Seroprevalence of Bovine Viral Diarrhoea (BVD) and Associated Risk Factors in Cattle in Selected Farms in Bachok, Kelantan

By

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Seroprevalence of Bovine Viral Diarrhoea (BVDV) and Associated Risk Factors in Cattle in Bachok, Kelantan

ABSTRACT

Bovine Viral Diarrhoea (BVD) is an infectious disease caused by the Bovine Viral Diarrhea Virus (BVDV). This pathogen is a small, enveloped, single-stranded RNA virus that belongs to the Pestivirus genus of the Flaviviridae Family. The cytopathic (CP) BVDV strains are mostly associated with mucosal disease outbreaks, while the non-cytopathic (NCP) strains are ubiquitous and commonly linked to severe acute infections. This contagious disease affects cattle worldwide, leading to reproductive disorders, immunosuppression, reduced productivity, and consequently major economic losses to the livestock industry. This study aims to detect the seroprevalence of BVDV in the blood serum from selected local cattle farms located in Bachok, Kelantan. A total of 40 blood samples were obtained from cattle of varying ages and breeds raised under smallholder farm systems. The samples were analyzed using a commercial BVDV rapid test kit based on the immunochromatographic detection of BVDV antibodies. However, there is still a lack of comprehensive studies in Malaysia on the seroprevalence and impact of BVDV in cattle, with only limited reports available in Selangor and Sabah, but little to no information in Kelantan. From the result, 33 samples (82.5%) were found to be seropositive for BVD, and 7 samples (17.5%) were negative shown by the rapid test kit. The high seroprevalence observed suggests active or past exposure of cattle to the virus within the study area. In conclusion, this study demonstrates the presence of BVDV in the blood serum of cattle, which indicates the potential exposure of the virus within the Bachok area. The findings indicate widespread circulation of BVDV among cattle populations in Bachok, emphasizing the need for improved surveillance, and routine diagnostic screening.

Keywords: BVDV, risk factor, rapid test kit, antibodies, cattle, Kelantan, Malaysia.

Seroprevalensi dan Analisis Faktor Risiko Bovine Viral Diarrhoea (BVDV) Antara Lembu in Bachok, Kelantan

ABSTRAK

Penyakit Bovine Viral Diarrhoea (BVD) adalah penyakit berjangkit yang disebabkan oleh Bovine Viral Diarrhea Virus (BVDV). Patogen ini adalah virus RNA beruntai tunggal yang bersampul dan kecil serta tergolong dalam genus Pestivirus dari keluarga Flaviviridae. Strain BVDV yang bersifat sitopatik (CP) kebanyakan dikaitkan dengan wabak penyakit mukosa, manakala strain bukan sitopatik (NCP) pula biasa dan sering dikaitkan dengan jangkitan akut yang teruk. Penyakit berjangkit ini menjejaskan lembu di seluruh dunia, menyebabkan gangguan pembiakan, penurunan imuniti, pengurangan produktiviti, dan akhirnya kerugian ekonomi yang besar dalam industri ternakan. Tujuan kajian ini adalah untuk mengesan seroprevalensi BVDV dalam serum darah daripada ladang lembu tempatan yang dipilih di Bachok, Kelantan. Sebanyak 40 sampel darah telah diambil daripada lembu pelbagai umur dan baka yang dibela di bawah sistem ladang kecil. Sampel-sampel tersebut dianalisis menggunakan kit ujian pantas komersial BVDV berdasarkan pengesanan imunokromatografi antibodi BVDV. Namun, kajian yang menyeluruh mengenai seroprevalens dan impak BVDV dalam lembu di Malaysia masih kurang, dengan laporan terhad hanya di Selangor dan Sabah, dan hampir tiada maklumat di Kelantan. Daripada keputusan, 33 sampel (82.5%) didapati positif BVD manakala 7 sampel (17.5%) negatif seperti yang ditunjukkan oleh kit ujian pantas tersebut. Seroprevalensi yang tinggi ini menunjukkan pendedahan aktif atau lepas lembu terhadap virus di kawasan kajian. Kesimpulannya, kajian ini membuktikan kehadiran BVDV dalam serum darah lembu yang menunjukkan kemungkinan pendedahan virus di Bachok. Penemuan ini menunjukkan kemungkinan penyebaran meluas BVDV dalam populasi lembu di Bachok dan menekankan keperluan untuk pengawasan yang lebih baik dan saringan diagnostik rutin.

Kata kunci: BVDV, faktor risiko, rapid test kit, antibodi, lembu, Kelantan, Malaysia.

It is hereby to certify that we have read this research paper entitled of "**Seroprevalence of Bovine Viral Diarrhoea (BVDV) and Associated Risk Factors in Cattle in Bachok, Kelantan**", by Aishah Irdina and in our opinion, it is satisfactory in terms of scope, quality and presentation as partial fulfillment of the requirements for the course of DVT 55204 – Research Project.

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Thank you kindly.

