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Research

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# Seroprevalence of Bovine Viral Diarrhea Virus Infection and Associated Risk Factors in Cattle in Selangor, Malaysia

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## ABSTRACT

The aim of this study was to investigate Seroprevalence of Bovine Viral Diarrhea Virus (BVDV) infection in cattle in the state of Selangor, Malaysia and associated risk factors. A total of 407 blood samples were collected from five selected farms within Selangor. Sampled animals were identified for their breed, age, lactation and pregnancy status. The plasma extracted from blood samples were used for detection of antibody against BVDV using an ELISA test kit (Prio-CHECK® BVDV antibody) following the manufacturer's protocol. Results demonstrated an overall 33.2% (135/407) prevalence of BVDV antibody; with four of the farms tested positive. Prevalence in one farm reached 75.9% (66/87) which was higher than the other four farms with a prevalence of 26.0% (66/254), 13.3% (2/15), 2.8% (1/36) and 0% (0/15). Animals grouped according to breed, age, lactation and pregnancy status showed significant variation in BVDV prevalence. Higher number of adults (36.7%) than young calves (15.2%), pregnant (42.9%) than non-pregnant (31.1%) and more lactating (51.1%) than non-lactating (25.8%) cows, were affected ($p<0.05$). Friesian-Sahiwal and Jersey cattle were the most affected while the local Kedah-Kelantan cattle were the least affected. In conclusion, the study revealed immense exposure of cattle in Selangor to BVDV infection that varied with breed, age, lactation, and pregnancy status of the animals. As to our knowledge, this is the first report on BVDV status in cattle in Malaysia and the seroprevalence result would serve as a baseline data for further investigation on the disease.

**KEYWORDS:** Bovine Viral Diarrhea Virus (BVDV); Cattle; ELISA; Prevalence; Risk factors.

**ABBREVIATIONS:** BVDV: Bovine Viral Diarrhea Virus; BVD: Bovine Viral Diarrhea; CP: Cytopathic; NCP: Non-cytopathic; MD: Mucosal Disease; PI: Persistently Infected; OIE: World Organization for Animal Health or Office International des Epizooitic; TPU: Taman Pertanian Universiti; PKC: Palm Kernel Cake; FMD: Foot and Mouth Disease; HS: Hemorrhagic Septicemia; OD: Optical Density; Pi: Percentage inhibitions; AI: Artificial Insemination; KK: Kedah-Kelantan.

## INTRODUCTION

Bovine Viral Diarrhea (BVD) disease is caused by BVD virus (BVDV) which is a small, enveloped, single-stranded RNA virus.[1] The virus belongs to the genus Pestivirus (Flaviviridae Family) and primarily infects cattle.[2] There are two known genotypes of BVDV (BVDV-1 and

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BVDV-2) that differ, in their antigenic and genetic properties. According to Houe,[3] either of the genotypes of BVDV is able to cause acute and persistent infection, but BVDV-2 causes much more severe and acute symptoms when infecting susceptible animals compared to BVDV-1. Based on cytopathogenicity, BVDV is further divided into two different biotypes: cytopathic BVDV (CP) and non-cytopathic BVDV (NCP). The CP BVDV is able to cause cell damage in a cell culture and shows vacuolization and cell lyses while NCP BVDV does not cause any changes in cell culture.

In nature, NCP BVDV is the most common biotype that causes damage while CP BVDV is responsible for mucosal disease (MD) in persistently infected (PI) animals. Pregnant cows which are exposed to NCP BVDV between 42 and 125 days of gestation may produce a PI calves if born alive.[4] Persistently infected calves basically are immunotolerant to the virus strain and perfect carriers that keep shedding the virus for their entire lives.[5] Infection to BVDV during the pregnancy period may also lead to early embryonic death where the animal will return to estrus cycle, calf born with congenital diseases, or born weakened. If naive non-pregnant cattle with no vaccination for BVDV come in contact with the agent, it may result in transient viremia that leads to short-term leucopenia, immunosuppression, agalactia, lymphopenia, pyrexia, and diarrhea. Antibodies against the virus are produced about 3 weeks post-infection and the animal may recover if no concurrent infection during that period, but the animal however still carries and continues to shed the virus but at much lower concentration as compared to what PI animal's do.[2]

