SEROLOGICAL DETECTION AND ASSOCIATED RISK FACTORS OF FOOT AND MOUTH DISEASE IN CATTLE IN BACHOK, KELANTAN

By

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SEROLOGICAL DETECTION AND ASSOCIATED RISK FACTORS OF FOOT AND MOUTH DISEASE IN CATTLE IN BACHOK, KELANTAN

ABSTRACT

Foot and Mouth Disease (FMD) is a highly contagious viral disease affecting cloven hoofed animals, which can cause significant economic losses to the livestock industry. The aim of this study was to detect seropositivity of FMD virus and determine the associated risk factors in cattle in Bachok, Kelantan. A total of 30 blood samples were collected from three cattle farms in Bachok, Kelantan, and the samples were tested using the Non-Structural Protein (NSP) Antibody Rapid Test Kit. The results show that a total of 17 samples (56.67%) were found to be seropositive. Age, sex, and source of the animal showed no significant association with FMD infection in this dataset. This is likely due to uneven group distribution and limited sample size. The use of the NSP rapid test kit provides an easy field detection, but confirmatory laboratory testing is needed. In conclusion, this study demonstrated the presence of FMDV exposure in cattle in Bachok, which needs proper control measures and continuous surveillance to prevent potential outbreaks and economic losses.

Keywords: Cattle, Foot and Mouth Disease, Kelantan, Risk factor, Seropositivity

PENGESANAN SEROLOGI DAN FAKTOR RISIKO BERKAITAN PENYAKIT KUKU DAN MULUT (FMD) PADA LEMBU DI BACHOK, KELANTAN

ABSTRAK

Penyakit Kuku dan Mulut (FMD) merupakan penyakit virus yang sangat mudah berjangkit yang menyerang haiwan berkuku belah dan boleh menyebabkan kerugian ekonomi yang ketara kepada industri ternakan. Kajian ini bertujuan untuk mengesan seropositiviti virus FMD dan menentukan faktor risiko berkaitan dalam kalangan lembu di Bachok, Kelantan dan diuji menggunakan Kit Ujian Antibodi Protein Bukan Struktur. Keputusan menunjukkan bahawa sebanyak 17 sampel (56.67%) adalah seropositif. Umur, jantina dan sumber haiwan menunjukkan tiada perkaitan yang jelas. Kebarangkalian untuk ini terjadi disebabkan oleh taburan kumpulan yang berbeza dan saiz sampel yang kecil. Kit ujian Antibodi Protein Bukan Struktur digunakan dalam kajian ini menjadikan pengesanan di lapangan menjadi mudah dan cepat tetapi ujian selanjutnya mesti dilakukan untuk kepastian. Kesimpulannya, kajian ini menunjukkan kewujudan pendedahan FMDV pada lembu di Bachok, sekaligus menekankan keperluan langkah kawalan yang sewajarnya serta pemantauan berterusan bagi mencegah potensi wabak dan kerugian ekonomi.

Kata kunci: Lembu, Penyakit Kaki dan Mulut, Kelantan, Faktor Risiko, Seropositiviti

CERTIFICATION

It is hereby to certify that we have read this research paper entitled of "Serological Detection and Associated Risk Factors of Foot and Mouth Disease in Cattle in Bachok, Kelantan", by Amir Aiman Bin Muhaidin and in our opinion, it is satisfactory in terms of scope, quality and presentation as partial fulfillment of the requirements for the course of DVT55204 - Research Project.

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LIST OF ABBREVIATIONS

DVS	Department of Veterinary Services
DIVA	Differentiating infected from vaccinated animals
ELISA	Enzyme-linked immunosorbent assay
FMD	Foot and mouth disease
FMDV	Foot and mouth disease virus
IBM SPSS	International business machines statistical package for the social sciences
ID	Identification
LBPE	Liquid phase blocking enzyme-linked immunosorbent assay
MFMDK	The National Foot and Mouth Disease Laboratory
NSP	Non-structural protein
PCR	Polymerase chain reaction
PVM	*Protocol Veterinar Malaysia*
RT-PCR	Reverse transcriptase polymerase chain reaction
ssRNA	Single-stranded ribonucleic acid
SAT	Southern African Territories
SP	Structural protein
SPCE	Solid phase competition enzyme-linked immunosorbent assay
UMK	Universiti Malaysia Kelantan
WOAH	World Organization of Animal Health
VNT	Virus neutralisation test
VRI	Veterinary Research Institute

LIST OF SYMBOLS

°C	Degree Celsius
rpm	Revolutions per minute
T	Test zone
C	Control zone
µL	Microliter
S	Sample
%	Percentage
>	Greater than
<	Lesser than
mg/kg	Milligram per kilogram
n	Number of observations
CI	Confidence interval
P-value	Probability value