DEDICATIONS

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BVD – Bovine Viral Diarrhoea

BVDV – Bovine Viral Diarrhoea Virus

CP – Cytopathogenic / Cytopathic

CPE – Cytopathogenic Effect

DVS – Department of Veterinary Services

DNA – Deoxyribonucleic Acid

ELISA – Enzyme-Linked Immunosorbent Assay

IgG – Immunoglobulin G

IgM – Immunoglobulin M

NCP – Non-Cytopathogenic / Non-Cytopathic

PCR – Polymerase Chain Reaction

PI – Persistently Infected

RNA – Ribonucleic Acid

RPM – Revolutions Per Minute

RT-PCR – Reverse Transcription Polymerase Chain Reaction

VNT – Virus Neutralization Test

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Chapter 1

INTRODUCTION

1.1 RESEARCH BACKGROUND

Bovine Viral Diarrhea (BVD) is a globally significant infectious disease in cattle, caused by the Bovine Viral Diarrhea Virus (BVDV), a single-stranded RNA virus of the *Pestivirus* genus under the *Flaviviridae* family (Wang et al., 2024). The virus exists in two main genotypes, BVDV-1 and BVDV-2, distinguished by genetic and antigenic variations (Qi et al., 2022). It also comprises two biotypes: non-cytopathogenic (NCP) and cytopathogenic (CP), which differ based on their ability to cause cellular damage. While CP strains are mainly associated with mucosal disease, NCP strains are widespread and linked to acute infections (Merwaiss et al., 2019; Ogujiofor et al., 2019). Transmission occurs primarily through direct contact with persistently infected (PI) animals, which continuously shed the virus via secretions such as saliva, nasal discharge, urine, feces, milk, and semen (Grünberg, 2024). Indirect transmission can also occur through contaminated equipment, clothing, and infected reproductive materials. Vertical transmission is of particular concern, as in utero infection during early gestation can result in PI calves that appear healthy but act as lifelong viral reservoirs (Grünberg, 2024).

In Malaysia, BVDV is recognized as a health and economic threat to the cattle industry, with confirmed cases reported in multiple states. However, Kelantan lacks specific data on BVDV prevalence.A recent cross-sectional study conducted in Selangor between 2021 and 2024 examined 596 ruminants consisting of cattle, goats, sheep, and deer, revealing notable differences in species susceptibility. The study reported a BVD seroprevalence of 29.54% in cattle, 11.3% in goats, 50% in sheep, and none in deer, with an overall herd-level seroprevalence of 57.89% (Abdul Rahman et al., 2025). Given the absence of data in Kelantan, conducting seroprevalence studies in this region is essential to evaluate local disease burden and inform effective control strategies.

1.2 RESEARCH PROBLEM

Bovine Viral Diarrhoea Virus (BVDV) has been reported globally in both dairy and beef cattle, which causes significant economic losses due to reproductive failure, immunosuppression, and persistent infections. However, there is still a lack of comprehensive studies in Malaysia on the seroprevalence and impact of BVDV in cattle, with only limited reports available in Selangor and Sabah, but little to no information in Kelantan. This highlights the need for further research to better understand the epidemiology and risk factors associated with BVDV in Malaysian cattle populations.

1.3 RESEARCH QUESTIONS

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

1.4 RESEARCH HYPOTHESIS

1. BVDV is serologically detectable in cattle in Bachok, Kelantan.
2. A significant association exists between BVDV serological detection in cattle including age, sex, breed, source of water, and herd management practices.

1.5 RESEARCH OBJECTIVES

1. To detect the presence of antibodies against BVDV in cattle in selected farms in Bachok district, Kelantan.
2. To determine the associated risk factors of BVDV in cattle in the targeted farms.

Chapter 2

LITERATURE REVIEW

2.1 AETIOLOGY

Bovine Viral Diarrhoea (BVD) is an infectious disease caused by the Bovine Viral Diarrhea Virus (BVDV). This pathogen is a small, enveloped, single-stranded RNA virus that belongs to the Pestivirus genus of the Flaviviridae Family (Wang *et al*., 2024) (Figure 1). Bovine viral diarrhea virus (BVDV) comprises two primary genotypes, BVDV-1 and BVDV-2, distinguished through their genetic sequences and antigenic profiles. This contagious disease affects cattle worldwide, thus affecting the cattle industry, leading to mass economic loss (Qi *et al*., 2022). However, BVDV can be classified into two biotypes, which are non-cytopathogenic (NCP) and cytopathogenic (CP), depending on whether it generates cytopathogenic effect (CPE) in infected cells (Merwaiss *et al*., 2019). The CP BVDV strains are mostly related to mucosal disease outbreaks, whilst NCP BVDV strains are ubiquitous and commonly related to severe acute infections. (Ogujiofor *et al*., 2019).