The virus causes significant economic losses to the farming industry due to its effect on reproductive performance of the infected animal and immunosuppression that leads to secondary infection.[6] The farmer also suffers from a severe economical impact as a result of repeat breeding problems, abortion, increased neonatal mortality, and increased death among young stock.[7,8] BVDV infection in ruminant has been reported by many countries worldwide and is listed by the World Organization for Animal Health or Office International des Epizooitic (OIE) as a notifiable and priority cattle disease for international trade due to its economic importance.[9,10] In Australia, the prevalence of BVDV exposed cattle herds was reported to be high varying from 82-100%.[11,12] Although reports varied across states, a seroprevalence of 75-85% of BVDV antibody in adult cows was documented in Australia.[13] Thailand and Argentina have also reported a BVDV prevalence of 73% and 70% respectively,[1,14] while a prevalence of 24.7% is reported recently from smallholder dairy units in India.[15] In Malaysia, despite partially restricted movement of cattle and importation from BVDV endemic countries such as Thailand and Australia, the disease seems to have been overlooked with no investigation and report on BVDV infection in domestic animals so far. Thus, the objectives of this study were to investigate the seroprevalence of the BVDV infection and risk factors in five cattle farms in Selangor, Malaysia.

## MATERIALS AND METHODS

### Animals and Management

A total of 407 cattle were sampled for BVDV prevalence from five farms in Selangor, Malaysia. The farms were Taman Pertanian Universiti (TPU) that belongs to the Universiti Putra Malaysia and four private farms adopted by the University for Student Practical training. For the purpose of reporting, the farms were identified as Farm A, B, C, D and E with a total estimated cattle population of 250, 70, 50, 80, and 300, respectively. The privately owned farms were involved in dairy production while TPU farm ran both beef and dairy units. Calves in the dairy farms were raised in cattle pens separated from their dams whereas in the beef unit, calves were allowed to run with their dams until weaning at 7 months of age. The predominant breeds of cattle found in the farms include Friesian, Friesian-Sahiwal and Jersey for dairy production and local Kedah-Kelantan, Brangus, Braford and Simmental for beef production.

Farms A, B and D were managed semi-intensively whereby cattle were allowed to graze during the day and housed during the night whereas farms C and E were managed under an extensive system whereby cows were left to graze on pasture in the field. Animals were also provided with feed supplements such as soybean and palm kernel cake (PKC) during the time of milking as well as with freshly cut Napier grass. Water was provided ad libitum. Animals in all the farms were vaccinated against Foot and Mouth Disease (FMD) and Hemorrhagic Septicemia (HS). There was no history of vaccination for BVDV and the owners did not know about the disease. All the animals at the time of sampling looked apparently healthy.

### Blood Sampling and Storage

A total number of 407 animals (87 from Farm A, 36 from Farm B,[15] from Farms C&D each, and 254 from Farm E) were used for blood sampling. Samples from the private farms were obtained randomly based on the total number of animals allowed for bleeding while almost all the animals from farm E were sampled. The samples from the private farms were obtained from November 2014 to January 2015 while majority of the samples from Farm E were obtained and stored earlier. The time length for both archived and newly collected samples is estimated to be one year. Animals were identified for their sex, age (as calves with less than 9 month old age or adults), breed, production type (dairy or beef), lactation and pregnancy status.

Blood samples were collected using EDTA tubes (BD Vacutainer®, USA) *via* venipuncture of either from the jugular vein or coccygeal vein. In adult animals, most samples were obtained from coccygeal vein due to lack of proper cattle restraining facility in the farms except farm E. Most of the blood samplings in calves however were obtained *via* the jugular vein. The fresh blood samples were transported to the laboratory on the same day in icebox and centrifuged at 1800 rpm for 10 minutes

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(Kubota, Japan) to separate the plasma from the whole blood. The collected plasma portion was stored at -20 °C in a refrigerator (Acson, Malaysia) in labeled microtubes (Eppendorf®, Malaysia) with individual animal ID.

