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SEROPREVALENCE OF BLUETONGUE AMONG DAIRY CATTLE IN SELECTED STATES IN MALAYSIA

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DOCTOR OF VETERINARY MEDICINE

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SEROPREVALENCE OF BLUETONGUE AMONG DAIRY CATTLE IN SELECTED FARMS IN MALAYSIA

By

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A research project submitted to the Universiti Malaysia Kelantan in partial fulfilment of the requirements for the degree of Doctor of Veterinary Medicine

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Dedication

I would be honoured to dedicate my dissertation work to my lecturer, family, and friends.

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List of abbreviations

%	Percent
℃	Degree Celsius
BT	Bluetongue
BTV	Bluetongue virus antibodies
cELISA	Competitive ELISA
DVS	Department Veterinary Services
EHDV	Epizootic Haemorrhagic Disease Virus
O.D.	Optical density
NS	Non-structural
RNA	Ribonucleic acid
SST	Serum separator tube
S/N	Sample-to-negative ratio
VP	Viral protein
WOAH	World Organization for Animal Health

Table of Content

SEROPREVALENCE OF BLUETONGUE AMONG DAIRY CATTLE

IN SELECTED FARMS IN MALAYSIA

Abstract

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

Bluetongue disease (BT) is a notifiable disease listed by World Organisation for Animal Health (WOAH) due to its significant impact on the ruminant industry. It is an arthropod-borne viral disease of domestic and wild ruminants such as sheep, goats, cattle, buffaloes, deer and most species of African antelope, and camelids as vertebrate hosts. Sheep being the major host and usually develop clinical signs, however, occasionally other ruminants can also acquire the same clinical sign. In Malaysia, the last known report was in 1995 and the latest outbreak was in 2009. The recent and updated information related to current prevalence of BT in Malaysia is limited. There is a lack of information related to the disease status with dairy cattle. Due to the lack of data on BT in Malaysia, this study aims to determine the seroprevalence of BT infection among dairy cattle in selected states in Malaysia, such as Perak, Pahang, Johor, and Sabah. Blood samples were collected randomly from dairy cattle regardless of its age, breed, and group. The blood serums were used to detect the antibody towards BT by competitive ELISA (cELISA). There is an increase in the seropositive level indicating the presence of BT antibodies among dairy cattle in Malaysia, with 100% herd prevalence in Johor and Sabah, 61.76% in Pahang and 89.66% in Perak. The increase in seroprevalence of BT in this study could be due to the fly population in the farm environment since flies are the potential vectors in the transmission of BTV.

Keywords: Bluetongue (BT), BTV antibodies, cELISA, seroprevalence, dairy cattle, flies

SEROPREVALESI PENYAKIT *BLUETONGUE* ANTARA LEMBU TENUSU DI NEGERI TERTENTU DI MALAYSIA

Abstrak

Abstrak daripada kertas penyelidikan dikemukakan kepada Fakulti Perubatan Veterinar, Universiti Malaysia Kelantan bagi memenuhi sebahagian daripada keperluan kursus DVT 55204 – Projek Penyelidikan.

Penyakit Bluetongue (BT) adalah penyakit yang telah sedia maklum disenaraikan oleh World Organisation for Animal Health (WOAH) disebabkan oleh impaknya yang besar kepada industri ruminan. Penyakit ini merupakan bawaan arthropoda yang viral merangkumi domestik dan ruminan liar seperti biri-biri, kambing lembu, kerbau, rusa dan sebahagian besar spesies antelop Africa dan unta sebagai hos vertebrata. Biri-biri sebagai hos utama dan kebiasaannya akan menghasilkan tanda klinikal, walaubagaimanapun, ada kalanya ruminan lain juga boleh memperoleh tanda klinikal yang sama. Di Malaysia, laporan terakhir yang dilaporkan adalah pada tahun 1995 dan kali terakhir ia berlaku adalah pada tahun 2009. Maklumat terbaru dan terkini berkaitan dengan penyakit BT di Malaysia ialah terhad. Terdapat kurang maklumat berkaitan dengan status penyakit ini dengan lembu tenusu. Oleh sebab kurangnya data berkaitan BT di Malaysia, kajian ini bermatlamat untuk menentukan seroprevalensi penyakit BT dalam kalangan lembu tenusu di negeri-negeri terpilih di Malaysia iaitu Perak, Pahang, Johor dan Sabah. Sampel darah dikumpul secara rawak dari lembu tenusu tanpa mengira umur, jenis baka dan kumpulan. Serum darah digunakan untuk mengesan antibodi terhadap BT melalui ujian cELISA. Terdapat peningkatan seroprevalensi menunjukkan kehadiran antibodi BT dalam kalangan lembu tenusu di Malaysia, dengan prevalensi kelompok 100% di Johor dan Sabah, 61.76% di Pahang dan 89.66% di Perak. Peningkatan seroprevalensi BT dalam kajian ini mungkin disebabkan oleh populasi lalat di ladang dimana lalat sebagai vektor yang berpotensi dalam transmisi virus BT.

