goats (90.9%) tested negative. There were 18 female goats (45% of the total sample) and all of the female goats tested negative for AKAV, resulting in a 0% positivity rate among female goats.

Table 4.4.1: Distribution of Akabane Virus Infection Among Goats Based on Sex

| Sex | Animals Free From Infected | Animals Infected | Total |
|---|---|---|---|
| **Male** | 20 | 2 | 22 |
| **Female** | 18 | 0 | 18 |
| **Total** | 38 | 2 | 40 |

### 4.4.2 Age

Thirty-five (n=35) goats (87.5%) were over one year old. Both AKAV-positive goats (D9 and D12) (Appendix B and C) were in this age group, yielding a 5.7% positivity rate among goats older than one year. Five goats (n=5) (12.5%) were under one year of age and all of the goats tested negative for AKAV, resulting in a 0% positivity rate in this younger age group.

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Table 4.4.2: Distribution of Akabane Virus Infection Among Goats Based on Age

| Age / Year old | Animals Free From Infected | Animals Infected | Total |
|---|---|---|---|
| **> 1** | 33 | 2 | 35 |
| **< 1** | 5 | 0 | 5 |
| **Total** | 38 | 2 | 40 |

### 4.4.3 Farm Management Practice

Twenty-three (n=23) goats (57.5%) were managed intensively, all of which tested negative for AKAV, resulting in a 0% positivity rate in this group. Seventeen goats (n=17) (42.5%) were managed under a semi-intensive system. Among these, both AKAV-positive goats (D9 and D12) belonged to this group, resulting in an 11.8% positivity rate for semi-intensive management.

Table 4.4.3: Distribution of Akabane Virus Infection Among Goats Based on Farm -Management Practices

| Farm Management | Animals Free From Infected | Animals Infected | Total |
|---|---|---|---|
| **Intensive** | 23 | 0 | 23 |
| **Semi Intensive** | 15 | 2 | 17 |
| **Total** | 38 | 2 | 40 |

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#### 4.4.4 Vector Control

Twenty-three goats (n=23) (57.5%) were reared on farms that employed vector control measures, including the use of mosquito killer lamps and fogging activities to reduce the presence of insect vectors. All goats from these farms tested negative for Akabane virus (AKAV), resulting in a 0% positivity rate within this group. Conversely, seventeen goats (n=17) (42.5%) were reared on farms that did not implement vector control measures. Among these, two goats (D9 and D12) tested positive for AKAV, indicating an 11.8% positivity rate for goats raised in environments without vector control measures.

Table 4.4.4: Distribution of Akabane Virus Infection Among Goats Based on Vector Control

| Vector Control | Animals Free From Infected | Animals Infected | Total |
| --- | --- | --- | --- |
| **Yes** | 23 | 0 | 23 |
| **No** | 15 | 2 | 17 |
| **Total** | 38 | 2 | 40 |



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### 4.5 Relationship Between AKAV Seropositive Levels with The Potential Risk Factors

4.5 table analyzes the relationship between Akabane virus (AKAV) seropositivity and potential risk factors. Among the variables, sex shows a significant association with AKAV seropositivity (p = 0.040), where males had a prevalence of 9.1% while females showed none. Other factors, including age (≤1 year: 0%; >1 year: 5.7%), farm practice (intensive: 0%; semi-intensive: 3.44%) and vector control (with: 0%; without: 4.76%), did not display significant associations with seropositivity (p > 0.05). This indicates that, apart from sex, none of the evaluated factors were statistically linked to AKAV seropositivity.

Table 4.5: The Statistical Relationship Between The Risk Factors and The Seropositivity AKAV

| Variables | Total Number of Samples (n) | Positive Samples (n) | Prevalence (%) | $x^2$ | P-value | Odd ratio | 95% CI |
|---|---|---|---|---|---|---|---|
| **Sex** | | | | 4.256 | 0.041 | 0 | 0.967 – 1.321 |
| Male | 22 | 2 | 9.1 | | | | |
| Female | 18 | 0 | 0 | | | | |
| **Age (Years)** | | | | 0.684 | 0.621 | 0 | 0.987 – 1.452 |
| <1 | 5 | 0 | 0 | | | | |
| >1 | 35 | 2 | 5 | | | | |
| **Farm Practice** | | | | 0.221 | 0.734 | | 0.124 – 17.432 |
| Intensive | 23 | 0 | 0 | | | 1.5 | |
| Semi Intensive | 17 | 2 | 3.44 | | | 1.0 | |

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| **Vector Control** | | | | | | | |
|---|---|---|---|---|---|---|---|
| Yes | 23 | 0 | 0 | 0.221 | 0.734 | 1.5 | 0.124 – 17.432 |
| No | 17 | 2 | 3.44 | | | 1.0 | |

*P-value <0.05 is considered significant*

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# CHAPTER 5

# DISCUSSION

This study aimed to assess the serological detection of AKAV antibodies and to explore risk factors associated with infection in goats from selected farms in districts of Kelantan, Malaysia. It is the first successful study of serological detection of antibodies against AKAV conducted by using competitive Enzyme-Linked Immunosorbent Assay technique in goats.

