# SEROLOGICAL DETECTION OF EPIZOOTIC HEMORRHAGIC DISEASE VIRUS (EHDV) AND ASSOCIATED RISK FACTORS IN GOATS IN KELANTAN, MALAYSIA.

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DOCTOR OF VETERINARY MEDICINE



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By

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2024

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# SEROLOGICAL DETECTION OF EPIZOOTIC HEMORRHAGIC DISEASE VIRUS (EHDV) AND ASSOCIATED RISK FACTORS IN GOATS IN KELANTAN, MALAYSIA



## ABSTRACT

Epizootic hemorrhagic disease virus (EHDV) is a notifiable, infectious but non-contagious hemorrhagic disease caused by a non-enveloped double-stranded RNA virus that belongs to the Reoviridae family (genus: Orbivirus). There are 7 known serotypes (EHDV- 1, 2, 4, 5, 6, 7 and 8). This virus is closely related to the Bluetongue virus, exhibiting similar clinical presentations and sharing the same transmission vectors. Nonetheless, these two viruses are genetically distinct. The common clinical signs that may be manifested by infected animals are facial oedema, hyperemia of the mucous membrane, excessive salivation, bloody diarrhea, ulceration in the oral cavity and others. Globally, the most reported and widely studied animal related to EHDV is the White-tailed deer as it is the most susceptible animal to EHDV while other species such as cattle may also be infected with less severe clinical signs. However, there are limited studies of EHDV in Malaysia, especially in goats. Therefore, the main objective of this study is to serve as a baseline study for the serological detection of EHDV in goats in Malaysia. For this study, competitive ELISA technique is used to detect the antibody against EHDV VP7 protein. This method is WOAH's current preferred diagnostic method as it does not cause cross -reactivity between EHDV and Bluetongue virus. In this study, blood samples from 2 farms (total: 40 goats) were collected for the detection of the antibodies in which 3 out of 40 goats were EHDV positive. EHDV is one of the economically important diseases in ruminants as it could lead to economic losses and reduced productivity among infected animals. Therefore, this study can be set as a preliminary study of EHDV status in goats especially in Malaysia for future study. In addition to that, early detection allows faster intervention to control this disease.

Keywords: EHDV, WOAH, c-ELISA, VP7 protein

**UJIAN SEROLOGI BAGI *EPIZOOTIC HEMORRHAGIC DISEASE VIRUS* DAN FAKTOR RISIKO YANG BERKAIT DALAM KALANGAN KAMBING DI KOTA BHARU DAN BACHOK, KELANTAN, MALAYSIA**

## ABSTRAK

*Epizootic hemorrhagic disease virus* (EHDV) ialah sejenis penyakit wajib lapor dan mudah berjangkit tetapi tidak mudah tular dari satu haiwan ke haiwan yang lain. Penyakit ini disebabkan oleh sejenis virus RNA berbenang kembar dan tidak bersampul yang merupakan salah satu ahli keluarga Reoviridae (genera: Orbivirus). Terdapat 7 serotype yang telah dikenal pasti iaitu EHDV-1,2,4,5,6,7 dan 8. Virus ini berkait rapat dengan virus Bluetongue dari segi tanda klinikal dan vektor penyebar. Walaubagaimanapun, kedua-dua virus ini berbeza dari segi genetik. Antara simptom yang mungkin ditunjukkan oleh haiwan dijangkiti adalah seperti bengkak di bahagian muka, kemerahan membran mukosa, penghasilan air liur secara berlebihan, cirit birit berdarah, ulser di dalam bahagian mulut dan sebagainya. Secara global, kes yang paling banyak dilaporkan ialah berkaitan dengan rusa (*Odocoileus virginianus)*. Namun, haiwan-haiwan lain juga boleh dijangkiti oleh virus ini seperti lembu dengan simptom klinikal yang tidak teruk. Kajian saintifik berkaitan dengan EHDV di Malaysia terutamanya yang melibatkan kambing adalah terhad. Oleh itu, objektif utama kajian ini ialah berfungsi sebagai kajian asas untuk mengesan EHDV dalam kalangan kambing di Malaysia. Bagi kajian ini, teknik ELISA kompetitif digunapakai untuk mengesan antibodi terhadap protein VP7 EHDV. Kaedah ini ialah kaedah diagnostik pilihan yang disarankan oleh WOAH kerana ianya tidak menghasilkan tindakbalas silang antara EHDV dan Bluetongue virus. Dalam kajian ini, sampel darah dari 2 ladang (jumlah: 40 ekor kambing) telah diambil bagi mengesan antibodi terhadap EHDV di mana 3 daripada 40 ekor kambing telah dikesan sebagai positif. EHDV ialah salah satu penyakit yang telah disenaraikan dalam penyakit penting dari segi ekonomi terutamanya dalam kalangan ruminan kerana ianya mampu mengakibatkan kerugian ekonomi kepada penternak serta penurunan produktiviti. Oleh yang demikian, kajian ini mampu untuk dijadikan sebagai kajian awal bagi mengetahui status EHDV dalam kalangan kambing di Malaysia bagi membantu kajian-kajian yang lebih mendalam pada masa akan datang. Tambahan pula, pengesanan awal penyakit ini membolehkan para penternak dan pihak berwajib untuk mengambil tindakan segera diambil bagi mengelakkan penyebaran penyakit ini.

Kata kunci: EHDV, WOAH, c-ELISA, VP7 protein

CERTIFICATION

This is to certify that we have read this research paper entitled **'Serological Detection of Epizootic Hemorrhagic Disease Virus and Associated Risk Factors in Goats in Kelantan, Malaysia'** by **Maria Fazira binti Mohd Zulkefli**, and in our opinion, it is satisfactory in terms of scope, quality, and presentation as partial fulfillment of the requirements for the course DVT 55204 – Research Project.

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This project is dedicated to my family, which has been my main support system from the beginning. To my beloved parents, Mohd Zulkefli and Che Mawarizal, for their sacrifices, support and prayers that has been my main source of motivation and strength. To the dean of Faculty of Veterinary Medicine, Prof. Madya Dr. Mohd Farhan Hanif and my supervisors, Dr. Intan Noor Aina, Dr. Mohammad Sabri and Dr. Dauda Goni, whose guidance, encouragement and wisdom have been my inspiration throughout my academic journey. I also dedicate this project to my friends and loved ones, who have been my good companions throughout this beautiful and memorable journey. May this project be a contribution to the advancement of knowledge in animal health and disease prevention.