Kata kunci: Bluetongue (BT), antibody virus BT, cELISA, seroprevalensi, lembu tenusu, lalat

Chapter 1

1.0 Introduction

Bluetongue (BT) is a viral disease that is closely related to the virus in the epizootic haemorrhagic disease virus (EHDV) serogroup which is classified in the genus of Orbivirus of the Reoviridae family. BT virus is known to be transmitted via *Culicoides* spp. (Maclachlan *et al*., 2018). The disease can result in both direct and indirect economic losses. The direct losses due to high morbidity and mortality with apparent clinical manifestation such as stillbirth, abortions, foetus defect, low birth weight in young, reduced milk production, reduced fertility rate, early culling, meat and fleece losses. While trade restriction regarding animal movement, vaccination, diagnosis, vector control and treatment of clinically pretentious animals are some of the indirect losses encountered due during the BT outbreak (Subhadra *et al*., 2023).

BT, once considered a disease of sheep confined to the southern African region and now becoming a global emergence. Other domestic and wild ruminants include goat, cattle, buffaloes, deer, most species of African antelope and other Artiodactyla such as camels have also been shown to be susceptible to BT (Hassani & Madadgar, 2021). Due to its impact on animal livestock, BT infection is regarded as a notifiable disease listed by the World Organization for Animal Health (WOAH). There are currently about 27 BTV serotypes that have been recognized all over the world, however, there are only 6 serotypes from cattle in Malaysia with BTV serotypes detected 1, 2, 3, 9, 16, 23 (Daniel *et. al*., 2004). In Malaysia, the first reported case of bluetongue infection outbreak in ruminants was in 1995 and the recent outbreak was detected in 2009 (Sharifah *et. al*., 1995).

In the past 25 years, no studies of BT among dairy cattle have been carried out in Malaysia, and therefore there is lack of information regarding BT status in the country prompting an urgent task to investigate the current seroprevalence within livestock in Malaysia especially in cattle. Additionally, BTV can replicate in many species of ruminants, however, signs are often subclinical or asymptomatically in cattle. Cattle are considered important and relatively long-term virus reservoirs (Hourrigan & Klingsporn, 1975). The recent outbreak of BTV-8 in Europe has shown remarkable differences in the clinical expressions of the disease, with clinical illness and reproductive disorders such as abortion, stillbirth, and foetal abnormalities (Pozzo *et al*., 2009). BTV-8 infection has been reported as the cause of infertility in dairy cattle and the BTV capable of transmitting vertically to the offspring (Santman-Berends *et al*., 2010).

Chapter 2

2.0 Research Statement

2.1 Research Problem

Since data on BT in Malaysia is limited, this study will be carried out to detect the seroprevalence of BT infection among dairy cattle in selected states that are among the highest dairy cattle population in Malaysia.

2.2 Research Question

1. What is the seroprevalence status of BT antibodies among dairy cattle in Malaysia?
2. Which of the selected states among dairy cattle present with BT antibodies in Malaysia?

2.3 Research Hypothesis

1. The seroprevalence level of BT among dairy cattle in Malaysia is low to moderate.
2. Johor presented the most with BT antibodies as being the state with the highest number of dairy cattle populations in Malaysia.

2.4 Research Objective

1. To determine the status of disease by the seroprevalence of BT antibodies among dairy cattle from the selected states in Malaysia.
2. To identify which of the selected states are with the most dairy cattle with seroprevalence positive for BT antibodies in Malaysia.

Chapter 3

3.0 Literature Review

3.1 Description of Bluetongue virus (BTV)

BT is an arthropod-borne non-enveloped double-stranded RNA virus of the genus Orbivirus belonging to the family Reoviridae. BTV is a 90nm in diameter non-enveloped virus with triple layered icosahedral protein capsid (Ali *et al*., 2020). The BTV genome consists of 10 segments which encodes seven structural proteins (VP1 until VP7) and four non-structural proteins (NS1, NS2, NS3/NS3A and NS4) proteins (Maan *et al*., 2012). The outer capsid consists of two major structural proteins (VP2 and VP5) where VP2 protein determine the serotypes of the virus and forms a continuous layer that covers the inner core that is composed of two major proteins (VP3 and VP7) and three minor enzymatic proteins (VP1, VP4 and VP6) as a virion particle (Bhattacharya & Roy, 2010).