CHAPTER 1

INTRODUCTION

1.1 Research Background

Foot and Mouth Disease (FMD) is a highly contagious viral disease affecting cloven-hoofed animals, caused by Aphthovirus of the Picornaviridae family. It has seven distinct serotypes, with four (O, A, C, Asia 1) present in Asia (Belsham *et al.,* 2025). The virus spreads through direct or indirect contact with infected animals, contaminated secretions, or airborne particles and enters the host via inhalation, ingestion, or broken skin and mucous membrane. Although not a public health concern, FMD poses a significant threat to livestock health and productivity (Belsham *et al.,* 2025). Infected animals may develop vesicles and lesions primarily in the oral cavity, feet, and teats. Some may become long-term carriers, especially cattle (Belsham *et al.,* 2025). A study in Peninsular Malaysia found high awareness among farmers of common clinical signs, such as drooling, inappetence, and blister formation (Abdullah & Shaari, 2022). Diagnostic approaches in Malaysia include clinical evaluation, PCR, virus isolation, and serological testing conducted by the National FMD Laboratory and supported by regional veterinary labs (Department of Veterinary Service, 2022). Therefore, by detecting the serology of FMD in cattle in Malaysia, specifically in Bachok, Kelantan, and its associated risk factors, economic loss in the ruminant industry can be prevented by organizing effective preventive and control measures based on the risk factors.

1.2 Research Problem

FMD is a highly contagious viral disease affecting livestock such as cattle, buffalo, goats, sheep, and pigs, with cases reported globally. In Southeast Asia, although FMD has been observed in various countries, only limited cases have been documented in cattle, specifically in Laos and Myanmar (Buckle *et al.,* 2021). In Malaysia, studies have been conducted in several states, including Kelantan, Pahang, Selangor, Melaka, Negeri Sembilan, and Johor (Abdullah & Shaari, 2022). However, these studies have largely focused on farmer awareness and knowledge rather than the actual prevalence of the disease. A significant gap remains in research specifically addressing the serological detection of FMD in cattle in the Kelantan region.

1.3 Research Question

1. Are antibodies against FMDV detectable in cattle in Bachok, Kelantan?
2. What are the risk factors associated with FMD in cattle in Kelantan?

1.4 Research Hypothesis

1. There is a detection of antibodies against FMDV in cattle in Bachok, Kelantan.
2. The cattle's vaccination status, rearing system, reproductive status, and source of the animal are the associated risk factors in FMDV serologically positive cattle.

1.5 Research Objective

1. To detect the presence of antibodies against FMDV in cattle in Bachok, Kelantan.
2. To determine the associated risk factors of FMDV in cattle in the targeted farm.

CHAPTER 2

LITERATURE REVIEW

2.1 Virus Structure

FMD is caused by Aphthovirus, a member of the family Picornaviridae. There are seven immunologically distinct serotypes of the virus, which are O, A, C, Asia 1, and Southern African Territories (SAT) 1, 2, and 3. 4 of the serotypes have been found in Asia, which are O, A, C, and Asia 1 (Belsham *et al.,* 2025). It is characterised by an icosahedral capsid approximately 25-30 nm in diameter. The virion contains a single-stranded positive-sense RNA(ssRNA) genome.

In addition to its structural protein, it encoded a non-structural protein (NSP) that helps in viral replication and host interactions. These include leader protease, which inhibits host protein synthesis. The 2C, 3A, and 3D proteins are for replication complex formation and RNA synthesis. The 3C acts as the main viral protease for polyprotein processing. Antibodies against NSP are commonly used in differentiating infected from vaccinated animals (DIVA) testing since they indicate natural infection rather than vaccination (Jamal & Belsham, 2013).