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## LIST OF ABBREVIATIONS

BT - Blue tongue

c-ELISA - Competitive ELISA

CI - Confidence interval

DIVA - Differentiating Infected from Vaccinated Animals

DVS - Department of Veterinary Service

EHDV - Epizootic Hemorrhagic Disease Virus

EIA- Enzyme immunoassay

ELISA - Enzyme linked immunosorbent assay

HD - Hemorrhagic disease

IACUC - Institutional Animal Care and Use Committee

n - number

OD - Optical Density

RNA - Ribonucleic acid

RT-PCR - Reverse Transcription Polymerase Chain

UVDC - UMK Veterinary Diagnostic Center

VP- Viral protein

WOAH - World Organization of Animal Health

## LIST OF SYMBOLS

% Percentage

°C Degree celsius

µL microliter

nm nanometre

$X^2$ Chi-square value

# CHAPTER 1

# INTRODUCTION

## 1.1 Research Background

Epizootic Haemorrhagic Disease Virus (EHDV) is a disease caused by a non-enveloped double-stranded RNA virus belonging to the family of Reoviridae and genus Orbivirus that consists of 7 known serotypes (EHDV- 1, 2, 4, 5, 6, 7 and 8) (Caixeta et al., 2024). EHDV is transmitted by biological vectors such as the biting midges, *Culicoides* spp. and predominantly infecting deer, specifically the White-tailed deer (*Odocoileus virginianus*) and cattle (Yang et al., 2020).

EHDV is listed as one of the notifiable diseases by the World Organisation of Animal Health (WOAH). Up to this date, there are still no isolated and documented cases of EHDV in Malaysia. However, there are reported cases of EHDV in neighboring countries such as Indonesia and other Asian countries such as China, Taiwan and Japan. Goats infected with EHDV may show clinical signs of edema of the head and neck, labored breathing due to pulmonary edema or pleural effusion, stomatitis with excessive salivation. Besides that, this virus also causes abortion, reduced milk production and infertility. EHDV causes a negative impact on the economical aspects hence why it is one of the important diseases. Therefore, economic loss can be prevented by determining the prevalence of EHDV in Malaysia, specifically in Bachok and Kota Bharu, Kelantan and its associated risk factors.

## 1.2 Research problem

Cases related to EHDV have been reported globally in white-tailed deer and cattle. However, to date, only a few cases of EHDV in goats have been reported globally. In Malaysia, there is still a lack of studies on EHDV, especially in goats.

## 1.3 Research questions

1. Is EHDV serologically detectable in goats in Kelantan?
2. What are the risk factors associated with EHDV in goats in Kelantan?

## 1.4 Research hypothesis

1. EHDV is serologically detectable in goats in Bachok and Kota Bharu, Kelantan.
2. The associated risk factors for EHDV in goats are sex, age, goat's origin, vector control and farm management .

## 1.5 Research objective

1. To detect the presence of antibodies against EHDV in goats in Bachok and Kota Bharu, Kelantan.
2. To determine the associated risk factors of EHDV in goats in the targeted farms.

# CHAPTER 2

# LITERATURE REVIEW

## 2.1 Agent description

EHDV is caused by an icosahedral capsid, non-enveloped double stranded RNA virus that belongs to the family Reoviridae and genus Orbivirus. According to Jiménez-Cabello et al., (2023), there are 3 layers of protein in the icosahedral which are the inner, intermediate and outer layers as described in Figure 2.1. The outermost layer is made up of VP5 and VP2 while the intermediate layer is constituted of the VP7 and VP3. Whereas the innermost layer is composed of RNA polymerase complexes (VP1, VP4 and VP6).

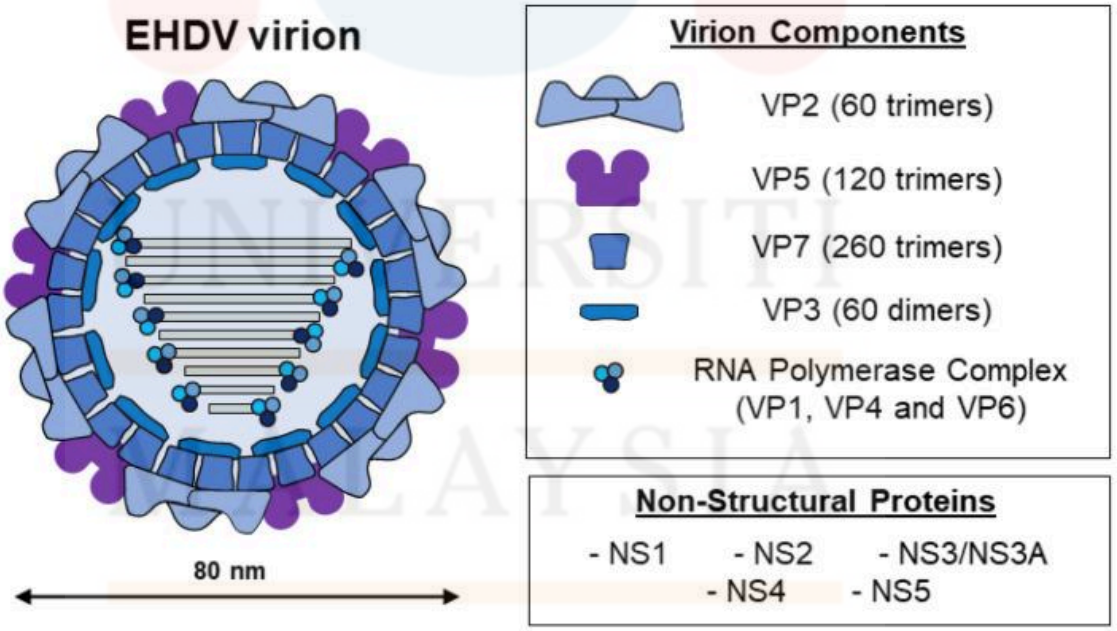



Figure 2.1 shows the 3 protein layers of icosahedral capsid

Source: Jiménez-Cabello et al., (2023)

The proteins responsible for initiating infection in the susceptible host by entering and attaching onto the host cells are VP2 and VP5. In addition, VP2 is mainly the protein used in molecular typing assays to identify EHDV serotype. The proteins that are involved in viral replication are VP1, VP4 and VP6. Diagnosis of EHDV cases by using enzyme-linked-immunosorbent assays (ELISA) test kit is mainly detecting protein VP7. There are also non-structural proteins that support the important viral process of Orbivirus such as control of immune response, the packaging of the genome, intracellular transport, capsid assembly and release of the virus.