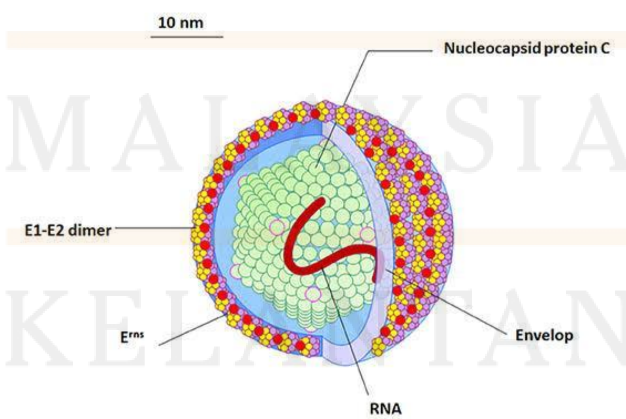

Figure 1: Bovine Viral Diarrhoea Virus

2.2 TRANSMISSION

BVDV is primarily transmitted among cattle through direct contact with infected animals, especially those that are persistently infected. These persistently infected animals act as lifelong carriers and shed large quantities of the virus in bodily secretions such as saliva, nasal discharge, urine, faeces, milk, and semen, making them the primary source of infection within herds (Grünberg, 2024). According to the Cornell University College of Veterinary Medicine (2020), indirect transmission of BVD can occur through contaminated equipment and clothing, as well as via the use of infected semen or embryos. However, vertical transmission is considered particularly significant as a pregnant cattle infected during early gestation may become immunotolerant to the virus towards the fetus and develop into a persistent infection in the calf (Grünberg, 2024). The calves are often seen as clinically normal, but the virus will continuously shed to the environment and pose a serious threat to the herd's health.

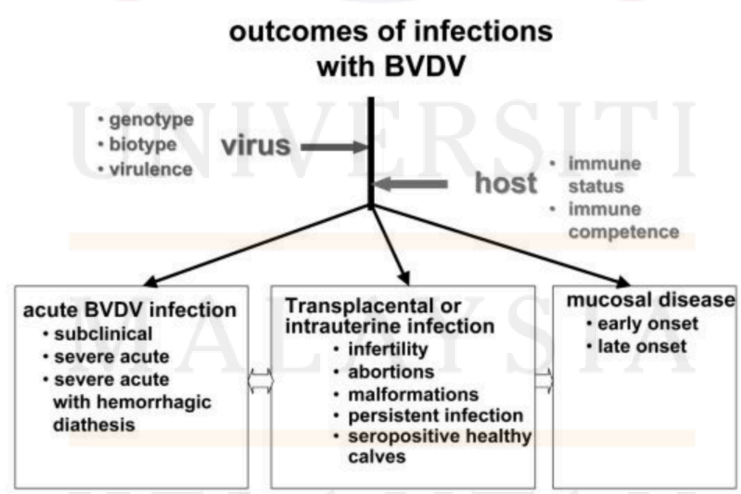

Figure 7.1. Outcomes and factors influencing the outcome of BVDV infections.

Figure 2: Factors that influence BVD infection (Liebler-Tenorio, E. M., 2005).

2.3 HOST AND CLINICAL MANIFESTATION

BVD primarily infects cattle, which are considered the principal natural host, but its host range extends to other even-toed ungulates including sheep, goats, swine, and wild ruminants (Ridpath, 2010). BVD infection presents a wide clinical spectrum influenced by viral strain, host immune status, and age. While subclinical infections are common, symptomatic animals may exhibit fever, depression, decreased milk production, anorexia, nasal and ocular discharges, diarrhea, and mucosal erosions (MSD Veterinary Manual, 2024). Severe cases, notably those involving BVD type 2, include hemorrhagic disease characterized by high fever, thrombocytopenia, mucosal ulcerations, and multisystemic hemorrhages, often resulting in significant mortality (Walz et al., 2020). Pregnant cattle infected early in gestation can produce persistently infected calves, which shed virus lifelong and serve as reservoirs, significantly impacting herd health (Ferreira et al., 2024). Moreover, BVD-induced immunosuppression predisposes affected animals to secondary infections, increasing morbidity and economic losses across herds (Nodar et al., 2023). The disease's clinical course varies from transient mild illness to fatal mucosal disease, necessitating early detection and control to mitigate losses (Wernike et al., 2025) (Figure 3).