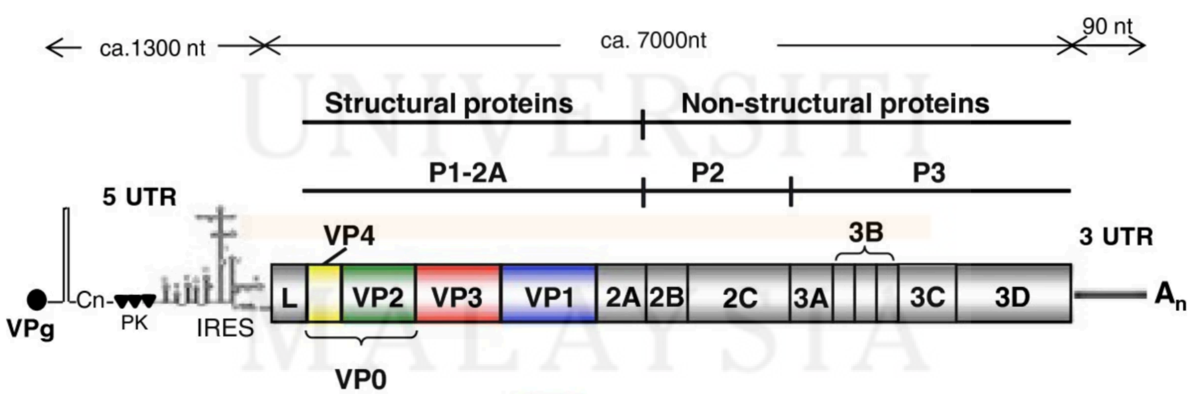

Figure 2.1: Genome organization of FMDV and the structure of the virus, adapted from Jamal & Belsham (2013).

2.2 Transmission

FMDV can be transmitted through direct contact with infected animals or indirect contact via contaminated secretions and excretion, including semen and milk. Airborne transmission is also possible, as the virus can travel long distances over land and water via wind. The primary routes of entry include inhalation, ingestion, or penetration through broken skin or mucous membrane (Belsham *et al.,* 2025). Additionally, breeding practices may serve as a route of transmission, particularly for the SAT serotypes. Farm workers may act as mechanical vectors by carrying the virus on clothing or equipment, however, FMD is not considered a public health concern (Belsham *et al.,* 2025).

2.3 Site of Infection

The primary site of infection and replication of FMDV is the pharyngeal mucosa, although the virus can also enter through skin lesions. Following entry, it spreads to the nearest lymph nodes and is distributed via the lymphatic system. Viral replication occurs in the epithelium of the mouth, muzzle, teats, feet, and other areas with damaged skin. Vesicles typically develop and rupture within 48 hours of infection. Approximately 50% of infected animals, including vaccinated animals, can become carriers, thereby harboring low levels of infectious virus (Belsham *et al.,* 2025). In cattle, the carrier state may persist up to 3.5 years, and the incubation period ranges from 2 to 14 days. (Belsham *et al.,* 2025).

2.4 Knowledge of Farm Owner on FMD in Peninsular Malaysia

A study was conducted in Peninsular Malaysia to assess farm owners' awareness and knowledge of FMD by administering a questionnaire focused on the common clinical signs observed in affected cattle. The study was carried out in Kelantan, Pahang, Selangor, Melaka, Negeri Sembilan, and Johor, involving 349 farmers. The finding revealed that most farmers were able to accurately describe the clinical signs of FMD in their cattle. The most commonly reported symptom was drooling (95.4%), followed by loss of appetite (77.7%) and the presence of blisters on the tongue, lips, mammary glands, around the mouth, and hooves (67.9%). Lameness was noted in 39.5%, while only 15.2% observed a reduction in milk production as a clinical sign (Abdullah & Shaari, 2022).

2.5 Diagnosis of FMD

Diagnosis of FMD according to the World Organization of Animal Health (WOAH) involves identification of the agent through virus isolation, ELISA, and RT-PCR. Other than that, detection can also be performed through serological tests. Serological tests are used to certify animals for trade, confirm suspected cases, provide evidence of freedom from infection, and assess vaccine effectiveness. There are two main types of tests, which are detecting antibodies to structural proteins (SP) in which involve serotype-specific and response to both infection and vaccination. The second type is those that detect antibodies to non-structural proteins (NSP), which generally indicate infections only. SP tests such as Virus Neutralisation Test (VNT), Solid Phase Competition ELISA (SPCE), and Liquid Phase Blocking ELISA (LBPE) are mainly used for trade and vaccination monitoring, with SPCE and LBPE being faster and easier than VNT.

NSP tests, on the other hand, detect antibodies to proteins like 3AB or 3ABC, allowing infection detection regardless of vaccination status, making them ideal for surveillance (WOAH, 2009).

On the other hand, in Malaysia, it involves clinical observation, virus detection, and serological testing. Clinically, FMD is identified by the presence of vesicles, ulcers, and lesions on the mouth, feet, and teats, which may lead to excessive salivation, nasal discharge, lameness, and reduced milk production. Laboratory confirmation includes virus isolation or PCR testing on appropriate samples, as well as NSP ELISA serology to detect current or past infections. The National FMD Laboratory (MFMDK) in Kelantan serves as the reference lab for live virus testing, while serological tests can also be conducted at regional veterinary labs and the Veterinary Research Institute (VRI) (Department of Veterinary Service, 2022).