The results indicated a low prevalence of AKAV antibodies (5.0%) across both sampled districts, with only two out of forty goats testing positive. Both positive cases were detected in Bachok district, which showed an 8.7% prevalence, while no positive cases were found in Kota Bharu. This finding aligns with previous studies that have found localized distribution of AKAV in certain areas due to varying environmental and management conditions (Kirkland, 2015).

The distribution of AKAV infection across sexes showed a significant association, with male goats exhibiting a 9.1% positivity rate and a statistically significant p-value of 0.041. No female goats tested positive for the infection. This difference value could potentially be attributed to sex-specific behavioral that predispose males to higher susceptibility to AKAV. For example, male goats may exhibit behaviors that increase their exposure to vectors, such as extended grazing times or increased number in activity, thereby heightening their risk of infection. This aligns with observations from previous studies, which propose that male hosts can often play a significant role in the maintenance of this arboviral infections in which presenting with subclinical or asymptomatic infections (Gao *et al.,* 2022). Further research is needed to confirm this hypothesis and determine the implications for disease control in goat populations.

In terms of age, all positive cases were detected in goats older than one year, with a 5.7% positivity rate in this age group, while younger goats (<1 year) showed no evidence of AKAV infection. This age-based difference, with a p-value of 0.621, indicates that there is no statistically significant association between age and AKAV seropositivity in this study. However, these findings are consistent with previous studies suggesting that older animals may have an increased



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likelihood of infection due to longer exposure to infected vectors (Oem *et al.,* 2012). The results imply that goats over one year old might have higher vulnerability or extended contact with vectors, which could elevate their risk of infection. Similarly, a study by Alsaad *et al.* (2017) observed a comparable pattern of age susceptibility in cattle, where older animals had higher seropositivity for AKAV.

Farm management practices also appear to influence AKAV transmission. Goats managed under a semi-intensive system showed a higher positivity rate (3.44%) compared to those in an intensive system, where no positive cases were detected. However, the p-value of 0.734 indicates no statistically significant association between farm management practices and AKAV seropositivity in this study. Intensive management typically involves stricter environmental controls, such as indoor housing, which may reduce exposure to *Culicoides spp.*, the primary vector for AKAV (Aiello *et al.,* 2016). In contrast, semi-intensive systems may increase outdoor exposure, thereby elevating the risk of contact with infected vectors. This finding aligns with studies suggesting that livestock kept under conditions with lower biosecurity measures and minimal vector control are more likely to be exposed to arboviruses (Ogawa *et al.,* 2007).

An important observation in this study is the relationship between vector control measures and AKAV seropositivity. Goats from farms without vector control exhibited a higher positivity rate (3.44%), whereas no positive cases were detected among goats from farms with vector control. However, the p-value of 0.734 indicates no statistically significant association between vector control measures and AKAV seropositivity in this study. Despite the lack of statistical significance, this finding aligns with prior research highlighting the importance of vector control, particularly against *Culicoides spp.*, in reducing the prevalence of arboviral infections in livestock populations (Gao *et al.,* 2022). Effective vector control remains a critical strategy for minimizing the risk of arbovirus transmission to livestock, especially in regions with known vector activity. The absence of such measures may increase the vulnerability of goat populations to AKAV infection.

Comparing these findings with other studies conducted in Asia, a similar seroprevalence pattern has been observed in countries with tropical climates, such as Indonesia and Japan, where

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AKAV infections are associated with vector exposure and farm management practices (Oem *et al.,* 2012 and Edi *et al.,* 2017). Studies from China and Korea have also reported regional variations in AKAV prevalence, often linked to Culicoides populations and favorable climate conditions for survival and reproduction (Gao *et al.,* 2022). For instance, research in Korea demonstrated a higher prevalence of AKAV in areas where livestock is kept outdoors or in semi-intensive systems, mirroring the current study's findings (Oem *et al.,* 2012).