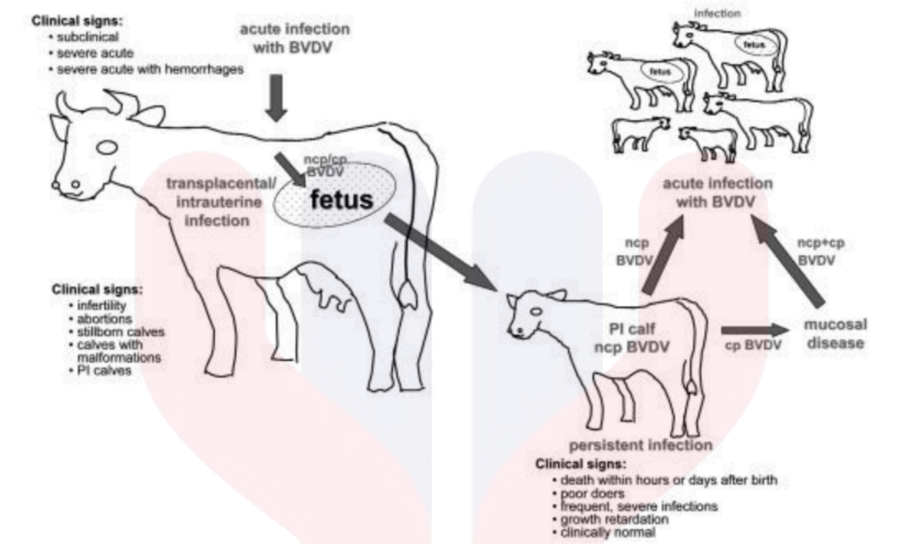

Figure 7.2. Circulation of BVDV in cattle populations.

Figure 3: The clinical manifestation and transmission (Liebler-Tenorio, E. M., 2005).

2.4 DIAGNOSIS OF BVD

Diagnosis of BVD is achieved through a combination of clinical evaluation and various laboratory techniques, each offering distinct advantages and limitations. Virus isolation remains the gold standard for confirming active infection but is time-consuming and requires specialized laboratories (Wang et al., 2024). Serological assays, such as enzyme-linked immunosorbent assays (ELISA), are widely used for detecting viral antigens or antibodies, offering rapid and high-throughput screening, particularly useful for identifying persistently infected animals (MSD Veterinary Manual, 2024). Molecular methods, particularly reverse transcription polymerase chain reaction (RT-PCR), offer high sensitivity and specificity by directly detecting viral RNA in blood, tissues, or secretions, thereby enabling early and accurate diagnosis (Wang et al., 2024; Jiang et al., 2024). Rapid test kits, based on lateral flow immunochromatographic assays, offer quick and easy field screening but serve only as preliminary diagnostic tools due to lower sensitivity compared to molecular and serological methods. Therefore, positive results from rapid kits require confirmation through more definitive laboratory assays to guide effective disease control and management (Wernike et al., 2024).

2.5 EPIDEMIOLOGY

BVD is a globally significant pathogen affecting cattle, with widespread reports of infection across both developed and developing countries. Its prevalence varies widely by region due to differences in surveillance, control programs, and farming practices. In Europe, coordinated eradication programs have significantly reduced BVD prevalence, with antibody prevalence around 46% and antigen prevalence approximately 0.2% (Wang *et al*., 2024). These variations are influenced by factors such as limited resources for disease control and inconsistent vaccination strategies. The economic impact of BVD is substantial, stemming from decreased productivity, reproductive losses, and increased susceptibility to secondary infections. Continued global surveillance and harmonized control efforts are essential to mitigate the burden of BVD on the cattle industry worldwide.

BVDV is classified as a notifiable disease under the Department of Veterinary Services (DVS) Malaysia, as stipulated in the Animal Act 1953 (Revised 2013), necessitating mandatory reporting of suspected or confirmed cases. This poses a significant threat to cattle health in Malaysia, with documented cases seen in various states. However, specific data on the prevalence of BVD in Kelantan is currently lacking. The absence of localised information highlights the need for targeted research in this region. A study conducted in Selangor has reported a BVDV antibody prevalence of 33.2% among 407 cattle screened, with one farm exhibiting serological detection as high as 75% (Admin, 2023). The identification of the BVDV-a subgenotypic in this region further emphasises the virus's presence in Malaysian cattle populations (Khalid et al., 2024). This may pose a potential for similar or varying prevalence rates in Kelantan, thus conducting serological detection studies in this state is crucial to contribute to the broader understanding of the virus's impact on Malaysia's cattle industry.

2.6 TREATMENT AND MANAGEMENT

Treatment and Management of Bovine Viral Diarrhea Virus (BVDV) primarily revolves around supportive care and strategic herd control measures, as there are no specific antiviral drugs available for clearing the virus. Supportive treatment involves addressing secondary bacterial infections, which are common due to immunosuppression caused by the virus, by administering antibiotics and providing fluids to prevent dehydration. Since BVD is highly contagious, management emphasizes preventing virus introduction and spread through strict biosecurity protocols, including quarantine of new animals and minimizing contact with potentially infected sources (MSD Veterinary Manual, 2024). Vaccination plays a crucial role in controlling BVD, with both modified-live and inactivated vaccines available. According to Cornell Vet (2013), vaccination protocols should be tailored to herd status, including the immunization of breeding animals to prevent reproductive losses and protect susceptible calves.

Moreover, herd management must prioritize identifying and removing persistently infected animals, as these calves constantly shed large amounts of virus and are a primary source of herd infection. Regular screening using serological or PCR-based testing, followed by culling of infected animals, can significantly reduce viral circulation within the herd (Walz et al., 2020). Implementing strict biosecurity measures, including controlled movement of animals, disinfection of equipment, and limiting farm access to essential personnel, further reduces risk (NADIS, 2016). Continuous monitoring, herd immunity maintenance, and prudent biosecurity are essential strategies to prevent reintroduction and sustain disease-free status.