## 2.2 Serotype of EHDV

According to Xin, et. al., (2023), there are 7 serotypes of EHDV have been identified and documented which are serotypes 1, 2, 4, 5, 6, 7, and 8. EHDV serotype 3 that was isolated from Nigeria (Nigerian strain Ib Ar 22619) was similar to serotype 1.

EHDV was first identified from North American white-tailed deer (*Odocoileus virginianus*) in 1955 in the USA. To date, this virus has spread throughout the globe, affecting the tropical and temperate regions. It has been identified in North and South America, Africa, Middle East, Oceania and Asia. Six out of seven serotypes (Serotype 1, 2, 5, 6, 7 and 8) have been detected in cattle in Australia (Noronha et al., 2021). In Japan, EHDV serotype 2 (Ibaraki) was first identified in cattle in 1959 followed by EHDV-7 in 2016 and EHDV-6 (Yamamoto et al., 2021). Moreover, EHDV-5 and EHDV-6 have been recently isolated from *Culicoides* sp. in Japan (Jiménez-Cabello et al., 2023).

According to Duan et al., (2022), EHDV has been detected in 14 out of 15 studied provinces in China, with different prevalence rates, Tibet being the only

province free from EHDV. Based on the same study, 5 serotypes of EHDV have been identified in China which are EHDV-1,-5,-6,-7,-8.

In 2006, EHDV serotype 6 was detected in cattle in Turkey, Morocco, Algeria and Jordan (Temizel et al., 2009). Based on a study conducted by Dhou et al., (2016), a significant morbidity in cattle was observed in early September 2006 in Tunisia. The result from the study revealed the identification of EHDV serotype 6 in Tunisia. In Egypt, a study was conducted in 2016 by Ahmed et al., (2019) and EHDV serotype 1 was identified in cattle that were having reproductive problems. According to a study by Mohammed et al., (1996), at least 2 serotypes are known to be enzootic among cattle in Sudan which were EHDV-5 and EHDV-318. EHDV-318 is an untyped EHDV (isolate 318) isolated from a sentinel calf herd at the Khartoum University farm. Even so, in 2009, Anthony et al., characterized then unclassified EHDV-318 to be a member of EHDV-6. The serotype isolated from Nigeria was first known as EHDV serotype 3. However, it was later known to be similar to EHDV-1 (Anthony et al., 2009). Hence, in Nigeria, the 2 serotypes successfully isolated were EHDV-3 (or EHDV-1) and EHDV-4 (Campbell & St George, 1986).

Until now, no reported and documented cases of EHDV in Malaysia. The nearest documented case of EHDV to Malaysia was from Indonesia in 1991. This case study was conducted to detect the prevalence of EHDV-5 in buffaloes, cattle, goats and sheep by using AGID. As a result, EHDV-5 was the highest in cattle (24%) followed by buffaloes (11%), goats (2%) and sheep 0% (Sendow et al., 1991).

## 2.3 Economical and welfare impact of EHDV

EHDV is not a zoonotic disease nor a food safety concern. However, EHDV is one of the diseases listed in the WOAH Terrestrial Animal Health Code due to its direct

and indirect economic impact and significant welfare implications to animals (Jiménez-Cabello et al., 2023). According to Kedmi et al., (2010), there was high correlation between EHDV seroprevalence and milk loss in dairy cattle where 50% seroprevalence resulted in about 133 kg milk loss while 100% seroprevalence led to 204 kg of milk loss. In this study, the average loss per cow due to EHDV was estimated at US$26.5 per cow (Kedmi et al., 2010).

In the aspect of welfare impacts, EHDV causes severe pain due to the clinical signs manifested such as oral, tongue and lips ulceration and erosion and lameness due to coronary band lesions. On the other hand, distress is associated with the presence of fever, anorexia and dehydration as the result of diarrhea. Besides that, poor welfare may also be indicated from reduced growth rate, decreased milk production and impaired fertility (Bøtner et al.,2009).

## 2.4 Transmission of EHDV

EHDV is transmitted mainly via arthropod vectors with the only known vector, Culicoides midges. According to Xin et al., (2023) there are 1400 *Culicoides* species, of which from the total, 30 known species are able to transmit EHDV. According to Wittmann et al., (2002), *Culicoides* sp. ability to transmit EHDV also depends on the genotype of the vector and virus. Few factors are associated with the competency of *Culicoides* as the vector of this disease based on the strain of EHDV and environmental factors such as the temperature and humidity (Xin et al., 2023). Since EHDV is transmitted by arthropod vector, transboundary transmission is a concern as it will further contribute to the spread of this disease. The transmission of EHDV by *Culicoides* spp. is illustrated in Figure 2.2.