Lastly, the geographical distribution of AKAV seropositivity in Kelantan suggests the possibility of localized transmission in Bachok due to environmental or ecological factors conducive to vector activity in this region. The exclusive detection of positive cases in Bachok aligns with reports from regions with similar climates, where AKAV transmission is sporadic and influenced by vector habitats (Kirkland, 2015). This localized presence highlights the importance of targeted surveillance and vector control interventions in areas with higher transmission risk.

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# CHAPTER 6

## CONCLUSION AND RECOMMENDATION

This study has provided important findings of Akabane virus (AKAV) antibodies in goat populations in Kelantan, Malaysia, revealing a low-level serological detection and highlighting potential risk factors associated with infection, such as semi-intensive farm management and lack of vector control. With two goats from Bachok testing positive, the results suggest that certain environmental and management practices may influence the exposure risk to AKAV. While this study offers a foundation for understanding AKAV's presence in Kelantan, it also highlight the need for further research to clarify these associations and develop effective preventive measures.

To enhance the accuracy of findings in future studies, it is recommended to expand the sample size and a wider range of locations across Kelantan and other regions in Malaysia be included. A larger sample from diverse districts would enable a more comprehensive assessment of AKAV prevalence and distribution, providing clearer insights into regional variations and risk factors. Additionally, employing multiple diagnostic methods, such as combining polymerase chain reaction (PCR) with serological testing, would improve detection sensitivity and specificity, offering a more complete picture of both past exposure and active infection rates among goat populations.

Longitudinal and seasonal studies are also advised to capture variations in AKAV transmission, which is the seasonal activity of *Culicoides* spp. Monitoring over different times of the year would identify peak risk periods, aiding in developing timely vector control strategies. These recommendations will strengthen future research on AKAV epidemiology in Malaysia, supporting more targeted disease control and contributing to the overall health management of small ruminants in the region.

In conclusion, the seroprevalence of AKAV among goats in Kelantan is relatively low, with a 5.0% overall positivity rate. However, cases in Bachok district suggest a localized risk for AKAV transmission, particularly in semi-intensive farms without vector control measures.

Implementing preventive strategies, such as vector management, intensive farm practices and targeted monitoring in high-risk areas, is crucial to control AKAV spread in Malaysia's tropical climate. These findings emphasize the need for proactive approaches to safeguard livestock health and support sustainable goat farming practices in the region.

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# APPENDIX

APPENDIX A

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FAKULTI PERUBATAN VETERINAR
Faculty of Veterinary Medicine

Ruj. Kami (*Our Ref.*) : UMK/FPV/ACUE/FYP/009/2024
Tarikh (*Date*) : 11th August 2024

**ASSOC. PROF. DR. MOHD FARHAN HANIF BIN REDUAN**
Main Supervisor
Faculty of Veterinary Medicine
University Malaysia Kelantan

Dear Assoc. Prof.,

**APPROVAL OF INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC) TO CONDUCT RESEARCH INVOLVING ANIMALS**

We are pleased to inform you that your application for approval to conduct research from Institutional Animal Care and Use Committee (IACUC), Faculty of Veterinary Medicine, University Malaysia Kelantan has approved. Please refer the table below for approval code:

| APPROVAL CODE | *UMK/FPV/ACUE/FYP/009/2024* |
| --- | --- |
| TITLE | SEROLOGICAL DETECTION AND RISK FACTORS OF AKABANE VIRUS IN GOATS FROM SELECTED FARMS IN KELANTAN, MALAYSIA |
| NAME OF STUDENT | IRFAN FAKHRI BIN AL-ITQAM<br>LEONG ZHI ZONG<br>MARIA FAZIRA BINTI MOHD ZULKEFLI |

You are advised to always follow "3R" (REDUCE, REFINE, & REPLACE) and all animal ethics and animal welfare principles to reduce suffering in animal.

Thank you.