Chapter 3

MATERIAL AND METHODOLOGY

3.1 ETHICAL CONSIDERATION

The ethical approval for the use and handling of animals in this study was retrieved from the Animal Ethics Committee of Universiti Malaysia Kelantan under the code (UMK.A06.800-1/2/8).

3.2 METHODOLOGY

3.2.1 STUDY AREA

The study area involved three cattle farms in Kelantan, Malaysia. Out of the eleven provinces in Kelantan, only three farms were selected from one province of Kelantan, which is Bachok. The selected farms included the UMK Teaching Farm and two private farms in Bachok.

3.2.2 STUDY DESIGN

This was a cross-sectional study design in which the selected serum samples were processed and tested for the presence of antibodies against BVD from the sample population at the point of sampling.

3.2.3 STUDY POPULATION

The study population consisted of cattle residing in Bachok, Kelantan, Malaysia. A total of 40 cattle were randomly sampled, including 14 from Farm A, 15 from Farm B, and 11 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 by simple random sampling, regardless of age, sex, breed, and health status.

3.3.2 EXCLUSION CRITERIA

There were no exclusion criteria 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 signalment was recorded in animal profile form prior to blood sampling (Table 3.1).

Table 3.1: Template Form of Physical Examination

Patient ID	Sex	Age	Breed	Water Source	Management Practice

3.4.2 SAMPLE COLLECTION

Forty blood samples were collected via the coccygeal venipuncture method using 1-inch 18-gauge vacutainer needles. The animal was restrained using a makeshift crush to restrict the movement of the cattle and minimize any unwanted trauma or injury to both cattle and personnel during blood collection. The tail was held firmly and raised to expose the ventral side of the tail for better access to the coccygeal vein. The coccygeal groove was palpated with the free hand by pressing the thumb or index finger firmly to identify the vein. The needle was inserted perpendicularly to the skin into the vein. Once drops of blood appeared, confirming vein access, a plain vacutainer tube was inserted into the holder and filled to approximately two-thirds of its capacity. The tube was then removed, followed by the Vacutainer holder and needle. All tubes were labeled with animal ID using a permanent marker and placed in an ice box for transport. Upon arrival at the laboratory, samples were stored in a chiller at 2–8 °C.

3.4.3 SAMPLE PROCESSING

Blood samples were centrifuged at 2500 rpm for 15 minutes to separate and remove clots. Following centrifugation, the serum was carefully extracted using a Pasteur pipette and transferred into labeled microcentrifuge tubes corresponding to each animal's identification number. The processed serum samples were stored at −20 °C until further analysis. Prior to testing with the rapid test kit, the samples were thawed at room temperature.

3.4.4 SEROLOGY DETECTION - RAPID TEST KIT

Serological detection of Bovine Viral Diarrhea Virus (BVDV) antibodies was performed using the commercially available ListoX BVDV P80 Antibody Rapid Test Kit (J&G Biotech Ltd, London, England). Each kit contains test cassettes, sample buffer tubes, and disposable pipettes. The test device includes a testing window with two zones: the test (T) zone and the control © zone. Serum or plasma samples were prepared and tested following the manufacturer's instructions. Approximately 40 µL of the prepared specimen was applied to the sample well labeled "S," followed immediately by two drops (approximately 80 µL) of assay buffer. Results were interpreted between 5 and 10 minutes; tests read after 15 minutes were considered invalid. Upon application of the treated specimen, the liquid migrates laterally by capillary action along the test strip and interacts with recombinant BVDV antigens pre-coated 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.

3.5 DATA COLLECTION

The data resulting from the serological detection of the Bovine viral Diarrhoea was manually recorded and tabulated using 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

4.1 RESULT

A total of 40 cattle serum samples were tested for the presence of Bovine Viral Diarrhoea Virus (BVDV) antibodies using a commercial rapid test kit. The results revealed that 33 out of 40 samples (82.5%) were seropositive, while 7 samples (17.5%) were seronegative. This finding indicates a high seroprevalence of BVDV antibodies among cattle in Bachok, Kelantan (Table 2).

Univariate analysis using the Chi-square test showed no significant association ($p > 0.05$) between BVDV seropositivity and risk factors such as age, sex, breed, deworming status, and herd management practices. This suggests that the widespread seropositivity may not be influenced by these individual factors but rather reflects general exposure to the virus within the study area. Among all tested variables, only the source of water showed a statistically significant association ($p = 0.007$), indicating that cattle drinking from different water sources had significantly higher odds of BVDV infection (OR = 42.018, 95% CI = 0.210–8415.662). Although the confidence interval was wide, this finding suggests that environmental exposure through contaminated water could be a potential route of transmission (Table 3).

Table 4.1: Seroprevalence of Bovine Viral Diarrhoea of Cattle in Bachok, Kelantan.