BTV can be transmitted between its ruminant host by adult female hematophagous midges that belong to *Culicoides* genus as the main vector (Roy, 2008). The *Culicoides* are small flies or known as “biting midges” that act as “incubator” to the viral variants where BTV replicates in the digestive system of the midges, and it will be released into the salivary glands. The whole cycle from infection to transmission takes between ten to fifteen days at temperature above 15 °C (Mellor *et al*., 2009). Biting midges capable of flying over a distance of two km due their small size (1mm to 3mm) as if uninterrupted from other factors (Ducheyne *et al*., 2007). The activity of midges is markedly influenced by ambient temperature, air humidity and total seasonal rainfall (Mullens et al., 1995; Wellby et al., 1996; Mellor, 2000). The recent global warming allows longer activity of biting midges in which longer periods of BTV transmission among the ruminant (Tweedle & Mellor, 2002).

3.2 Clinical Manifestation of Bluetongue virus in cattle

The clinical outcome of BTV infection may vary among ruminant species. Although sheep are more apparent, cattle can be the main mammal reservoir of the virus and are critical in the disease epidemiology. Cattle are particularly in the epidemiology of disease due to prolonged viremia that occurs following the infection, even while not necessarily showing outward signs of infection. The clinical signs typically appear after an incubation period of about 4 to 8 days, or sometimes longer (WOAH, 2021). The appearance of clinical signs of bluetongue infection may vary among species and depending upon viral strains, such as goats generally show few or no clinical signs. While affected cattle may develop signs of illness similar to that in sheep, however, cattle are usually limited to fever, increased respiratory rate, lacrimation, salivation, stiffness, oral vesicles and ulcers, hyperesthesia, vesicular and ulcerative dermatitis (Andrea, 2022). According to Andrea (2022), subclinical infected cattle can become viraemic 2 to 4 days post infection. Following the bite of the insect vector, the virus spreads through lymphatic vessels and localised in the vascular endothelial cells resulting in the destruction of the vessel walls. Hence, the manifestation of haemorrhage, exudation, tissue edema and vascular occlusion subsequently give rise to hypoxia and induce other epithelial lesions.

Venereal transmission from infected bulls is also a possible method of disease transmission (Subhadra *et al*., 2023). BTV can present in semen causing structural changes in spermatozoa. The BTV can also cross the placental barrier to invade the foetus and death of the foetus will be due to hepatic necrosis and depression of the hematopoietic system of the foetus. Hence, susceptible cattle infected during pregnancy may abort or deliver

malformed calves (Andrea, 2022). In addition, the virus can also spread mechanically through surgical equipment and needles (Subhadra *et al*., 2023).

Although clinical signs in cattle are rare and limited, exposure to BTV can influence international trade and movements of serologically positive animals. The disease can have a considerable economic impact due to the morbidity and mortality of livestock as well strict movement restriction and control measures will be required.

3.3 Serology Detection and Identification of Bluetongue virus

IDScreen® Bluetongue Competition assay, ID Vet can be used to detect the antibody of bluetongue virus in the serum samples collected from the dairy cattle. The wells are coated with the antigens and the sample antigen is incubated with the unlabelled primary antibody. After first incubation, antigen-antibody complexes are then added to the ELISA plate which has been pre-coated with the same antigen. Once complete incubation, any unbound antibody is washed away. Antibodies in tested serum will block the reaction between the antigen specific monoclonal antibody resulting in reduction in colour. The result of colouration depends on the quantity of specific antibodies present in the serum tested which will directly reveal the status of disease either being positive or negative.

By detecting the presence of bluetongue virus, this information will serve as a valuable reference to estimate the likely seroprevalence of BTV antibodies among dairy cattle in Malaysia. If it goes undetected, an import of infected animals is likely to result in a large-scale outbreak of BTV infection, regardless of the time of year the import was made. Besides, this study may serve as a guideline for future reference for reporting to the Department of Veterinary Services (DVS) to impose appropriate control measures in preventing the possible spread in the farm and to other ruminant livestock.

3.4 Economic impact of Bluetongue virus

Although BT disease has no zoonotic potential to humans, BT has significant economic losses of livestock (Douangngeun *et al*., 2016). According to Animal Health Australia (2015), cost would arise from the control measure, while production losses due to the effect of BT disease and affects on market. Other direct costs that will be associated such as due to morbidity of sick animals, abortion, reduced milk yield, reduced meat efficiency production. While indirect cost mostly because of restrictions to the export of semen, live animals, and some products such as bovine foetal serum (Acevedo *et al*., 2016). The cost for surveillance, vector control strategies, mass vaccination of susceptible animals and associated veterinary cost also contributes to economic loss (Wilson and Mellor, 2009).