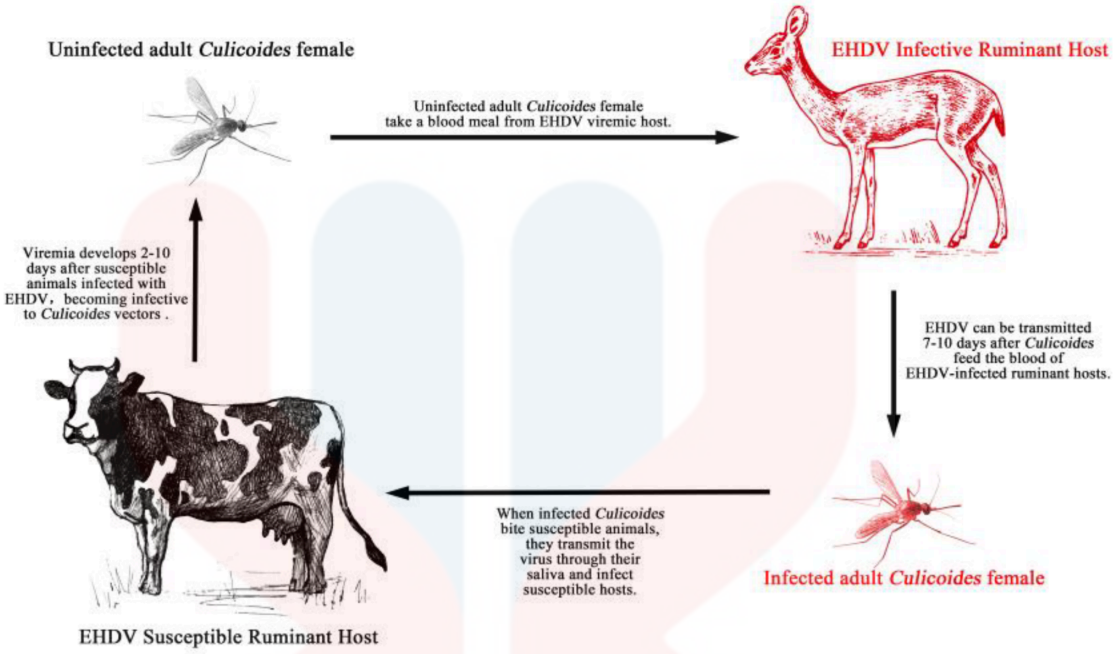



Figure 2.2: The transmission of EHDV by *Culicoides* spp.

Source: Xin et al., (2023)

## 2.5 Laboratory diagnosis of EHDV

According to WOAH, EHDV can be diagnosed by several recommended diagnostic workups. Among these are Reverse Transcription Polymerase Chain Reaction (RT-PCR), competitive enzyme-linked immunosorbent assays (C-ELISA) or sandwich ELISA. RT-PCR is useful in the detection of RNA viruses as it helps to make a double-stranded DNA from a RNA template before amplifying it (Coffin et al., 1997). The competitive ELISA is the test to detect the presence of an antibody that is specific for an antigen presence in the test serum. It uses 2 types of specific antibodies in competitive ELISA (an enzyme-conjugated antibody and antibody present in the test). The presence of color change on the test kit indicates a negative result, while the absence of color change indicates a positive result in C-ELISA. On the other hand, sandwich ELISA in the test consists of an antigen that has been "sandwiched" between 2 layers of antibodies from the capture and detection antibodies. Sandwich ELISA has

the highest sensitivity compared to all the other types of ELISA including the competitive ELISA (Alhajj et al., 2023).

Other serological laboratory diagnoses can be used such as the agar immunodiffusion and indirect ELISA. However, these tests have their limitations as they are not able to differentiate between antibodies being produced by EHDV or Bluetongue Virus (WOAH, 2021).

## 2.6 Treatment of EHDV

There is no treatment for EHDV and it is treated with only supportive care to improve the quality of life, reduce discomfort and pain of the infected animals (Thompson & Goodrich, 2018).

## 2.7 Prevention and control

EHDV can be prevented by vaccination. According to Jiménez-Cabello et al., (2023), few types of vaccine against EHDV have been commercialized. For example, in Japan, 2 types of vaccine against EHDV-2 are available (monovalent live attenuated vaccine and inactivated bivalent vaccine). Nevertheless, there is a need for the production of next-generation vaccines against EHDV that is able to overcome the disadvantages of current vaccines. The new vaccines to be produced must allow the differentiation between naturally infected and infection in vaccinated animals (DIVA) and it should be able to give a better range of protection against multiple serotypes of EHDV (Jiménez-Cabello et al., (2023). Additionally, controlling the vector is also one of the important ways to control the spread of this virus (Thompson & Goodrich, 2018). *Culicoides* biting midges can be controlled by the application of insecticides and pathogens to the breeding sites, elimination of the breeding sites and the usage of repellent or kairomones to kill adult midges (Carpenter et al., 2008).

# CHAPTER 3

# RESEARCH METHODOLOGY

## Sampling method and procedure

### 3.1 Animal ethics statement

This study has acquired the animal ethics approval from the Institutional Animal Care and Use Committee (IACUC) of Universiti Malaysia Kelantan with the approval reference: UMK/FPV/IACUC/FYP/009/2024.

### 3.2 Sample collection

Blood samples were collected into plain blood tubes from 40 goats via jugular venipuncture from 2 farms: Farm A, 23 samples while Farm B, 17 samples. These 2 farms are located in Kelantan at Melor and Bachok respectively. Following that, the blood samples were temporarily stored in a polystyrene filled with ice packs for transportation from the farm to UMK Veterinary Diagnostic Center (UVDC). Upon arrival at the laboratory, the blood samples were stored in the freezer at -20 °C.

### 3.3 Sample and wash solution preparation

For this study, a competitive ELISA diagnostic kit from IDVet, France was used to detect the antibodies against VP7 protein of EHDV. C-ELISA is currently the preferred diagnostic tool for EHDV detection, as recommended by WOAH.

Firstly, the frozen blood sample was thawed at room temperature Subsequently, the blood samples were centrifuged at 2500 rpm for 3 minutes. Next, 200 µL of the

blood serum was carefully harvested by using a pipette and transferred into the labeled microcentrifuge tube before it was stored in the freezer at -20 °C.

On the next day, the frozen blood serum was thawed and all the reagents were allowed to come to room temperature at 21°C (±5°C). Meanwhile, the wash solution (1X) was prepared by diluting the Wash Concentrate (20X) in distilled water according to the calculation 3.1 as the following:

$$300\ \mu L \times 4 \text{ times wash} \times 96 \text{ well} = 115{,}200 \quad (3.1)$$

$$\text{Convert } \mu L \text{ to mL} = 115{,}200 \times 0.001$$

$$= 115.2 \text{ mL}$$

Assume 200 mL of distilled water is needed

$$M1V1 = M2V2 \quad (3.2)$$

$$20\ (V1) = 1\ (200)$$

$$V1 = 10$$

Therefore, 10mL of wash concentrate will be mixed into 190 mL of distilled water to get 1X Wash Solution.