Farm	Positive BVDV Antigen (Frequency)	Negative BVDV Antigen (Frequency)	Total
A	8	6	14
B	14	1	15
C	11	0	11
Total	33	7	40
Total Percentage	82.5%	17.5%	100%

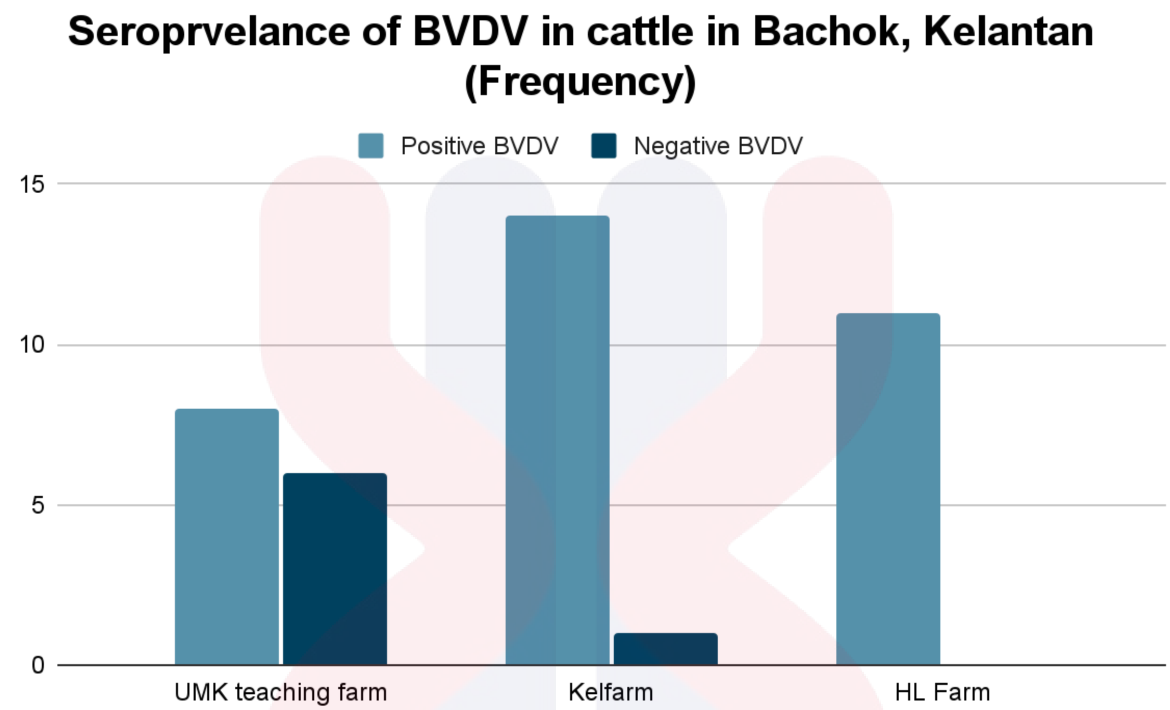

Figure 4: The number of seropositive and seronegative cattle for BVDV in Bachok, Kelantan.

Table 4.2: Statistical analysis of the associated risk in BVDV infected cattle

Variables	Tested (n)	Positive (n)	Prevalence	Odd Ratio	95% CI	P Value*
Age (Years)				0.417	0.094 - 1.848	0.067
< 2	13	9	69.23%			
3 - 5	21	19	90.47%			
> 6	6	3	50%			
Sex				1.413	0.039 - 51.391	0.875
Male	5	4	80%			
Female	35	29	82.85%			
Breed				0.568	0.103 - 3.149	0.667
Kedah Kelantan	15	12	80%			
Friesian Sahiwal	3	2	28.57%			
Charolais	8	7	87.5%			
Brahman	6	6	100%			
Angus	6	5	83.33%			
Cross breed	2	2	100%			
Sources of Water				42.018	0.210 - 8415.662	0.007
Kelantan water	14	9	64.28%			
Underground	15	14	93.33%			
Both	11	11	100%			
Herd Management				1.222	1.041 - 1.435	0.567
Semi-intensive	34	27	79.41%			
Extensive	6	6	100%			

**P-value <0.05 is consider significant*

4.2 DISCUSSION

BVDV is one of the most economically important viral pathogens affecting cattle worldwide. The disease is associated with a wide range of clinical manifestations including reproductive failure, abortion, congenital deformities, growth retardation, mucosal disease, and immunosuppression that predisposes affected animals to secondary bacterial or viral infections (Peterhans et al., 2010). Infected herds often experience substantial financial losses due to reduced fertility, decreased milk yield, increased calf morbidity and mortality, and higher veterinary costs (Gunn et al., 2004). BVDV is also important from an epidemiological standpoint, as it creates persistently infected animals that act as a continual source of viral shedding and contribute to continuous transmission within and between herds (Lanyon et al., 2014). To assess the potential risk in Kelantan, the study was conducted to investigate the seroprevalence of BVDV among bovine serum samples collected from Bachok.