During the BT outbreak, there will be resistance in both export and domestic markets which may result in reduced value of the livestock in general. The impacts of quarantine and movement controls tend to limit the animal movement and thus reduce the options to gain access to the market, which subsequently increase in the cost of livestock consumption and the cost to export, which also due to cover the cost of testing for the presence of disease (Animal Health Australia, 2015). Cattle that tested BT positive are not allowed to be moved from the outbreak areas due to have prolonged viraemia, where they tend to be persistently infected in the population without having clinical symptoms (Tabachnick *et al*., 2020).

3.5 Prevention and control of Bluetongue virus

To date, there is no specific therapy for animals affected with BT. Affected animals mainly will be managed with symptomatic therapy including gentle handling of affected animals, providing stabling rest, provision of soft feed, and basically with good husbandry. In the ruminant industry, the priority measure during BT outbreak is by immediate ban on animal import from countries with BT, followed by the monitoring of farms raising domestic ruminants which include clinical examination, with serological and virological testing, and monitoring of insect vectors (Sperlova & Zendulkova, 2011). Since there is no direct host to host transmission, there is no justification to perform stamping out where to slaughter all infected or exposed animals, since the amplification of virus mainly carried in the vector itself. However, some animals may need to be removed for those severely affected with BT disease due to the welfare concerns (Sperlova. & Zendulkova, 2011).

Before any control strategy for BT begins, the initial outbreak must be investigated thoroughly. The most critical control of BTV infection is by the control of vectors in the farm to reduce the transmission of BTV vectors, although it is not possible to completely eradicate the BT vectors in the environment. The control of adult midges can be carried out using approved insecticides, to the stable or directly to the susceptible animals (Schmahl *et. al*., 2009). The control by the usage of synthetic pyrethroids such as deltamethrin, cyfluthrin and permethrin can provide protection against adult midges for 3 to 5 weeks, which can be given by spray on or by impregnated ear tags (Mehlhorn *et. al*., 2008).

Chapter 4

4.0 Material and Methodology

4.1 Ethics Statement

No ethics application is required for this study, as serum is obtained from archive samples from a previous research project with an approval code from FPV Animal Ethics Committee: UMK/FPV/ACUE/RES/002/2023.

4.2 Methodology

4.2.1 Study area

There are a total of four selected states involved in this study. The four selected states involved in this study are Perak, Pahang, Johor, and Sabah. These states were included based on its high number of dairy cattle populations in Malaysia.

4.2.2 Study design

This study is a cross sectional (seroprevalence) study design where dairy cattle are randomly selected with either presence or absence of bluetongue at a point of time.

4.2.3 Study population

A total of thirty dairy cattle regardless of age, breed and group were collected randomly for this study from each four states selected with a total number of 120 dairy cattle. A total of 34 samples each were collected from several farms in Johor and Pahang, with 29 samples were collected from several farms in Perak, and 23 samples were collected from several farms in Sabah, of which a total of 120 serum samples were collected.

4.3 Selection criteria

4.3.1 Inclusion criteria

The inclusion criteria for this study are dairy cattle and were randomly selected with no bias in terms of age, breed, sex, body weight and its health status.

4.3.2 Exclusion criteria

There are no exclusion criteria for this study since all samples were randomly collected from dairy cattle in each selected state as in one population at a time.

4.4 Sampling method and Procedure

4.4.1 Coccygeal vein blood sampling

Equipment required for the procedure include a pair of gloves, alcohol swab, vacutainer holder, vacutainer needle (18G), serum separator tube (SST), icepack, icebox. The procedure was according to the guideline provided by the University of Bristol (2020) as reference. Basically, gloves were worn before starting the blood collection procedure. A vacutainer needle size 18G, a plastic vacutainer holder and SST were selected. The position was by standing behind the cow and slightly to one side of the tail. Followed by the tail was held approximately a third of the way from the base with the non-dominant hand and lifted until the ventral aspect of the tail was visible. The groove which was situated in the midline on the ventral aspect of the tail was located and palpated, where coccygeal vein and artery are run in this groove. The back of the fingers then rested against the base of the tail with the vacutainer holder held in the dominant hand to steady the hand. The needle was inserted into the midline groove at 90° to the tail surface and continued to advance to approximately halfway along its length. The vacutainer holder was stabilised with the thumb, index and middle fingers and held with the 4th and 5th fingers and against the palm. Once stabilized, the SST was pushed onto the needle to allow the needle to pierce the bung. The SST was removed when blood filled in about ⅔ of tube, and so the vacutainer holder and needle were removed afterwards. The needle was recapped and discarded. Most importantly, blood samples collected were all stored in ice box containing of ice packs.