## 3.4 Serological Detection

Following that, all the reagents were homogenized by using the vortex mixer. Next, 50µL of the dilution buffer was added into each well. Then, 20 µL of positive control was added into well A11. A12, B11 and B12. Negative control was then added to wells C11, C12, D11 and D12. After that, 20µL of each sample to be tested were

added to the remaining wells except for the wells located at column 11 and 12. Each sample was added into 2 wells adjacent to one another and the average result will be calculated. The plate was then covered with aluminum foil before being incubated in the incubator for 1 hour at 21°C.

After 1 hour, the wells were emptied by carefully inverting the plate vertically. Then, each well was washed 2 times with 300 µL wash solution (a 5 minute resting interval in between the first and second wash should be applied). During the 5 minutes interval, the conjugate (1X) was prepared by diluting the concentrated conjugate (10X) with dilution buffer 3 based on the calculation 3.2 below:

100 µL (per well) conjugate x 96 well = 9600 µL (3.3)

Conversion of µL to mL= 9600 µL x 0.001

= 9.6 mL

Assume 10 mL of dilution buffer required

M1V1=M2V2 (3.4)

10 (V1) = 1(10)

V1 = 1ml of concentrated conjugate

Therefore, 1mL of concentrated conjugate was mixed with 9mL of Dilution Buffer 3 to produce 10mL of conjugate (1X).

Thereafter, 100 µL of conjugate (1x) was added into each well except for G11 and G12. The plate was then covered with aluminum foil and incubated at 21 °C for 30 minutes. This was followed by emptying and washing the wells again before 100 µL of substrate solution was added to each well except for wells H11 and H12. The plate was

then covered again and incubated in a dark environment for 15 minutes to allow the reaction to take place. Next, 100 µL stop solution was added to each well to stop the reaction and lastly the plate was placed in the ELISA plate reader at 450 nm and the Optic Density (OD) was recorded. The methodology conducted in this study was summarized in Figure 3.1.

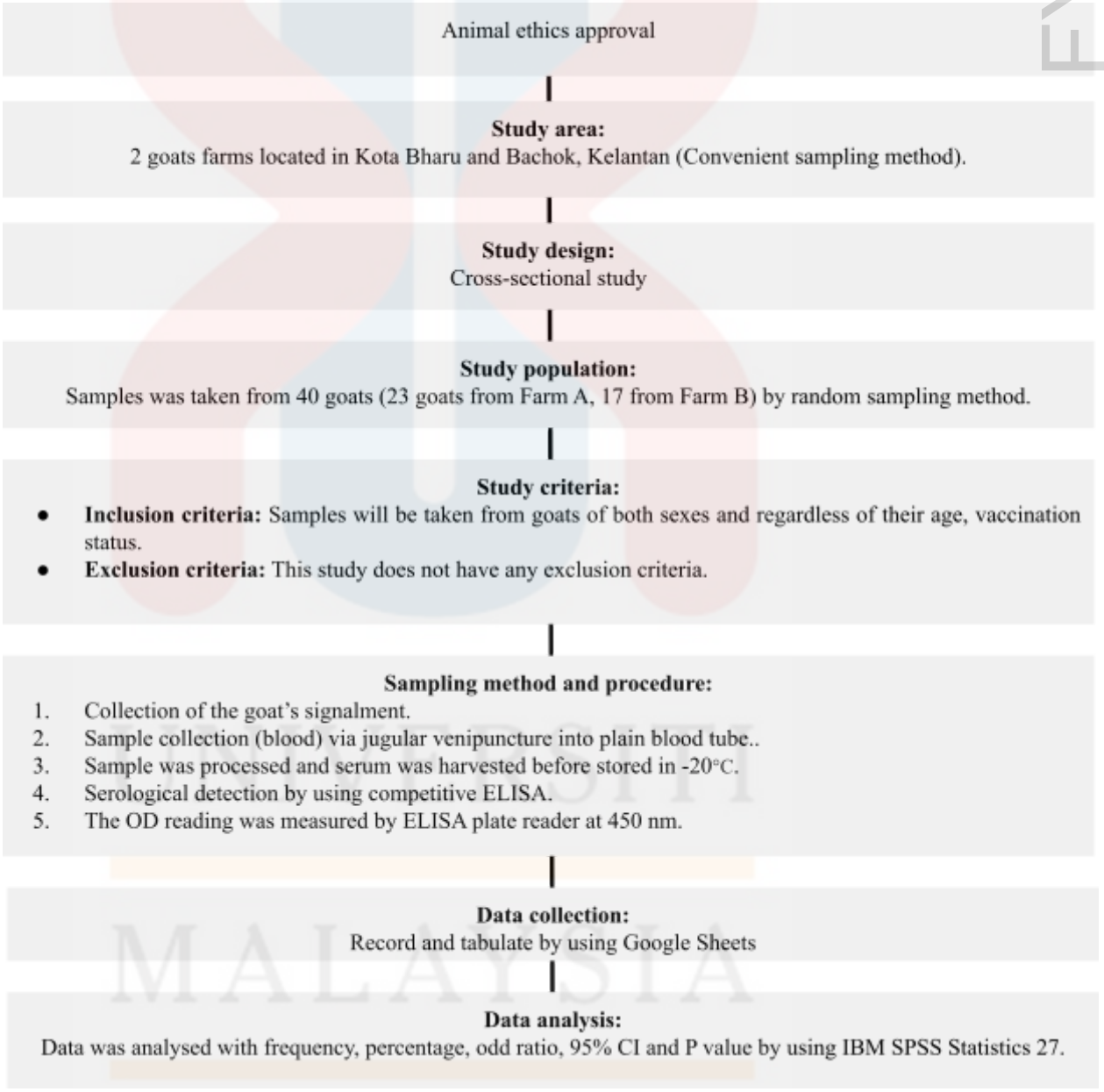



Figure 3.1: Summary of the methodology conducted in this study

# CHAPTER 4

# RESULTS AND DISCUSSION

## 4.1 Result

Based on the result obtained, Farm A showed two positive samples while Farm B showed one positive sample. Out of the three positive samples, the true positive result was only one sample from Farm A, whereas the other two samples (one from each farm) were doubtful. The data is represented in Table 1 and Figure 1.