### Detection of Antibody using ELISA

The ELISA procedure to detect antibody against BVDV was done according to the protocol provided by the manufacturer (PrioCHECK® BVDV Ab, Prionics AG, Switzerland). The test kit is an inhibition ELISA method giving a signal that is reciprocal to the sample antibody concentration. The test employs two monoclonal antibodies (mAb) that recognize two different epitopes, which are found, located at the highly conserved non-structural protein NS-3 (p80) of BVDV. One of the mAb was coated to the plate while the other mAb used as a conjugate.

A 50 µl of samples were incubated with the inactivated BVDV antigen in the wells that came coated with the first mAb. After incubation for one hour and washing, the second mAb conjugated with an enzyme that generated a color signal was added. Then, subsequent to a second incubation for one hour and washing, the chromogen 3,3', 5,5; -tetramethylbenzidine (TMB) substrate was dispensed to all wells. After 15 minutes of incubation, color development was stopped by addition of sulfuric acid that acted as a stop solution for the TMB reaction. The color signal was measured by using ELISA microplate reader (TECAN, Switzerland) at 450 nm using data analysis software Magellan™.

Each of the optical density (OD) result of the sample was recorded and the percentage inhibitions (Pi) of the optical density were calculated by using the formula given by the manufacturer's protocol as indicated below for interpretation of the result.

$$\text{Percentage inhibition} = 100 - \left[\frac{\text{corrected OD}_{450}\ \text{test sample}}{\text{corrected OD}_{450}\ \text{Max value}}\right] \times 100$$

A Pi result <50% was considered as test negative with absence of BVDV specific antibody in the sample indicating that the animals are either free from the agent or immunotolerant. While results with Pi≥50% were considered as test positive indicating the presence of BVDV – specific antibody in the samples reflecting that the animals' current or previous infection by the virus. The sensitivity and specificity of the test kit were 98% and 99% respectively.

### Data Analysis

Data obtained were analyzed for the seroprevalence of the virus infection for the total sample population and at individual herd level according to Bonita et al[16] by dividing the total number of animals tested positive to the total number of animals tested, multiplied by 100%. A non-parametric statistical tests (Kruskal-Wallis and Mann-Whitney U test) using a Statistical Package for Social Sciences (SPSS) v.20 (IBM Inc, USA) were used to compare the differences in BVDV seroprevalence among breeds while a chi-square test was conducted to determine the association between the BVDV status of the animals and the risk factors (age, sex, production type, pregnancy and lactation status). Differences among groups of each factor were considered significant at $p<0.05$ for all parameters tested.

## RESULTS

Overall and individual farm seroprevalence of BVDV disease in cattle in the study area are shown in Table 1. The overall prevalence of BVDV antibodies found was 33.2%. Looking at individual farm level, farm A showed the highest prevalence with 75.9% of the animals tested were seropositive against BVDV infection. Farm A accounted for almost half of the BVDV seropositive samples out of the total 407 animals investigated; it represented 66 samples out of 135 total seropositive BVDV samples found. This is followed by farm E, C and B with prevalence rate of 26%, 13.3% and 2.8%, respectively. However, there was no any detectable antibody against BVDV was found in samples collected from farm D (0%).

During sampling, animals were identified according to their sex, age, breed, pregnancy status, lactation status, and purpose to investigate their association with BVDV prevalence. As shown in Tables 2 and 3, results showed significant effects of named risk factors on the seroprevalence of BVDV. It was found that higher ($p<0.05$) number of females (35.5%) than males (16.3%), adults (36.7%) than young calves (15.2%), dairy cattle (52.6%) than beef cattle (7.9%); pregnant (42.9%) than non-pregnant cows (31.1%), lactating (51.1%) than non-lactating (25.8%) cows, were sero-positive to antibody against BVDV (Table 3).