4.4.2 Jugular vein blood sampling

Some blood samples were also collected using the jugular vein blood sampling method. The procedure was according to the guideline provided by the University of Bristol (2020) as reference. Basically, gloves were worn before starting the blood collection procedure. A vacutainer needle size 18G, a plastic vacutainer holder and serum separator tube (SST) were prepared. The jugular groove was palpated with the free hand to determine the location of the jugular vein. The jugular vein was raised with the free hand by firmly pressing the thumb into the jugular groove. The thumb position was far enough down the groove caudally to leave enough space to insert the needle and collect the sample. The needle was positioned over the jugular groove cranial to the thumb and held at 45° to the skin surface. The needle was pushed through the skin and into the jugular vein following the direction of the jugular with the needle. The vacutainer holder was stabilised between the thumb and index finger of the hand that was raising the vein. The free fingers of the free hand continued to maintain the jugular vein. The SST was then inserted into the vacutainer holder with the index and middle fingers over the base of the holder and the palm of the hand was used to push the blood tube up into the holder and firmly onto the needle. While the other hand maintained the position of the needle in the jugular vein by stabilising the vacutainer holder. The SST was removed once it was ⅔ filled with blood and the vacutainer holder and the needle were removed afterwards. The needle was recapped and discarded. All blood samples were stored in ice box containing of ice pack after collection for transportation. Upon arrival at the laboratory, all blood samples were stored at refrigerator at -20℃.

4.4.3 Detection of antibodies using cELISA

Competitive ELISA test kit for the detection of antibodies against BTV (ID Screen, Bluetongue Competition, France) was used to test the serum samples obtained from the blood samples collected. The components provided in the kits included reagents, microplate coated with VP7 recombinant protein, concentrated conjugate, positive control and negative control, dilution buffer 2, wash concentrate (20X), substrate solution and stop solution (0.5M). Additional equipment such as mono-channel and multi-channel pipettes that capable of delivering volumes of 5µL, 100µL and 500µL, disposable tips (10µL, 200µL and 500µL), 96-well pre-dilution microplate, distilled or deionized water, manual wash system and 96-well microplate reader.

The procedure done was according to the guidelines provided by cELISA test kit. Begins with sample preparation, a 96-well plate containing test and control specimens was prepared before transferring them into an ELISA microplate using a multichannel pipette to avoid difference in incubation between specimens. Next, wash solution preparation, (a) the wash concentrate (20x) was brought to room temperature and mixed thoroughly to ensure the wash concentrate was completely solubilized. Followed by the wash solution (1x) was prepared by diluting the Wash Concentrate (20x) in distilled/deionized water. The calculation was done as stated below:

$M_1V_1 = M_2V_2$

(20) V = (1) (500)

$V1$ = 25 mL

* 25 mL of Wash Concentrate (20x) is diluted in 500 mL distilled water to get Wash Solution (1x)

300µL x 3 x 96 x 2 = 172 800 µL

= 1728 mL of Wash Solution is required for 3 wash steps

Next, the testing procedure, where all reagents were allowed to come to room temperature (21℃ ± 5℃) before use. All reagents were homogenised by inversion or vortex. All samples were tested at a final dilution of 1:50. All these components were added in a 96-well pre-dilution microplate, with 50µL of Dilution Buffer 2 to each well, 50µL of the Positive Control to wells A12 and B12, 50µL of the Negative Control to wells C12 and D12, and 50µL of each sample to be tested in the remaining wells except for wells E12, F12, G12 and H12. All these components were transferred into the ELISA microplate, with that 100µL of the pre-diluted Negative Control to wells C12 and D12, then 100µL of the pre-diluted Positive Control to wells A12 and B12, and 100µL of each pre-diluted sample to be tested in the remaining wells except for wells E12, F12, G12 and H12. The plate was covered and incubated for 45 minutes at 21℃ ± 5℃.

In the meantime, the conjugate 1x was prepared by diluting the Concentrated Conjugate 10x to 1:10 in Dilution Buffer 3. Calculation was done as stated below:

$M_1V_1 = M_2V_2$

(10) V = (1) (20)

$V1$ = 2 mL

* 2 mL of Conjugate Concentrate 10x is diluted in 20 mL of Dilution Buffer to get Conjugate 1x

$M_1V_1 = M_2V_2$

(10) (60) = (1)

$V2$ = 600 mL

100 µL x 96 well x 2 plates = 19 200 µL

= 19.2 mL of Conjugate 1x is required

100µL of the Conjugate 1x was added to each well. The plate was covered and incubated for 30 minutes at 21°C ± 5°C. The wells were emptied, and each well was washed 3 times with 300µL of the Wash Solution. Ensure wells were not dried between washes. 100µL of the Substrate Solution was added to each well. k. The plate was covered and incubated for 15 minutes at 21°C ± 5°C in the dark. 100µL of the Stop Solution was added to each well, in the same order as in step in (h), to stop the reaction. The optical density (O.D.) at 450 nm was read and recorded. Before interpretation of result, validation was determined. The test was validated as if mean value of the Negative Control optical density (O.D.) is > 0.7, and ratio of the mean values of the Positive and Negative Control O.D. is < 0.3.