Table 4.1: Frequency table for positive and negative EHDV in Farm A and B

| Farm | No. of sample | No. of positive (Frequency) | No. of negative (Frequency) |
|---|---|---|---|
| A | 23 | 2 | 21 |
| B | 17 | 1 | 16 |
| Total | 40 | 3 | 37 |
| Percentage (%) | | 7.5 | 92.5 |

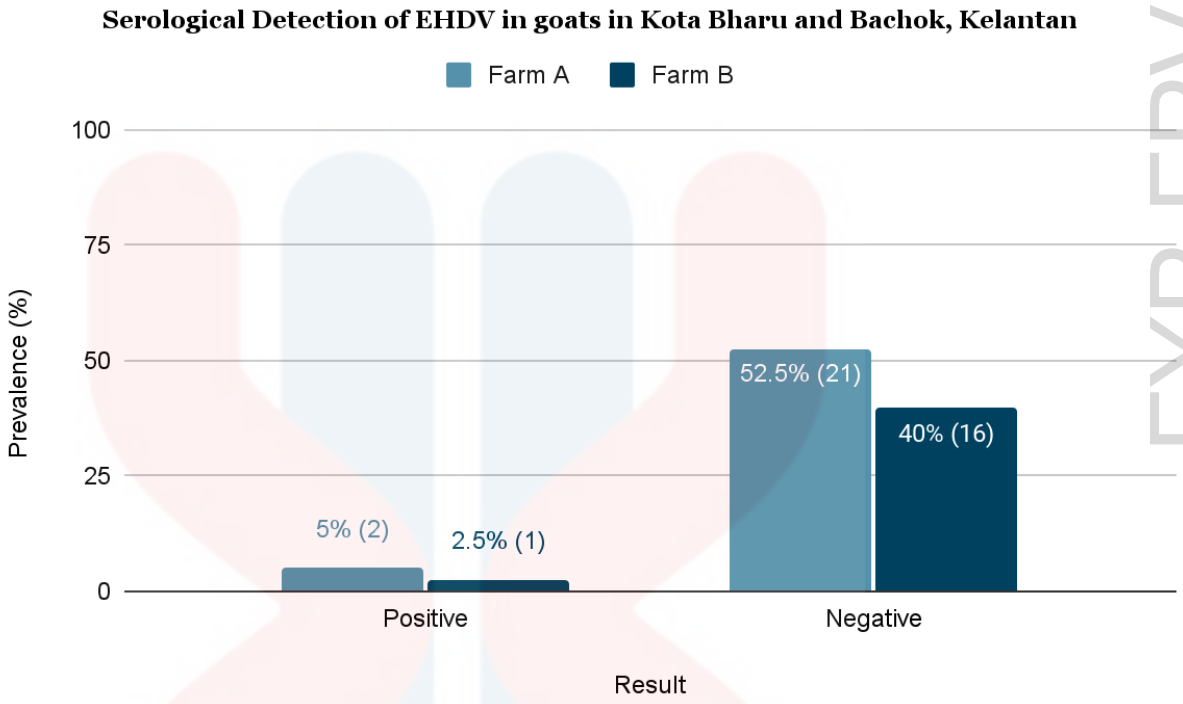



Figure 4.1: The bar chart representing the percentage of serological detection of EHDV in Farm A and Farm B

The results were further broken down into groups of each variable of possible risk factors as represented in Table 2. The age was separated into two groups, which are less than a year and 1 to 6 years old with three positive cases. As for the origin of the goats, Farm A acquired the goats from external suppliers, presented with two positive cases while the goats in Farm B were mostly locally bred on the farm itself (native) and showed one positive goat. Farm A practices some extent of vector control (two positive cases) while Farm B does not practice vector control (one positive case). The last variable is the farm management where two positive cases were from intensive while the other one was from semi-intensive farm management.

Table 4.2: The statistical relationship between the risk factors and the seropositivity to EHDV

| Variables | Number of animal (n) | Positive (n) | Prevalence (%) | $\chi^2$ | P value | Odd ratio | 95% Cl |
|---|---|---|---|---|---|---|---|
| Age (years) | | | | 0.463 | 0.496 | 0 | 0.988 - 1.211 |
| <1 | 5 | 0 | 0 | | | | |
| 1-6 | 35 | 3 | 7.5 | | | | |
| Sex | | | | 4.388 | 0.036 | 0 | 0.974 - 1.513 |
| Male | 22 | 0 | 0 | | | | |
| Female | 18 | 3 | 16.7 | | | | |
| Origin | | | | 0.112 | 0.738 | | 0.127 - 18.324 |
| Acquired | 23 | 2 | 8.70 | | | 1.5 | |
| Native | 17 | 1 | 5.88 | | | 1 | |
| Vector control | | | | 0.112 | 0.738 | | 0.127 - 18.324 |
| Yes | 23 | 2 | 8.70 | | | 1.5 | |
| No | 17 | 1 | 5.88 | | | 1 | |
| Farm management | | | | 0.112 | 0.738 | | 0.127 - 18.324 |
| Intensive | 23 | 2 | 8.70 | | | 1.5 | |
| Semi-intensive | 17 | 1 | 5.88 | | | 1 | |

*P-value <0.05 is considered significant*

## 4.2 Discussion

This study was conducted to detect the seroprevalence and the associated risk factors of goats in Kelantan. It is the first successful study of serological detection of antibodies against EHDV VP7 protein conducted by the using competitive Enzyme-Linked Immunosorbent Assay (C-ELISA) technique in goats in Malaysia. The primary purpose of conducting this study is to establish a baseline assessment of EHDV status in goats, especially in Kelantan, Malaysia.

According to Alhajj et al., (2023), ELISA uses the technique of heterogeneous enzyme immunoassay (EIA) which involves bound components (to the microplate) and free components. There are four main types of ELISA available: Direct, Indirect, Sandwich and Competitive ELISA. For this EHDV serological detection, Competitive ELISA or c-ELISA is used to detect the antibody against EHDV VP7 protein. The concept behind c-ELISA is by utilizing two types of antibodies which are the enzyme-conjugated antibody and antibody in the sample. These two antibodies will compete among themselves for binding to the bounded antigen. As a result, the higher the concentration of antibodies in the sample, the more it will bind to the bounded antigen. Hence, the weaker the signals are produced (Sakamoto et al., 2018). According to Bréard et al., (2020), the measured specificity of this c-ELISA is 100% with excellent sensitivity by which the antibodies are detectable as early as 7 to 15 days post-infection.