Moreover, as shown in Table 3, breed was also found to affect significantly ($p<0.05$) the seroprevalence of BVDV

| Farm | Total No. of Animals Tested | No. of Animals Tested Seropositive | Prevalence of BVDV exposure |
|---|---|---|---|
| A | 87 | 66 | 75.9% |
| B | 36 | 1 | 2.8% |
| C | 15 | 2 | 13.3% |
| D | 15 | 0 | 0% |
| E | 254 | 66 | 26.0% |
| Total | 407 | 135 | 33.2% |

**Table 1:** Overall and individual farm prevalence of BVDV exposure in cattle in Selangor.

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| Risk Factors | Group | Total | Test Result | | BVDV Ab Prevalence (%) | *P*-value (*P*<0.05) |
|---|---|---|---|---|---|---|
| | | | Positive | Negative | | |
| **Sex** | Male | 49 | 8 | 41 | 16.3 | 0.008 |
| | Female | 358 | 127 | 231 | 35.5 | |
| **Age Group** | Adult | 341 | 125 | 216 | 36.7 | 0.001 |
| | Calf | 66 | 10 | 56 | 15.2 | |
| **Lactation Status** | Lactating | 135 | 69 | 66 | 51.1 | 0.000 |
| | Non-lactating | 217 | 56 | 161 | 25.8 | |
| **Pregnancy Status** | Pregnant | 133 | 57 | 76 | 42.9 | 0.025 |
| | Non-pregnant | 219 | 68 | 151 | 31.3 | |
| **Production Type** | Beef | 177 | 14 | 163 | 7.9 | 0.000 |
| | Dairy | 230 | 121 | 109 | 52.6 | |

**Note:** *p* values <0.05 indicate significant difference between the groups under the same category.
**Table 2:** Prevalence of BVDV exposure and its association with grouping factors.

| Breeds | Total No. of Animals Tested | Tested Positive | Tested Negative | BVDV Ab Prevalence (%) |
|---|---|---|---|---|
| Braford | 36 | 5 | 31 | 13.9[a] |
| Friesian-Sahiwal | 133 | 60 | 73 | 45.1[b] |
| Simmental | 33 | 2 | 31 | 6.1[ac] |
| Brangus | 42 | 5 | 37 | 11.9[ac] |
| Kedah-Kelantan | 66 | 2 | 64 | 3.0[c] |
| Friesian | 52 | 27 | 25 | 51.9[b] |
| Jersey | 45 | 34 | 11 | 75.6[d] |

**Note:** values with different superscripts across column varies significantly ($p<0.05$).
**Table 3:** Distribution of BVDV prevalence among the different breeds of cattle investigated.

found among the different breeds of cattle investigated. According to the result, the highest prevalence of BVDV is found in Jersey breed of cattle (75.6%) followed by the Friesian (51.9%), Friesian-Sahiwal (45.1%), Braford (13.9%), Brangus (11.9%), Simmental (6.1%), while the lowest prevalence was recorded in Kedah-Kelantan (KK) (3.0%).

## DISCUSSION

The overall prevalence of BVDV infection obtained from the present study is 33.2% which is a little higher, compared to a report from Saudi Arabia (26%)[8] and Kerala, India (24.7%)[15] but less compared to other places such as Thailand (73%)[14] and Australia (75-85%) varying according to states.[12,13] The finding is also in agreement with previous reports of worldwide BVDV antibody prevalence in cattle ranging from 0-90%.[9]

All of the grouping factors which were investigated for their interaction with the BVDV prevalence in the current study have shown significant association. These factors include sex, breed, age, lactation and pregnancy status of the animals. It was found that significantly more females (35.5%) than males (16.3%), more adults (36.7%) than young calves (15.2%), more pregnant (42.9%) than non-pregnant (31.1%) cows, and more lactating (51.1%) than non-lactating (25.8%) cows were affected. Although the actual mechanism by which these factors biologically interact with BVDV infection needs a more detail investigation, the data obtained suggests that these factors might have important role in BVDV occurrence and hence they may be considered as risk factors. Risk factors to BVDV occurrence in cattle might vary from place to place. For example, a study by Almeida et al[17] in Southern Brazil had hypothesized that artificial insemination (AI) technicians as contributing factors to introduce the virus into farms through clothes, shoes and contaminated equipment. However, this factor is only important in countries where AI practice is very popular such as most Western countries, but not in Malaysia's cattle industry where AI service is very limited. While a study by Humphry et al[18] reported that vaccination, suspicion of BVD by the farmer, housing pregnant cows with calves, herd size and proportion of herd that is dry are all associated with higher percentage of seropositive result.