***"ISLAM MEMBIMBING, RAJA MEMIMPIN, NEGERI DIBERKATI"***
***"MALAYSIA MADANI"***
***"BERKHIDMAT UNTUK NEGARA"***

Yours sincerely,

**(DR. MOHAMMED DAUDA GONI)**
Chairman
Institutional Animal Care and Use Committee
Faculty of Veterinary Medicine

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APPENDIX B

**Table of S/N (%) values and corresponding AKAV infection status in goats from selected farms in Kelantan, Malaysia**

| No. | ID Number | S/N (%) | Result | No. | ID Number | S/N (%) | Result |
|---|---|---|---|---|---|---|---|
| **1.** | N3 | 63.50 | Negative | **21.** | N24 | 98.56 | Negative |
| **2.** | N4 | 93.68 | Negative | **22.** | N25 | 46.55 | Negative |
| **3.** | N5 | 47.70 | Negative | **23.** | N26 | 65.52 | Negative |
| **4.** | N6 | 97.70 | Negative | **24.** | D1 | 99.13 | Negative |
| **5.** | N7 | 97.41 | Negative | **25.** | D2 | 59.20 | Negative |
| **6.** | N8 | 96.84 | Negative | **26.** | D3 | 116.95 | Negative |
| **7.** | N9 | 72.70 | Negative | **27.** | D4 | 85.34 | Negative |
| **8.** | N10 | 37.36 | Negative | **28.** | D5 | 62.07 | Negative |
| **9.** | N11 | 48.85 | Negative | **29.** | D6 | 85.06 | Negative |
| **10.** | N12 | 72.41 | Negative | **30.** | D7 | 104.06 | Negative |
| **11.** | N13 | 37.36 | Negative | **31.** | D8 | 127.59 | Negative |
| **12.** | N14 | 55.46 | Negative | **32.** | D9 | 9.48 | **Positive** |
| **13.** | N15 | 99.80 | Negative | **33.** | D10 | 81.32 | Negative |
| **14.** | N16 | 72.41 | Negative | **34.** | D11 | 53.74 | Negative |
| **15.** | N18 | 48.85 | Negative | **35.** | D12 | 5.52 | **Positive** |
| **16.** | N19 | 36.78 | Negative | **36.** | D13 | 49.98 | Negative |
| **17.** | N20 | 43.39 | Negative | **37.** | D14 | 54.45 | Negative |
| **18.** | N21 | 66.67 | Negative | **38.** | D15 | 63.78 | Negative |
| **19.** | N22 | 35.89 | Negative | **39.** | D16 | 81.60 | Negative |
| **20.** | N23 | 93.39 | Negative | **40.** | D17 | 105.17 | Negative |

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APPENDIX C

**Table of Demographic data of goats sampled in Kelantan, Malaysia (n = 40)**

| No. | ID Number | Sex | Age | Farm Management | Vector control | Place (District) |
|---|---|---|---|---|---|---|
| **1.** | N1 | Male | > 1 | Intensive | Yes | Kota Bharu |
| **2.** | N2 | Male | > 1 | Intensive | Yes | Kota Bharu |
| **3.** | N3 | Male | > 1 | Intensive | Yes | Kota Bharu |
| **4.** | N4 | Male | > 1 | Intensive | Yes | Kota Bharu |
| **5.** | N5 | Male | > 1 | Intensive | Yes | Kota Bharu |
| **6.** | N6 | Male | < 1 | Intensive | Yes | Kota Bharu |
| **7.** | N7 | Male | > 1 | Intensive | Yes | Kota Bharu |
| **8.** | N8 | Male | < 1 | Intensive | Yes | Kota Bharu |
| **9.** | N9 | Male | > 1 | Intensive | Yes | Kota Bharu |
| **10.** | N10 | Male | < 1 | Intensive | Yes | Kota Bharu |
| **11.** | N11 | Male | > 1 | Intensive | Yes | Kota Bharu |
| **12.** | N12 | Female | > 1 | Intensive | Yes | Kota Bharu |
| **13.** | N13 | Female | > 1 | Intensive | Yes | Kota Bharu |
| **14.** | N14 | Female | > 1 | Intensive | Yes | Kota Bharu |
| **15.** | N15 | Female | > 1 | Intensive | Yes | Kota Bharu |
| **16.** | N16 | Female | > 1 | Intensive | Yes | Kota Bharu |
| **17.** | N18 | Female | > 1 | Intensive | Yes | Kota Bharu |
| **18.** | N19 | Female | > 1 | Intensive | Yes | Kota Bharu |
| **19.** | N20 | Female | > 1 | Intensive | Yes | Kota Bharu |
| **20.** | N21 | Female | > 1 | Intensive | Yes | Kota Bharu |