Bluetongue (BT) and EHDV are closely related as both are within the same genus, Orbivirus (family: Reoviridae) but distinct species (Ward, 2011). These two viruses may exhibit similar clinical signs and lesions; the syndrome caused by BT and EHDV is known as hemorrhagic disease (HD) (Rivera et al., 2021). Despite the similarities, they have discrete genetic makeup and do not lead to serologically cross-reactivity (Brown-Joseph et al., 2018). Referring to the Terrestrial Manual issued by WOAH in 2021, cross reactivity may occur between EHDV and BTV that lead to misinterpretation of BTV positive as EHDV positive in the event of immunological methods in which weak immunofluorescence reaction with polyclonal anti-EHDV antiserum occurs. According to WOAH (2021) which is consistent with Brown-Joseph et al., c-ELISA is the current preferred diagnostic method that prevents cross-reactivity between EHDV and BTV by using monoclonal antibodies capable of detecting the EHDV serogroup-specific antibodies.

Based on the result, 3 from 40 samples (7.5%) were positive while the others were negative of antibodies against EHDV. Of the three positive samples, only one was confirmed positive with the competition percentage (SN %) falling in the range of less than or equal to 30% whereas the other two fell in the doubtful range (30-40%). However, in this study, doubtful samples were considered weak positives and further actions are required to deal with these samples which will be further discussed later in this chapter. More than or equal to 40% indicate that the sample is negative.

The samples evaluated in this study showed three seropositive goats that are more than 1 year old. However, based on the chi-square, age as one of the risk factors influencing EHDV positivity was insignificant (P value >0.05). The result is aligned with the findings of a study conducted by Cottingham et al., (2021), where they found that there was no significant difference of EHDV in terms of age. Based on the result in this study, there is a significant association between sex and EHDV status. Considering BTV can be transmitted vertically, it is contrary to EHDV as there is no evidence it could be passed from mother to their offspring vertically. Even so, a few abortion cases have been reported in EHDV-positive cattle with EHDV-6 isolated from the aborted fetuses and placentas (Golender et al., 2021). In addition to that, Lv et al., (2023) also stated, cattle that are infected by specific serotypes of EHDV may develop clinical signs of abortion or stillbirth. Limited information is available about age and sex-related susceptibility to EHDV in goats owing to goats being less susceptible to EHDV.

The main route of transmission for EHDV is via the biting of *Culicoides* sp. Therefore, in this study, the origin of the goat, vector control and type of farm management are included as one of the risk factors that could influence the spread of EHDV in these two farms. In farm A, they mostly sourced their goats from other local breeders (outsourced) and also by importing these goats from other countries such as Thailand and Australia. While Farm B breeds their goats from local sources. As the

Department of Agriculture and Fisheries of Australia indicated, 6 out of 7 serotypes have been isolated in Australia (EHDV-1,2,5,6,7,8). No confirmed EHDV cases in Thailand have been reported but a study conducted in 2021 by Fujisawa et al., (2021) has stated the presence of bluetongue virus detected from four species of *Culicoides*. Since EHDV and BTV are transmitted by *Culicoides* and closely related to one another, further study may be required to detect EHDV. Based on this study, it was proven that more serologically positive cases are detected in farms that acquired their goats from imported sources compared to the farms that practice closed-herds.

Vector control in this study is only limited to the practice of red-light lamps in Farm A as it is believed that *Culicoides* spp. are less likely to be attracted to red light. This statement is supported by González et al., (2016), *Culicoides* is more attracted to green, blue or ultraviolet (UV) light compared to red light as these lights resemble the natural moonlight. Whereas red light has a longer wavelength ranging from 620 - 750 nm, does not attract the insect to the surrounding area and can be beneficial to livestock farmers. However, based on this study, more positive cases were detected on the farm practicing red light. Few factors may contribute to this result such as limited effectiveness of the red lamp when used as a single pest control method. The effectiveness of red lamps can be enhanced when practiced together with environmental management such as reducing the breeding sites and proper waste management (Marzoli et al., 2021). Besides that, the usage of insecticide in the high breeding areas and also the installation of UV light traps to reduce the *Culicoides* spp. population on the farm.

There is no published research on the correlation between intensive farming and susceptibility to EHDV. However, the intensive farming practice may contribute to higher serological detection of EHDV aligned with the result of this study considering the stocking density of goats is commonly higher in intensive practice, and the space

between goats is lesser compared to semi-intensive or extensive farming (high stocking density). This situation increases the chance for a single infected vector to infect multiple goats besides the naive vector feeding on the infected goat and passing the virus to other naive goats. In addition to that, intensive farming often causes more stress to the animals which could lead to weaker immune systems and increase the susceptibility to the disease. Moreover, improper management of intensive farming tends to lead to the presence of standing water and the accumulation of manure that could be the breeding site of the vector.

In any suspected case of EHDV based on the clinical manifestations, the Department of Veterinary Service (DVS) has set protocols to manage EHDV that align with WOAH guidelines and involve main preventive measures such as screening and diagnostic testing in the animal population. Serological tests such as c-ELISA used in this study can be used for screening while PCR can be used for serotype identification using serotype-specific primers before conducting sequencing (Sailleau et al., 2011; WOAH Terrestrial Manual, 2021). When the farmers noticed any of the clinical signs, they should immediately seek veterinarian assistance or report to the DVS.

EHDV commonly infect wild animals in nature such as deer, but a number of experimental studies have been conducted on other animals, especially livestocks including cattle, sheep, goats and pigs to investigate the susceptibility of EHDV and the clinical signs manifestation in these animals. Deer and cattle are known to be the main ruminant host of EHDV (WOAH, 2021). On the other hand, goats are less susceptible to this disease as discussed in a study conducted by Duan et al., (2022) which concluded that the serological-positive goats were not more than 50% compared to the seropositivity rate of cattle that ranged from 0 to 100%. There is limited scientific research that discusses the resilient factors of goats against EHDV. Nonetheless, according to Jiménez-Cabello et al., (2023), one theory is that the goat's immune system may play an important role in which their innate immune response could recognize EHDV faster or exhibit a more efficient response in eliminating the virus antigen.