The higher prevalence of BVDV in female cattle compared to males might be due to most of the Ladang Angkat farms in this study are dairy farms which depend on imported breeds of cattle such as Jersey and Friesian from disease endemic countries such as India and Brazil with BVDV prevalence of 16.3-24.7% and 56% respectively,[15,19] as well as from neighboring country like Thailand.[20] Australia with high seroprevalence of BVDV as documented by Taylor et al[13] could also be one of the contributing factors as most Jersey cows were imported from there. Moreover, in dairy cattle production, male calves are not usually kept longer within the farm as the farmers usually sell them as veal.[21]

The higher seroprevalence of BVDV found in adult animals compared to young calves (<9 months) is consistent with a recent report from Ireland[10] who also reported higher prevalence of BVDV antibody in cattle beyond 270 days old age compared to younger calves. An increase in seroprevalence from 10% in heifers to 75-85% in cows aged 10 years has been also reported possibly due to an increase in an animal's risk of having been

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exposed to BVDV over time.[12,13] The lower seroprevalence in calves could also be due to some of the calves investigated might be PI animals which are known to be immunotolerant to the virus and do not produce antibody against the virus to be detected by the ELISA Ab test. According to Fulton et al[4] the prevalence of PI animals in South Central United States was revealed to be 0.55%, while Houe et al[22] recorded the prevalence of PI animals in Michigan (USA) to be 0.13%. The prevalence of PI animals worldwide has been reported between 0.13% and 2%.[23] Further systematic investigation using antigen-based ELISA should be conducted to determine the prevalence of PI animals in Selangor, which are very important sources of infection.

The significantly higher prevalence of BVDV found in pregnant cows compared to non-pregnant cows might be attributed to peripartum immunosuppression effect[24] that can be associated with the change in the stress hormone (cortisol) level in the body which is well known to increase about few weeks before parturition. But, this explanation only applies to cattle about a few weeks before parturition. In the present study, attention was not given to determine the exact stage of pregnancy. Hence, further study on the association considering the various stages of pregnancy is needed to explain the difference in prevalence of BVDV in term of pregnancy status of the animal. The possible explanation for the observed significant association between seropositivity and lactation might be due to the higher risk of getting infection from the workers that milked the cows as the virus can easily be transmitted through fomites such as contaminated cloth or equipment[17,25] which are used during the milking process.

Furthermore, the difference in prevalence from farm to farm might be attributed to the difference in management of the farms, the source of animals, as well as the type of production. For example, farm A has the highest prevalence of seropositive BVDV (75.9%) and according to the owner, most of the animals were imported from endemic countries such as Thailand and Australia. According to Salina et al[20] and the Malaysian Department of Veterinary Services, the list of countries from which Malaysia imports cattle either for breeding or slaughtering includes, Thailand, Myanmar, Australia, India, New Zealand and Brazil. Each of these countries has reported variable degree of BVDV prevalence in their cattle population. Moreover, there is no also any BVDV specific restriction on importation of cattle to Malaysia currently. Therefore, if the farm imports more animals from Thailand with prevalence of 73%, it is likely to have higher BVDV seropositive animals in the farm. On the other hand, if importation of cattle is from countries like India where BVDV prevalence is relatively lower (15.3-24.7%), it might result in low prevalence of BVDV.[15,19] While farm D, with no detected seropositive animals found, it has been noted that the owner mostly relies on the farm itself to breed and rear its own replacement heifers instead of importation from outside. Trade is known as one of the epidemiological determinants for the introduction and spread of BVDV in cattle herds.[26] In addition to animal source, farm size could also be another factor for the difference in seroprevalence as higher numbers of seropositive animals have been reported to be detected in larger herds.[10,18] Additionally, larger herds have more susceptible animals available to maintain infection and herd size is a cluster variable for several biosecurity risks such as increased purchase of animals and increased visitors (veterinary practitioners, technicians, contract workers), all of which will increase the risk of disease introduction and maintenance.[10]