2.6 Treatment

There is no specific treatment for FMD. However, symptomatic treatment is usually applied to infected animals to relieve clinical signs and prevent secondary infection, using antibiotics and anti-inflammatory agents (Radostits, Gay, Blood, & Hinchcliff, 2000). The suggested supportive treatment includes administering enrofloxacin at 2.5mg/kg twice daily for 5 days and meloxicam at 0.5mg/kg once daily, both given intramuscularly (Dilawar, 2025).

2.7 Control of FMD

The World Organization of Animal Health (WOAH) outlines several measures that could be implemented to control FMD. It starts with vaccination and surveillance. Countries or zones may be classified as FMD-free either with or without vaccination. To achieve FMD-free status, it requires systematic surveillance and the absence of the infection for at least 12 months. Next, establishing a protection zone is recommended. This involves implementing strict biosecurity to separate susceptible animals from potential sources of infection. Animals intended for import must undergo an isolation period prior to entry. Additionally, containment zones in areas linked to an outbreak are needed to minimise further disease spread (WOAH, 2024).

The control and prevention of FMD in Malaysia is under the responsibility of the Department of Veterinary Services (DVS), which can be referred to via *Protokol Veterinar Malaysia* (PVM) entitled *Penyakit Kuku dan Mulut Edisi Ketiga* (Department of Veterinary Services, 2022). PVM outlines thirteen strategies, which are mandatory disease reporting, strategic vaccination, strict import controls, quarantine, regulation of animal movement, disinfection procedures, preparedness planning, enforcement, public awareness campaigns, maintenance of records, and continuous surveillance. Movement restrictions are enforced in outbreak zones, where there is a prohibition on transferring the animal for 28 days following the last detected case. An infected herd may be submitted for supervised culling, with the carcass being deboned and deglanded. The carcass will then be placed in the chiller for 24 hours before local distribution is permitted. Additionally, the usage of disinfectants such as sodium hydroxide, sodium carbonate, citric acid, hypochlorite, and peroxymonosulphate compounds enhances biosecurity. Active and passive surveillance programs are also conducted within a one to five km radius of the outbreak site.

CHAPTER 3

MATERIAL AND METHODOLOGY

3.1 Ethical Consideration

The ethical approval for the use and handling of animals in this study was obtained from the Animal Ethics Committee of Universiti Malaysia Kelantan under the code (UMK/FPV/ACUE/FYP/008/2025).

3.2 Methodology

3.2.1 Study Area

The study involved three cattle farms in Bachok, Kelantan. The selected farms included the UMK Teaching Farm and two private farms.

3.2.2 Study Design

This study was conducted as a cross-sectional study at the UMK Teaching Farm and two selected private farms in Kota Bharu and Bachok, Kelantan. A cross-sectional study design involved the simultaneous measurement of both exposure and outcome variables in the study subjects. This means that data on FMD serological detections and associated risk factors were collected at a single point in time.

3.2.3 Study Population

A total of 30 cattle were randomly sampled, including nine (9) from Farm A, fifteen (15) from Farm B, and six (6) from Farm C.

3.3 Study Criteria

3.3.1 Inclusion Criteria

Three cattle farms in Bachok, Kelantan, were chosen by convenience sampling. The cattle on the selected farms were chosen regardless of age, sex, breed, or health status.

3.3.2 Exclusion Criteria

No exclusion criteria were listed in this study.

3.4 Sampling Method

3.4.1 Animal Profile

The physical examination of the selected cattle was performed, and the patient's signalment was recorded in animal profile form prior to blood sampling.

Patient ID	Sex	Age	Vaccination Status	Rearing System	Source of Animal	Presence of Clinical Sign

Table 3.4.1: Animal Record

3.4.2 Sample Collection

Thirty blood samples were collected through coccygeal venipuncture using 1-inch, 18-gauge vacutainer needles. Each animal was restrained in a makeshift crush to limit movement and reduce the risk of injury to both cattle and handlers during the procedure. The tail was raised and stabilized to expose the ventral surface, providing easier access to the coccygeal vein. The vein was located by palpating the coccygeal groove with the thumb finger. The needle was then inserted perpendicularly into the vein. Once blood appeared at the needle hub, confirming successful venous entry, a plain vacutainer tube was attached and filled to approximately two-thirds of its volume. The tube was removed first, followed by the vacutainer holder and needle. Each tube was labeled with the corresponding animal ID using a permanent marker and kept in an ice box during transport. Upon arrival at the laboratory, the samples were kept at 2–8 °C until further analysis.