After the interpretation, the competition percentage was calculated by S/N (%) in order to determine the status of dairy cattle against BT antibodies either to be positive or negative. Formula to determine **S/N % = OD sample / OD $_{NC}$ x 100**

Table 4.1: Interpretation of status by S/N %

Result	Status
S/N % < 40%	Positive
S/N % ≥ 40%	Negative

Chapter 5

5.0 Result

In this study, a total of 120 blood samples of dairy cattle were collected randomly from four selected states in Malaysia specifically Pahang, Sabah, Johor and Perak. Blood serums were used for the detection of BTV antibody using an ELISA Bluetongue test kit. The ELISA test result revealed 104 samples out of 120 total samples tested in this study were detected positive, with 16 samples were detected negative against BTV antibodies.

Overall, in all selected states, the seroprevalence of BTV antibodies are generally moderate to high among the dairy cattle population. Dairy cattle in Sabah and Johor showed 100% positive against BTV antibodies in all its tested serum samples (Table 5.1).

Result	**Pahang**	**Sabah**	**Johor**	**Perak**	**Total**
Negative	13	0	0	3	16
Positive	21	23	34	26	104
Total samples	34	23	34	29	120

Table 5.1: Result of cELISA test kit for the detection of antibodies against BTV in serum samples of dairy cattle from farms in selected states in Malaysia

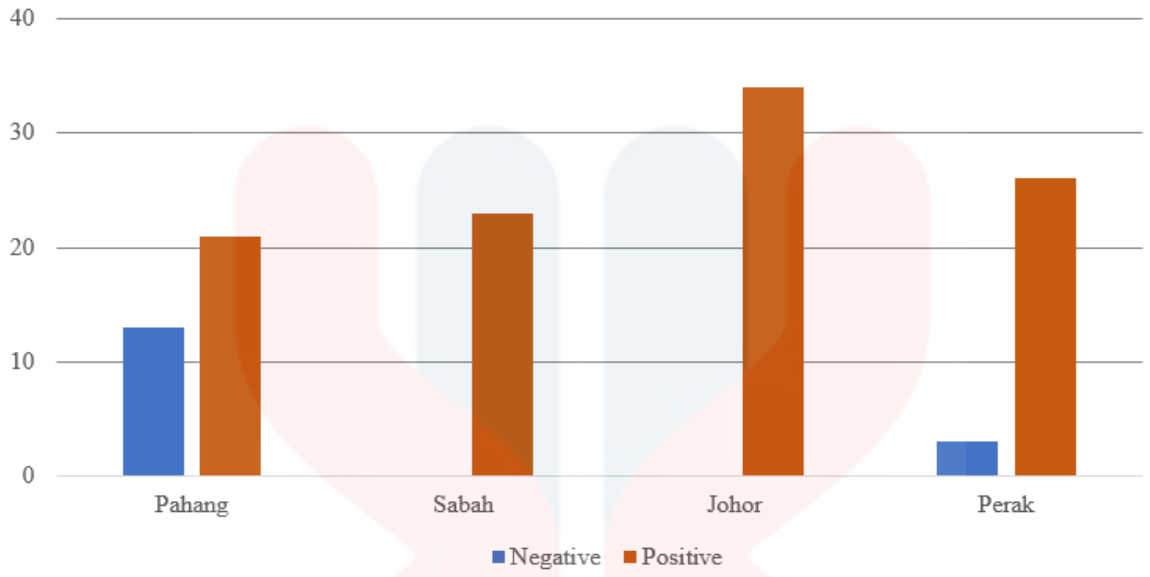

Figure 5.1: Bar Chart representing result of cELISA by interpretation of negative and positive for the detection of antibodies against BTV in serum samples of dairy cattle from farms in selected states in Malaysia.

State	Herds Free from Infection	Herds Infected	Total Herds	Herd Prevalence (%)
Pahang	13	21	34	61.76
Sabah	0	23	23	100
Johor	0	34	34	100
Perak	3	26	29	89.66
Total	16	104	120	86.66

Table 5.2: Herd prevalence by % of BT by states in a BT seroprevalence study

Figure 5.2: Bar Chart in herd prevalence (%) of BT by state

Based on Figure 5.2, the total samples of positive against BTV antibodies among the dairy cattle population was observed high respectively in all states. However, Johor and Sabah recorded to have the highest herd prevalence (%) in which suggesting an increase in the seroprevalence of BTV antibodies among the dairy cattle population in Johor and Sabah.