Breed of cattle investigated has shown to have also a significant association with BVDV prevalence in the current study. Based on the data obtained, dairy breeds such as the Jersey breed (75.6%), Friesian (51.9%) and Friesian-Sahiwal (45.1%) breeds showed higher prevalence rates. Most of these dairy breeds were imported which might reflect the importance of importation as a possible contributing factor to the observed prevalence of BVDV in the State of Selangor. Meanwhile, for beef cattle which comprises mainly the local Kedah-Kelantan (KK) breed showed the least prevalence (3%). The low prevalence in KK breeds might imply that the animal's freedom of the disease originally but get infected as a result of rearing them in contact with other imported cattle breeds that showed higher prevalence. This also might explain why farms with dairy production which consists of Jersey and Friesian breeds have showed higher prevalence (which is up to 75.9%) compared to the other breeds of cattle. However, this assumption could be explained better by extending the study further to other states of Malaysia.

The use of PrioCHECK® BVDV antibody testing in this study generally revealed the first evidence that the BVDV exposure is prevalent in the state of Selangor, Malaysia. However, based on the current study it is not possible to confirm PI status and tell the genotype of BVDV that might be predominant, whether BVDV-1 or BVDV-2. Knowing the genotype and sub-type of BVDV is very important in term of control of the infection *via* vaccination approaches. BVDV distribution reported globally has shown variation in genotype and sub-type. For example, the study by Lanyon et al[2] stated that BVDV type 1 is predominant in Australia with subtype 1c being the most prevalent, while in a study by Fulton et al[4] revealed that the most prevalent BVDV subtype in affected beef cattle in south central of USA is type 1b followed by subtype 1a and 2a.

## CONCLUSION

The study revealed a high rate of exposure of cattle in Selangor to BVDV as demonstrated by a seroprevalence of 33.2%. Further study needs to be done to evaluate and determine the overall prevalence status of BVDV in different states of Malaysia. This will help to evaluate the extensiveness and impact of the disease to the cattle industry, as well as to other ungulates that can be cross infected by the virus. This study also suggests the importance of sex, age, breed, production type (dairy or beef), lactation status, and pregnancy status as contributing factors to the prevalence of BVDV. Despite some of the explanations pro-

vided, full understanding of the biological association between these risk factors and BVDV prevalence requires further detail investigations. Breed differences in the sero-positivity of BVDV demonstrated by higher prevalence among the dairy cattle that mainly comprises imported Friesian and Jersey breeds compared to the beef cattle breeds that were mainly composed of the local Kedah-Kelantan breed, might reflect the importance of importation as crucial route of introduction of the disease to the country while the indigenous population could have been free or at a low rate of occurrence.

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**ANIMAL ETHICS AND USAGE**

All samples were collected under the supervision of veterinarians. The study was conducted following the guidelines as stated in the Code of Practice for Care and use of Animals for Scientific Purposes as stipulated by Universiti Putra Malaysia (Ref: UPM/IACUC/FYP-2014/FPV.018), complied with the current guidelines for the care and use of animals, and was approved by the Animal Care and Use Committee (ACUC), Faculty of Veterinary Medicine, Universiti Putra Malaysia.

**ARTICLE HIGHLIGHTS**

- Overall cattle BVDV seroprevalence of 33.2% in Selangor, Malaysia.
- A highest seroprevalence of 75.9% found in one farm.
- Seroprevalence influenced by breed, age, sex, lactation and pregnancy status.

**CONFLICTS OF INTEREST**

The authors declare that there are no conflicts of interest that could be perceived as prejudicing the impartiality of the research reported.

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