This study is the first to successfully detect BVDV exposure in cattle from Kelantan, with a high seroprevalence of 82.5% observed in Bachok. Since the assay used in this study was an antibody detection test, the interpretation of these findings indicates previous immunological exposure to BVDV antigens rather than confirmation of active viremia. Antibodies against BVDV may persist for long periods following natural infection, and high seroprevalence values have been described in herds where pestivirus circulation is endemic (Fulton et al., 2016). Therefore, confirmatory diagnostics such as antigen ELISA or RT-PCR would be required to differentiate animals that are persistently infected, actively shedding, or simply seropositive after past exposure.

The seroprevalence value recorded in this study was notably higher than values reported in other Malaysian localities. For example, Daves et al. (2016) reported 33.2% in Selangor, while 29.5% was recorded in mixed ruminant herds in another study (Yimer et al., 2021). Variation in seroprevalence between farms and regions has been linked to differences in husbandry systems, population mixing, and biosecurity practices (Peterhans et al., 2021). Most herds sampled here were semi-intensively managed, and several farms practiced mixed-species husbandry where cattle and goats were kept in close proximity. These practices allow increased animal contact rates, enhance the potential for virus spread, and could explain the higher exposure pressure in Bachok (Abdul Rahman et al., 2025). Furthermore, the movement of animals, equipment and personnel between multiple holdings under the same farm operator can accelerate between-herd transmission and help maintain pestivirus circulation (Cardenas et al., 2021).

In this study, BVDV positivity showed a significant association with the type of water source used in the farms. This is biologically logical because BVDV can be shed into the environment via nasal secretions, saliva, urine and faeces from infected animals, especially persistently infected cattle, and contaminated water troughs or shared ponds can act as indirect transmission points within and between herds. Previous studies have similarly reported that indirect exposure via shared water sources contributes to BVDV spread and that viral particle may survive longer in moist, organic-rich environments, which increases the probability of infection when multiple animals access the same untreated water source (Nascimento et al., 2017; Nelson et al., 2018). Therefore, water hygiene and biosecurity around communal drinking points are important risk factors to consider. Farms relying on open or shared water should prioritise closure or treatment of water systems and implement routine cleaning and disinfection of troughs, alongside detection and removal, to reduce the risk of continuous BVDV circulation.

In this study, age, sex, and breed did not show statistically significant associations with BVDV seropositivity. Although cattle aged 3–5 years recorded the highest seroprevalence (90.47%), followed by those under 2 years (69.23%) and those above 6 years (50%), the difference between age groups was not significant (P = 0.067). This suggests that BVDV exposure was relatively widespread across all age categories, despite numerical variations. Similarly, seroprevalence was comparable between males (80%) and females (82.85%), with sex showing no significant effect on infection status (P = 0.875), indicating similar exposure risks for both sexes within the herd. Regarding breed, although certain breeds like Brahman and crossbreeds exhibited 100% seropositivity while Friesian Sahiwal had the lowest prevalence (28.57%), the association between breed and BVDV status was not statistically significant (P = 0.667). This could be attributed to small sample sizes within specific breeds and uniform environmental exposure across different breeds in the study population.

The high seropositivity of 82.5% obtained in this study suggests that a good number of cattle in Bachok have been exposed to BVDV antigens. However, this does not confirm active infection, as antibody detection represents exposure to virus infection rather than current viremia. Several factors may account for the high seropositivity detected: Firstly, this higher seroprevalence may be related to cross-reactivity between BVDV and other pestiviruses such as BDV or HoBi-like viruses since antigenic epitopes may be shared among viruses and can therefore give false-positive reactions following interactions with a detecting antibody (Becher et al., 2003). Secondly, it is important to note here that the rapid test kit used in this study was more than six months beyond the expiration date set by its manufacturer. This could affect the sensitivity and specificity of the assay since expired diagnostic reagents degrade over time, reducing accuracy and increasing the possibilities of both false positives and false negatives (OIE, 2021). Hence, while the overall seroprevalence indicates prior infection with pestivirus antigens, the actual magnitude of the infection within this population should be interpreted with caution due to potential diagnostic limitations.

The diagnostic approach adopted for this study used a rapid immunochromatographic test kit designed for the detection of BVDV antibodies. While these kits present advantages in terms of simplicity, speed, and field applicability, they generally possess lower analytical sensitivity and specificity compared to laboratory-based assays such as ELISA or VNT (OIE, 2021). The use of a rapid test kit coupled with its expired status at the time of testing may have reduced the accuracy of the test, with resultant possibilities of overestimation or underestimation of the true seroprevalence. In contrast, other studies conducted in Malaysia using ELISA-based assays reported markedly lower seroprevalence rates. For instance, Abdul Rahman et al. (2025) reported a prevalence of 47.2% in cattle from Selangor, while Ismail et al. (2021) detected only 26.3% in Pahang. Such observed differences could, in part, be related to variation in the diagnostic methods, test sensitivity, sample size, and regional management practices. According to Fulton et al. (2004), ELISA remains one of the most reliable methods for BVDV antibody detection, offering higher diagnostic accuracy and reproducibility than the rapid tests. In this respect, although these findings do indicate that there is widespread exposure to BVDV within Bachok, the true infection prevalence may indeed differ from the observed rate due to diagnostic limitations with the rapid test method.