3.4.3 Sample Processing

Blood samples were centrifuged at 2500 rpm for 15 minutes to separate the serum from the clots. After centrifugation, the serum was gently aspirated using a Pasteur pipette and transferred into pre-labeled microcentrifuge tubes matching each animal's ID. The serum samples were then stored at −20 °C until analysis. Before performing the rapid test, the samples were thawed at room temperature for at least one hour.

3.4.4 Serology Detection - Rapid Test Kit

Serological detection of Foot and Mouth Disease (FMD) Virus was performed using the commercially available ListoX Foot and Mouth Disease Virus Non-Structural Protein Antibody Rapid Test Kit. Each kit contains test cassettes and disposable pipettes. The test device includes a testing window with two zones: the test (T) zone and the control (C) zone. Serum or plasma samples were tested following the manufacturer's instructions. Approximately 120 µL of the sample was applied to the sample well labeled “S". Results were interpreted between 5 and 10 minutes; tests read after 15 minutes were considered invalid. Upon application of the sample, the liquid migrates laterally by capillary action along the test strip and interacts with pre-coated FMDV 3ABC antigens on the membrane. A visible colored line appearing in both the T and C zones indicates a positive result, while the presence of only the C line denotes a negative result.

Figure 3.4.4: Test kit showing result interpretations, from left to right (Positive, Negative, Invalid)

3.5 Data Collection

The data from the serological detection of Foot and Mouth Disease were manually recorded and tabulated in Microsoft Excel.

3.6 Data Analysis

The collected data were analyzed using IBM SPSS Statistics version 31. Categorical variables were summarized as frequencies and percentages. The results were presented in frequency tables and bar charts to facilitate comparison and visualization.

CHAPTER 4

RESULT AND DISCUSSION

4.1 Result

A total of 30 cattle serum samples were tested for the presence of Foot and Mouth Disease (FMD) virus antibodies using a commercial rapid test kit. The results revealed that 17 out of 30 samples (56.67%) were seropositive, while 11 samples (36.67%) were seronegative and 2 samples (6.67%) were invalid. The invalid result will be excluded from the data analysis. The findings indicate a high seroprevalence of FMDV antibodies among cattle in Bachok, Kelantan.

Univariate analysis using Chi-square test revealed no significant association between the age ($p>0.05$), sex ($p>0.05$), rearing system ($p>0.05$), source of animal ($p>0.05$), and clinical sign ($p>0.05$) of the animal with seropositivity of FMD, suggesting that the observed differences are likely due to the few number of samples collected.

Farm	Positive FMDV (Frequency)	Negative FMDV (Frequency)	Invalid Result	Total
A	5	4	-	9
B	8	5	2	15
C	4	2	-	6
Total	17	11	2	**30**
Total Percentage	**56.67%**	**36.67%**	**6.67%**	**100%**