# CHAPTER 5

# CONCLUSION AND RECOMMENDATION

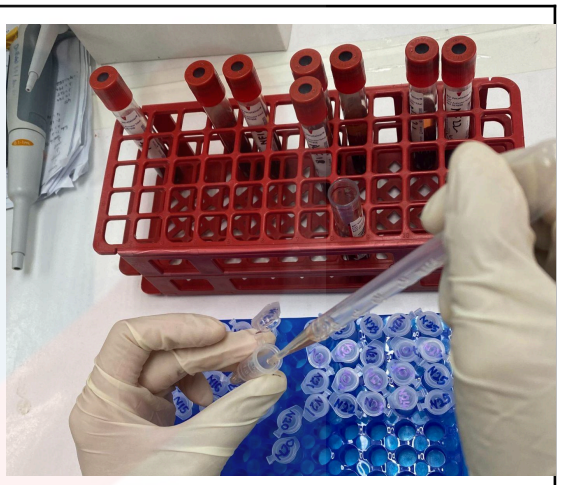
## 5.1 Conclusion

In conclusion, three out of 40 goats were serologically positive for EHDV by using the c-ELISA method that detects the presence of antibodies against EHDV VP7 protein. Sex was the only statistically significant risk factor associated with EHDV in this study.

## 5.2 Recommendations for future work

This study serves as the preliminary study for the detection of EHDV in goats in Malaysia. For future studies, increasing the number of samples helps obtain more reliable results and reduces the bias. Besides that, adding more farms and diversifying the study location will provide a more comprehensive result. Lastly, to improve the impact of the study, the choice of risk factors should be helpful in further understanding EHDV besides enabling better disease control and management.

Blood sample collection via jugular venipuncture

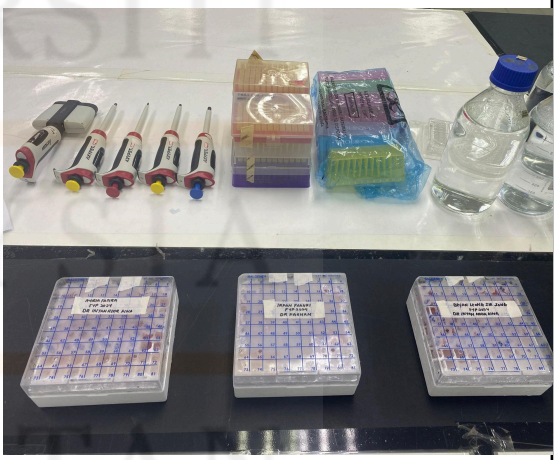


Transfer of serum into microcentrifuge tube

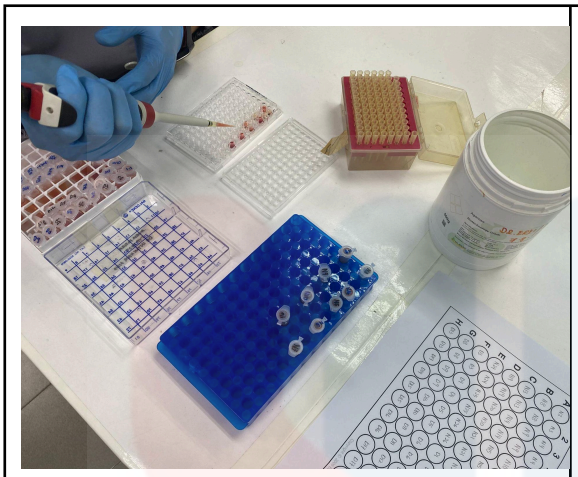
Serum transfer process


The total of 40 serum to be tested

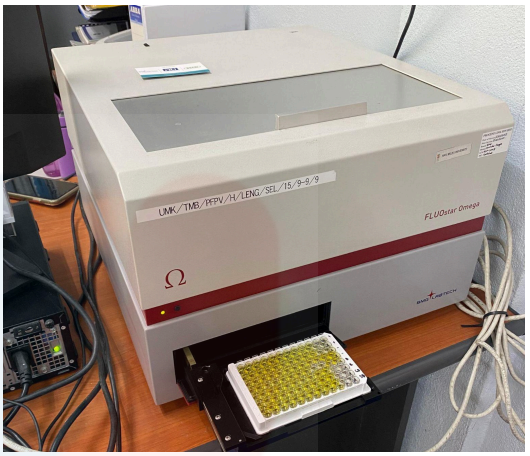
The reagents used in c-ELISA



Working station with prepared instruments



Pipetting of sample into plate




Placement of ELISA plate into the plate reader

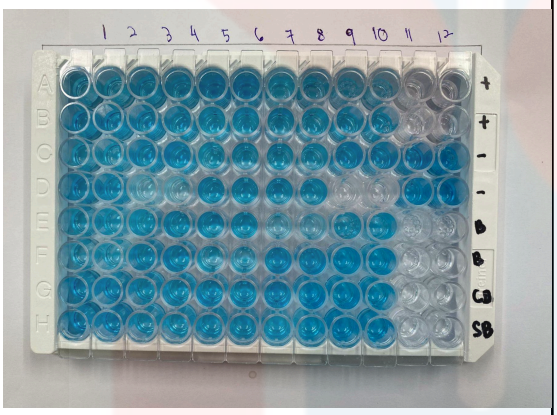



Plate before the addition of stop solution

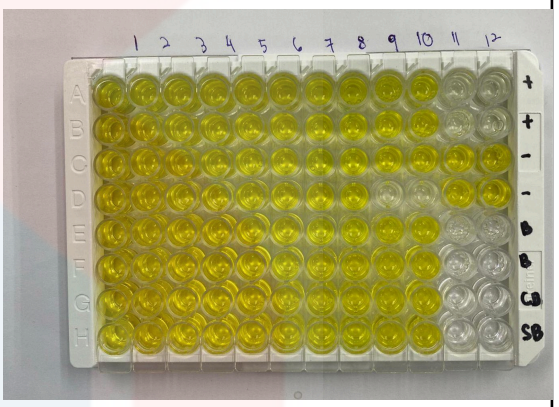



Plate after adding Stop Solution and color changed was observed

Picture with supervisor of this study

Picture with classmates working together in the laboratory

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