Although rapid test kits are advantageous in their ease of use, affordability, and applicability for field screening, there are a number of limitations to these methods, which significantly influence diagnostic reliability. Besides that, antibodies and reagents inside the kit may decay due to improper storage or long expiration time-this is particularly possible if kits are used past the expiration time, as was the case in this study. It can result in a reduction in the efficiency of antigen-antibody binding and will reduce both sensitivity and specificity of the performed assay (Tao et al., 2013). The rapid test kit adopted for this study identifies IgG antibodies against BVDV, which indicate previous exposure or long-term immune response. Similarly, most conventional assays performed using indirect ELISA also target IgG but with greater analytical sensitivity, offering quantification of antibody titres. IgM antibodies, reflecting recent or acute infection, are transient and not measured in the course of standard serological surveillance regarding BVDV infection (Lanyon et al., 2014). Thus, even though the rapid kit provides a qualitative measure of exposure, it cannot differentiate active, past, or persistent infections.

A high seroprevalence, 82.5%, detected in this study indicates that BVDV is likely circulating at high rates within the cattle population in Bachok. In addition, it has serious consequences in both animal welfare and herd productivity. Although most infections are subclinical, BVDV causes a wide range of health problems, including immunosuppression, reproductive failure, congenital defects, and increased susceptibility to secondary bacterial or viral infections (Ridpath, 2010; Lanyon et al., 2014). PI cattle, in particular, experience chronic ill-thrift, reduced growth performance, and a shortened lifespan, all of which have negative effects on animal welfare and economic efficiency (Fulton et al., 2004).

Furthermore, from an industrial point of view BVD is considered one of the most economically important viral infections of cattle worldwide, with substantial losses arising from reproductive wastage, reduced milk yield, higher mortality among calves, and increased veterinary expenditure (Huser et al., 2021). The presence of BVDV-positive animals in a herd can also interfere with trade and movement based on control and eradication programs that are usually certified free from the disease (Lindberg & Alenius, 1999). On mixed-species farms, there is also the possibility of spillover to small ruminants, further complicating disease control and potential establishment of pestivirus reservoirs in other livestock species. Although BVDV is not zoonotic itself, its impact on herd health and productivity makes it an indirect threat to food security and rural livelihoods of major proportions. Thus, the high seroprevalence recorded in the current study provides a warning signal about the pressing need for efficient surveillance, control, and farmer education programs in Kelantan in order to alleviate welfare concerns and reduce the long-term economic burden associated with the disease.

Chapter 5

5.1 CONCLUSION

This is the first serological investigation of BVDV exposure amongst cattle in Bachok, Kelantan. A high percentage of seroprevalence at 82.5% was detected using a rapid antibody test kit, indicating that the infection of BVDV or related pestiviruses is widespread in the local cattle population. These results suggest that the infection may be endemic within this region due to semi-intensive management, mixed-species rearing of animals, and frequent inter-farm contact by communal farm management. Even though the findings of the study revealed considerable antibody detection, the interpretation must be made with caution, as several of the diagnostic kits utilized had surpassed their expiration date and would have affected their sensitivity and specificity. Moreover, antibody detection merely accounts for previous infection rather than the true current infection status. Therefore, the actual infection rate in the population could be different from the seroprevalence observed.

The present study showed that BVDV has significant health and economic impacts on the local cattle industry. The high seropositivity indicates a call for increased awareness among farmers and veterinarians on matters of biosecurity, monitoring of herd health, and implementation of national control programs. Continuous surveillance is highly vital in preventing further viral spread to improve the productivity and welfare of Malaysian cattle herds.

5.2 FUTURE WORK

Future research should aim to confirm the presence of active or persistent BVD infections through more specific diagnostic approaches such as antigen detection, ELISA or reverse transcription polymerase chain reaction (RT-PCR). These methods would enable differentiation between animals that are seropositive due to previous exposure and those that are persistently infected, thereby providing a more accurate understanding of infection dynamics. It is also recommended that subsequent studies employ validated and unexpired diagnostic kits, such as ELISA or virus neutralization tests, to enhance diagnostic precision and reliability.

In addition to testing animals, future work should include environmental sampling such as testing of water sources, feed, handling equipment, and communal areas to identify potential reservoirs or fomites that may contribute to indirect transmission of the virus within and between farms. Molecular characterization and genotyping of BVDV strains should also be conducted to determine the circulating subtypes and assess possible sources or transmission pathways. Expanding surveillance to encompass a larger geographic area and including other ruminant species such as goats and sheep would further aid in evaluating cross-species transmission risks, especially in mixed-farming systems. Lastly, future research should investigate herd-level risk factors such as management practices, animal movement, vaccination status, and reproductive performance to clarify the determinants of BVDV persistence. The establishment of coordinated regional surveillance programs, farmer education initiatives, and enhanced biosecurity and vaccination strategies will be essential to effectively mitigate the impact of BVDV on Malaysia's cattle industry.

Appendix

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