Table 4.1: Frequency table for positive and negative FMD in Farm A, B, and C

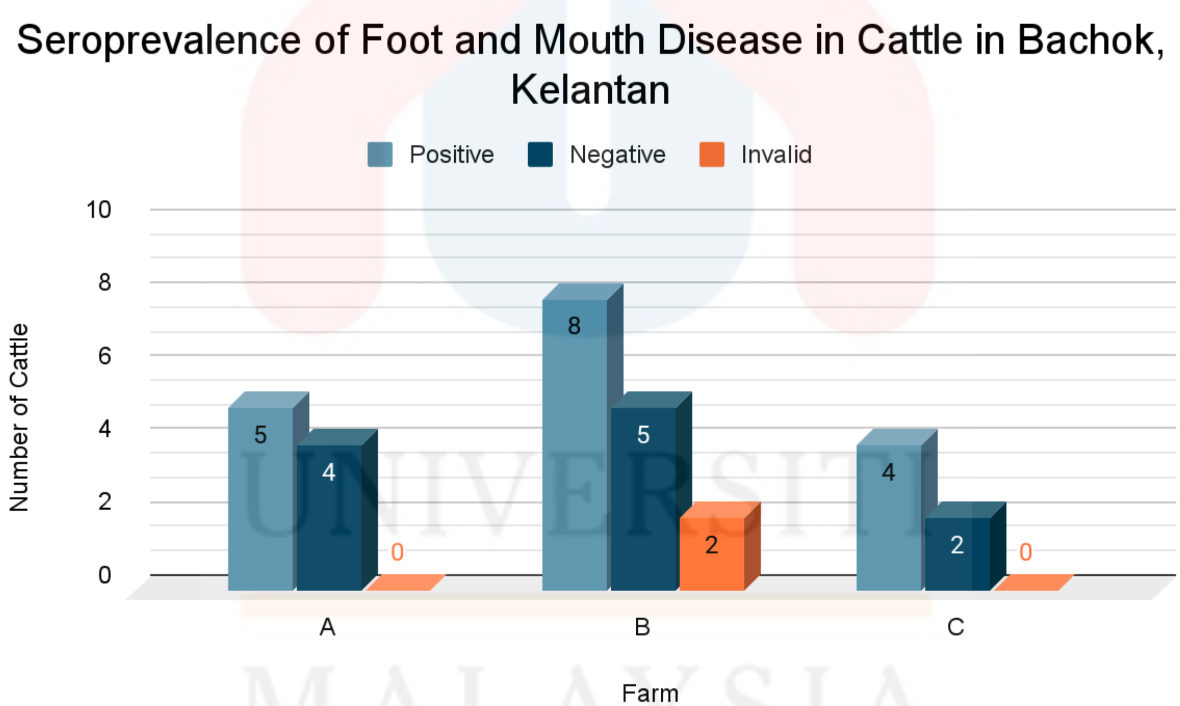

Figure 4.1: The number of serologically positive and negative cattle for FMDV

Variables	Tested (n)	Positive (n)	Frequency	Odd Ratio	95% CI	P-value
Age (Years)						0.463
< 2	8	4	50.00%			
> 3	20	13	65.00%	2.853	0.373 - 21.828	
Sex						0.330
Male	5	4	80.00%	0.228	0.012 - 4.375	
Female	23	13	56.52%			
Rearing System						0.330
Semi intensive	23	17	73.91%			
Extensive	5	4	80.00%	3.288	0.241 - 44.840	
Source of Animal						0.387
Local	10	5	50.00%			
Import	18	12	66.66%	1.954	0.238 - 10.685	
Clinical Sign						0.413
Presence	1	1	100.00%	-	-	
Absence	27	16	59.26%			

Table 4.2: Statistical analysis of the associated risk factors in FMD-infected cattle

Remark:

The invalid result is excluded from the subsequent testing.

P-value < 0.05 is considered significant.

4.2 Discussion

Foot-and-mouth disease (FMD) is a highly contagious viral disease affecting cloven-hoofed animals. It is caused by Aphthovirus, a member of the family Picornaviridae. There are seven immunologically distinct serotypes of the virus, which are O, A, C, Asia 1, and Southern African Territories (SAT) 1, 2, and 3. 4 of the serotypes have been found in Asia, which are O, A, C, and Asia 1 (Belsham *et al.,* 2025). The disease primarily affects domesticated livestock such as cattle, pigs, sheep, and goats but has also been reported in wild species, including bison, giraffes, camels, and antelope (Belsham *et al.,* 2025). In order to figure out the seropositive and associated risk factors of FMD, a study was carried out and described the serological detection of FMD infection among cattle in the year of 2025 within range of June until October specifically in Bachok, Kelantan.

The overall detection rate was 56.67%, which is considered high compared with the finding reported by Ramanoon (2016), who reported a seroprevalence of 24.2% for FMD in cattle. This difference might be due to our study focusing on one specific district during a recent period, while the previous study covered a wider area and a longer time frame, which were from 2001 to 2011.

In this study, serological detection of FMD virus was performed using the commercial ListoX Foot and Mouth Disease Virus Non-Structural Protein Antibody Rapid Test Kit. The advantage of using the NSP antibody assay is that it detects antibodies generated after viral replication rather than vaccination, because non-structural proteins are absent in purified vaccine preparations (King *et al.,* 2015). The NSP test enables the DIVA approach. However, the test kit results are not definitive, and further confirmation is needed. The official confirmation tests, as per the Department of Veterinary Services (DVS), primarily involve Real-Time Reverse Transcriptase-Polymerase Chain Reaction (RT-qPCR) and FMD Virus Antigen ELISA tests in a government-accredited laboratory. Additionally, the rapid test kit provides fast results, is easy to use, and is suitable for field conditions without the need for advanced laboratory equipment.

A study by data on prevalence, distribution, and risk factors for Foot and Mouth Disease in grazing cattle in haor areas of Bangladesh (Chowdhury *et al.,* 2019) it was found that older cattle have a significantly higher risk of FMD infection compared to younger cattle, and male cattle were three times more likely to be infected than female with p-value of <0.001. In our study, neither age nor sex showed a significant association with FMD, indicating that neither older age nor male cattle had a higher risk. This could be due to our study not having uniform exposure towards the age and sex groups of cattle. Additionally, in our study, male cattle were used for meat production, whereas in other studies, higher seropositivity was observed in male cattle because they are commonly used for draught purposes, which can lead to skin irritation and damage (Hossain *et al.,* 2019).