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| No. | ID Number | Sex | Age | Farm Management | Vector control | Place (District) |
|---|---|---|---|---|---|---|
| **21.** | N22 | Female | > 1 | Intensive | Yes | Kota Bharu |
| **22.** | N23 | Female | > 1 | Intensive | Yes | Kota Bharu |
| **23.** | N24 | Male | > 1 | Intensive | Yes | Kota Bharu |
| **24.** | D1 | Male | > 1 | Semi-Intensive | No | Bachok |
| **25.** | D2 | Male | < 1 | Semi-Intensive | No | Bachok |
| **26.** | D3 | Male | > 1 | Semi-Intensive | No | Bachok |
| **27.** | D4 | Male | < 1 | Semi-Intensive | No | Bachok |
| **28.** | D5 | Male | > 1 | Semi-Intensive | No | Bachok |
| **29.** | D6 | Male | > 1 | Semi-Intensive | No | Bachok |
| **30.** | D7 | Female | > 1 | Semi-Intensive | No | Bachok |
| **31.** | D8 | Female | > 1 | Semi-Intensive | No | Bachok |
| **32.** | D9 | Male | > 1 | Semi-Intensive | No | Bachok |
| **33.** | D10 | Female | > 1 | Semi-Intensive | No | Bachok |
| **34.** | D11 | Male | > 1 | Semi-Intensive | No | Bachok |
| **35.** | D12 | Male | > 1 | Semi-Intensive | No | Bachok |
| **36.** | D13 | Male | > 1 | Semi-Intensive | No | Bachok |
| **37.** | D14 | Male | > 1 | Semi-Intensive | No | Bachok |
| **38.** | D15 | Male | > 1 | Semi-Intensive | No | Bachok |
| **39.** | D16 | Female | > 1 | Semi-Intensive | No | Bachok |
| **40.** | D17 | Female | > 1 | Semi-Intensive | No | Bachok |

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*Figure 1: Goat farm in Kota Bharu District*

*Figure 2: Forty (n=40) Plain Red Tubes filled with Goat Blood Sample*

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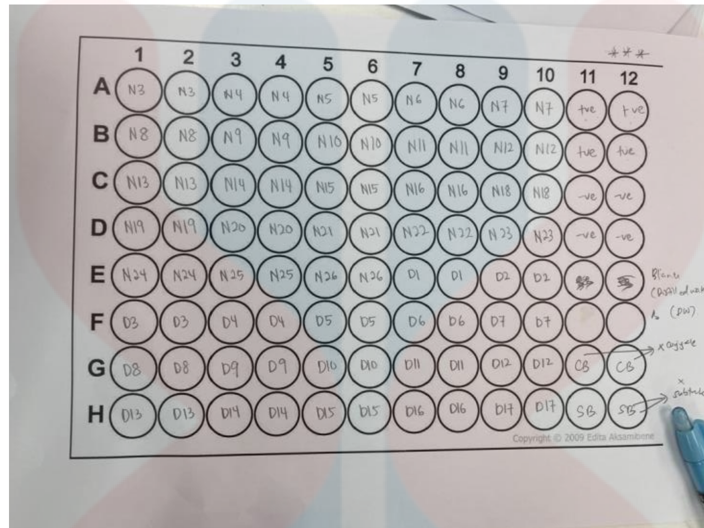


*Figure 3: Flowchart of Sample Arrangement prior (ELISA) process*

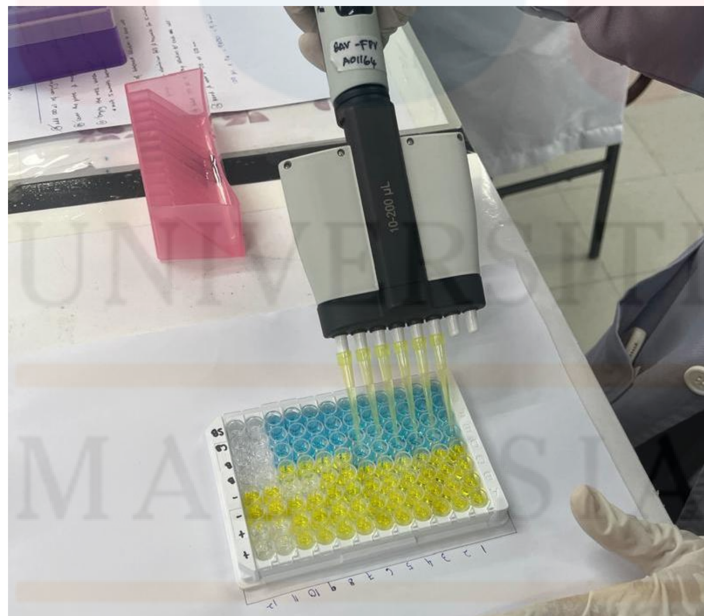


*Figure 4: Samples Transfer into Competitive ELISA Plate Using Channel Micropipette*

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# CHAPTER 8

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