Chapter 6

6.0 Discussion

This study was conducted to detect seroprevalence of BTV antibodies in the population specifically among dairy cattle in Malaysia. The cELISA test result showed significant increase in the seroprevalence of BTV antibodies among dairy cattle in Malaysia. It is speculated to be due to the environment of the farms itself with high fly population that influenced the seroprevalence of BTV antibodies detected by cELISA. Flies are the main vector involved in the transmission of BTV (Socha *et. al*., 2022). The life cycle of BTV vector has relation with climatic conditions, where *Culicoides* rely on warmth and moisture for its breeding and feeding (Chanda *et al*., 2019). In addition, the climate in Malaysia is equatorial, where weather occurs to be hot, humid, and rainy throughout the year, like Indonesia, Singapore, Brazil, Colombia, Kenya, Nigeria and Central African Republic. The temperature in Malaysia is usually high but stable ranging from 21°C to 32°C with rainfall between 2,000 mm to 2,5000 mm annually (B. T. Tan *et al.*, 2021). The high seroprevalence rate of BTV antibodies may indicate favourable climatic condition for breeding and survival of various stages of Culicoides vectors in the region (Elmahi *et al*., 2020). It probably persists due to the hot and humid climate which favours the proliferation of *Culicoides* spp. in the environment. Based on Roy (2008), BTV tends to remain in the infected *Culicoides* throughout its life. Therefore, there is a possibility for the BTV antibodies to be presented in the dairy cattle population in this study, leads to an increase seroprevalence against BTV antibodies in the herd.

However, BTV does not spread directly from host to host, but it can spreads based on the fly's movement and distribution with the assist of wind pattern that carries it (Aguilar-Vega *et. al*., 2019). The biting flies when flying at high to higher altitudes may easily be carried away by the flowing of air for dozens of kilometres, which thus explained the expansion ability of arbovirus

many kilometres away from a source. Most biting flies exhibit their abundance and increase its activity to be active at most during dusk or cloudy weather (Kočišová *et al*., 2021).

Despite being the less likely, the cattle may be due to acquired immunity secondary to recurrent natural or maternal exposure to BTV, whereby it is then highly likely for the herd to have developed antibodies in response to the past exposure or vaccination. A seroprevalence study in France in 2016 suggested the antibody longevity of BTV group-specific antibodies for at least 5 to 6 years after natural infection or vaccination. The booster vaccination prolonged the antibody longevity of vaccine induced antibodies and the number of serologically positive cattle (Ries & Beer, 2019). The possibility that insects could acquire vaccine virus by feeding on vaccinated cattle and transmit it to other cattle cannot be eliminated (Murray & Eaton, 1996).

The effort that can be done to control or prevent BTV infection is by having vector control and surveillance programs (O'Brien, 2022). It is crucial to control the fly's population in the environment of farms by removing the potential breeding site of the vector population. The use of insecticide can be considered for environmental control for adult flies. The application of larvicide Abate containing 5% temephos granulated with gypsum on the midge breeding ground can be an option to kill off larvae (Schmahl *et al.*, 2009). On top of that, close monitoring should be carried out intensively for the early control, eradication, and prevention of the possible spread of the BT infection. Our Department of Veterinary Services (DVS) also has prepared an action to be done based on WOAH guidelines if there is a BT outbreak in our country (DVS, 2014). In addition, the regulatory governing the importation of cattle by DVS ensures that all imported cattle meet the required health and safety standards (Azmi & Associates, 2023).

The cELISA test results showed 100% seropositive against BTV antibodies among the dairy cattle population in Johor, similar in Sabah. Cattle are readily susceptible to BTV infection, where they can be a source of infection for *Culicoides* species that biologically transmit BTV to other ruminant population (Elhassan *et al*., 2014d). In this study, the presence of BTV antibodies in tested serum samples may suggested that the dairy cattle population may have had previous BT exposure, whereby their immune system had produced the BT antibodies as part of the immune response to the BTV at some point in the past (Rodríguez-Martín *et al*., 2021b). Another possible reason for the high seroprevalence BTV antibodies could be due to the BT antibodies were passed as maternal antibodies though colostrum to the calves by the mother. If the mother was vaccinated against BTV, the possibility of maternal vaccination could also provide passive immunity to the offspring through antibody transfer by the colostrum intake after birth (Rojas *et al*., 2021).