On the other hand, the source of the animal did not show a significant association with FMD infection, as the p-value was greater than 0.05. This means that cattle that were imported were not likely to be infected, and increased the risk of FMD infection compared to locally raised cattle. However, this contradicts Iriarte Vera (2023), who reported that the source of the animal can affect and increase the risk of FMD infection. One possible reason for this difference is that the number of imported animals in our sample was small, which could have affected the statistical results. Additionally, it is possible that, in our study, vaccination status and herd immunity differ from those in the earlier study.

A positive NSP test indicates that the animal has been naturally infected with FMDV. This is significant because it suggests that the infected animal can become a carrier up to seven months post-infection (Tenzin *et al.,* 2008). This could lead to the transmission of antigen to the herd even though the possibility is low (Parthiban *et al.,* 2015). It is important for the farmer to take extra precautions and to follow routine vaccination, as these can reduce the risk of infection.

A study by Mohamad *et al.* (2023) reported that FMD causes major economic losses in Malaysia, including reduced productivity, treatment costs, and losses from control measures. This shows how serious the disease is for farmers and the livestock industry. Therefore, this study is important because it provides local data that can help with early detection, enabling early prevention steps.

There were a few limitations that may have affected the accuracy and representativeness of the results. The sample size was small and only a few farms were included, which can reduce the strength of the findings and make it harder to detect true associations with risk factors. The study was also conducted in one province, so the results may not reflect all cattle farms across Kelantan.

CHAPTER 5

CONCLUSION AND RECOMMENDATION

5.1 Conclusion

In short, this study demonstrated a high seropositivity rate of Foot and Mouth Disease, at 56.67%, among cattle in Bachok, Kelantan. This indicates that FMD remains a concern in this province. Although no significant associations were found between age, sex, rearing system, source of animals, and clinical signs with FMD seropositivity, this may be influenced by the small sample size and limited study area. The finding highlights that FMD virus exposure continues to occur. Thus, this study provides a baseline understanding of the current situation in Bachok and highlights the need for preventive measures to protect the livestock sector.

5.2 Recommendation

Based on the findings, it is recommended that future studies include a larger sample size and broader farm coverage to improve accuracy and strengthen the statistical power of the results. In addition, Receiver Operating Characteristic Curve analysis should be performed to assess the sensitivity, specificity, and overall diagnostic accuracy of the rapid test kit. Additional laboratory tests, such as RT PCR and ELISA, should be performed to validate the results. This is done because molecular detection can identify specific antigens, is sensitive, and can detect even when only a few antigens are present (Wong *et al.,* 2020). Thus, it can confirm the animal's infection status. Continuous education and awareness programs are crucial for reducing the risk of FMD outbreaks and minimizing future economic losses.

APPENDICES

Figure 6.1: Briefing prior to sampling

Figure 6.2: Post sampling

Figure 6.3: Sample processing and interpretation

REFERENCES

Belsham, G. J., Bøtner, A., & Lohse, L. (2025, April 3). Foot-and-Mouth disease in animals. MSD Veterinary Manual. https://www.msdvetmanual.com/infectious-diseases/foot-and-mouth-disease/foot-and-mouth-disease-in-animals

Buckle, K., Bueno, R., McFadden, A., Van Andel, M., Spence, R., Hamill, C., Roe, W., Vallee, E., Castillo-Alcala, F., Abila, R., Verin, B., Purevsuren, B., Sutar, A., Win, H. H., Thiha, M., Lwin, K. O., Khounsy, S., Phonthasy, S., Souriya, V., . . . Mioulet, V. (2021). Detection of Foot-and-Mouth disease virus in the absence of clinical disease in cattle and buffalo in Southeast Asia. Frontiers in Veterinary Science, 8. https://doi.org/10.3389/fvets.2021.691308

Chowdhury, M. S. R., Ahsan, M. I., Khan, M. J., Rahman, M. M., Hossain, M. M., Harun-Al-Rashid, A., Ahmed, S. S. U., & Uddin, M. B. (2019). Data on prevalence, distribution and risk factors for Foot and Mouth Disease in grazing cattle in haor areas of Bangladesh. *Data in Brief*, *28*, 104843. https://doi.org/10.1016/j.dib.2019.104843

Clinical Cases of Foot and Mouth Disease (FMD) in Cattle Recorded at Upazila Veterinary Hospital, Kulaura, Bangladesh. (2016). In www.jakraya.com/journal/vri. Veterinary Research International. https://www.researchgate.net/profile/Md-Mukter-Hossain/publication/332037163_Clinical_Cases_of_Foot_and_Mouth_Disease_FMD_in_Cattle_Recorded_at_Upazila_Veterinar y_Hospital_Kulaura_Bangladesh/links/5c9c86c892851cf0ae9c8ada/Clinical-Cases-of-Foot-and-Mouth-Disease-FMD-in-Cattle-Recorded-at-Upazila-Veterinary-Hospital-Kulaura-Bangladesh.pdf

Dilawar, A. (2025, November 7). *Clinical Pattern and Therapeutic Approaches in Dairy Cattle Affected with Foot and Mouth Disease Virus (FMDV) Serotype O/2021-22 during an Outbreak in Kashmir, India*. https://www.arccjournals.com/journal/indian-journal-of-animal-research/B-5486

FOOT AND MOUTH DISEASE ON CATTLE IN PENINSULAR MALAYSIA: TOWARDS A SUSTAINABLE LIVESTOCK. (2022). JOURNAL OF SUSTAINABILITY SCIENCE AND MANAGEMENT, VOLUME 17(NUMBER 5). https://jssm.umt.edu.my/wp-content/uploads/sites/51/2022/06/Article-12-JSSM-Volume-17-Number-5-May-2022.pdf

FOOT AND MOUTH DISEASE. (2009). WORLD ORGANIZATION OF ANIMAL HEALTH (WOAH). https://www.woah.org/fileadmin/Home/eng/Animal_Health_in_the_World/docs/pdf/2.01.05_FMD.pdf

INFECTION WITH FOOT AND MOUTH DISEASE VIRUS. (2024). OIE. https://www.woah.org/fileadmin/Home/eng/Health_standards/tahc/2023/chapitre_fmd.pdf

IMPACT OF FOOT AND MOUTH DISEASE ON CATTLE PRODUCTION IN PENINSULAR MALAYSIA (2023). JOURNAL OF SUSTAINABILITY SCIENCE AND MANAGEMENT, VOLUME 18 (NUMBER5). https://jssm.umt.edu.my/wp-content/uploads/sites/51/2023/10/10-JSSM-Volume-18-Number-5-May-2023-FINAL.pdf

Iriarte, M. V., Gonzáles, J. L., Gil, A. D., & De Jong, M. C. M. (2023). Animal Movements and FMDV Transmission during the High-Risk Period of the 2001 FMD Epidemic in Uruguay. *Transboundary and Emerging Diseases*, *2023*, 1–11. https://doi.org/10.1155/2023/8883502

Jamal, S. M., & Belsham, G. J. (2013). Foot-and-mouth disease: past, present and future. *Veterinary Research*, *44*(1), 116. https://doi.org/10.1186/1297-9716-44-116

PENYAKIT KUKU DAN MULUT (FOOT AND MOUTH DISEASE, FMD) (3RD ED.). (2022). JABATAN PERKHIDMATAN VETERINAR KEMENTERIAN PERTANIAN DAN INDUSTRI MAKANAN MALAYSIA. https://www.dvs.gov.my/dvs/resources/user_1/2024/BKPBV/PVM/01_-_DVS_-_INSIDE_FMD_(_147MM_X_210MM_)_-_SET.pdf

Parthiban, A. B. R., Mahapatra, M., Gubbins, S., & Parida, S. (2015). Virus Excretion from Foot-And-Mouth Disease Virus Carrier Cattle and Their Potential Role in Causing New Outbreaks. PLoS ONE, 10(6), e0128815. https://doi.org/10.1371/journal.pone.0128815

Ramanoon (2016). The epidemiology of foot and mouth disease in Malaysia. https://researchportal.murdoch.edu.au/esploro/outputs/991005539992707891

The seroprevalence of foot-and-mouth disease in the sedentary livestock herds in four districts of Bhutan. (2011). ScienceDirect, 100(3–4), 231–236. https://doi.org/10.1016/j.prevetmed.2011.04.013

THE USE OF NON-STRUCTURAL PROTEINS TO DIFFERENTIATE BETWEEN VACCINATED AND INFECTED ANIMALS. (2013). *MIDDLE EAST – OIE REGIONAL COMMISSION*. https://www.woah.org/fileadmin/Home/eng/Publications_&_Documentation/docs/pdf/TT/2015_MO2_King.pdf

Tenzin, N., Dekker, A., Vernooij, H., Bouma, A., & Stegeman, A. (2008). Rate of Foot‐and‐Mouth Disease Virus Transmission by Carriers Quantified from Experimental Data. Risk Analysis, 28(2), 303–309. https://doi.org/10.1111/j.1539-6924.2008.01020.x

Wong, C. L., Yong, C. Y., Ong, H. K., Ho, K. L., & Tan, W. S. (2020). Advances in the diagnosis of Foot-and-Mouth Disease. Frontiers in Veterinary Science, 7, 477. https://doi.org/10.3389/fvets.2020.00477