Last but not least, according to Hamblin (2004), it is crucial to consider cross-reactivity when interpreting serological test results for BT. The presence of antibodies against BTV may not always indicate a direct infection with BTV, whereby the antibodies detected in the test could be generated as a response to a different virus of reovirus origin, however, it still shows some reactivity with BT. This is due to the cross-reactivity of the secondary antibody to the adsorbed antigen, which could increase the background noise. Confirmatory testing may be necessary to address any uncertainties arising from the potential cross-reactivity issues to reduce likelihood of false positives. Specific real time RT-PCR have recently been designed for some BTV serotypes that are circulating in given geographic areas, or to differentiate between vaccine and field trains of the BTV, or able to recognize all 24 BTV reference strains (Polci A. *et al*., 2007).

Chapter 7

7.0 Conclusion

The findings in this study suggested that BTV antibodies were prevalent among dairy cattle in all the selected states. The contact with sheep or other ruminants or transmission media such as the biting flies may increase the seroprevalence of BT in cattle (Gong *et al*., 2021). Although bluetongue virus is not a zoonotic disease, it is an important issue in international trade and can cause significant economic losses due to losses in production, with regards to the fact that biting flies cause enormous economic loss. Overall, the data obtained and interpreted in this study does provide a baseline information regarding the current seroprevalence of BTV antibodies among dairy cattle in Malaysia which would serve as part of surveillance for herd health status of dairy cattle in Malaysia. Generally, it is concluded that the BTV still exists and is circulating among our ruminant livestock industry in Malaysia. It is suggested to have continuous and consistent monitoring and surveillance programs of BTV in our ruminant's industry to prevent the potential outbreak of BTV in the future. As if it goes undetected and unaware, an import of BT infected animals is highly likely to contribute to a large-scale outbreak regardless of the time of year.

Chapter 8

8.0 Recommendation

In future, holistic study of seroprevalence is needed by adopting a bigger sample size involving different species of ruminants such as sheep, goats, deer, buffalo, and meat cattle for the surveillance of BTV to better predict the overall status of BTV antibodies in Malaysia.

9.0 Appendix

Figure 9.1: Sample preparation

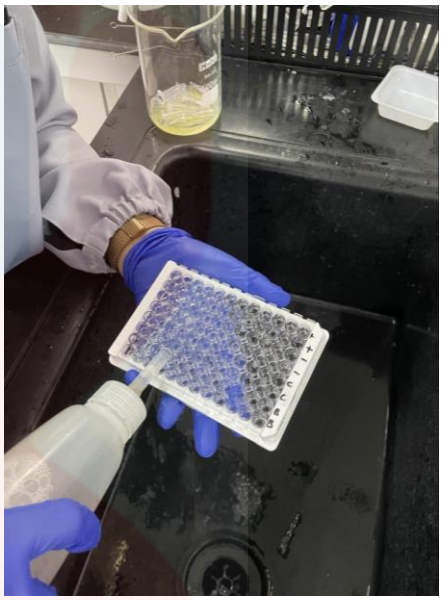

Figure 9.2: Washing step

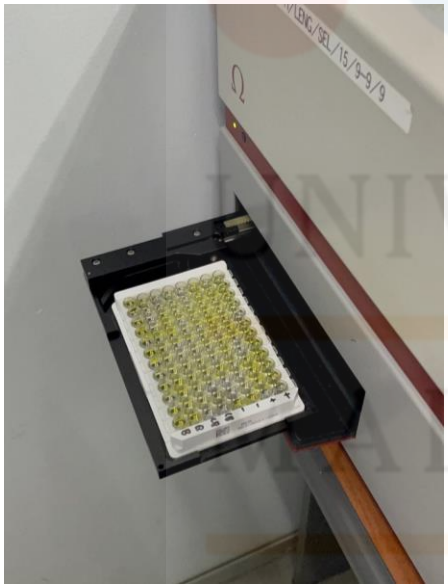

Figure 9.3: Loading sample into ELISA machine

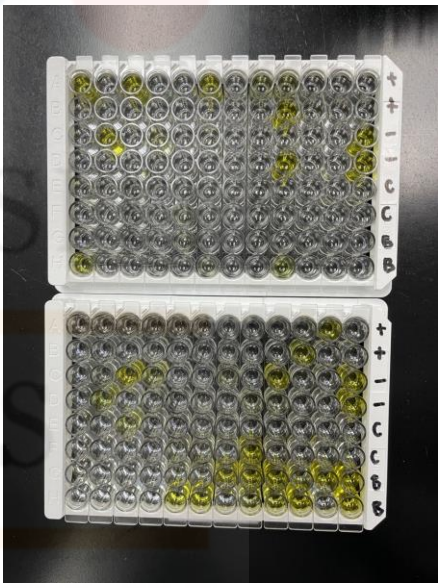

Figure 9.4: cELISA test kit result

10.0 Reference

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