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PREVALENCE OF BOVINE VIRAL DIARRHEA VIRUS (BVDV) INFECTION IN CATTLE POPULATION IN THE STATE OF SELANGOR

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PREVALENCE OF BOVINE VIRAL DIARRHEA VIRUS (BVDV) INFECTION IN CATTLE POPULATION IN THE STATE OF SELANGOR

LARRY DAVES

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CERTIFICATION

It is hereby certified that we have read this project paper entitled "PREVALENCE OF BOVINE VIRAL DIARRHEA VIRUS (BVDV) INFECTION IN CATTLE POPULATION IN THE STATE OF SELANGOR" by LARRY DAVES and in our opinion it is satisfactory in terms of scope, quality and presentation as partial fulfilment of the requirement for the course VPD 4999 – Final Year Project.

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ABSTRAK

Kekerapan Jangkitan Bovine Viral Diarrhea Virus (BVDV) Dalam Lembu Di Dalam Negeri Selangor

Oleh

Larry Daves

2015

Penyelia: Dr. Nurhusien Yimer

Kajian ini adalah bertujuan untuk menyiasat prevalen jangkitan BVDV pada populasi lembu di ladang terpilih di Selangor serta interaksi dengan factor risiko yang berkemungkinan. Sejumlah 407 sampel darah telah diambil dari lima ladang Angkat terpilih UPM dan Taman Pertanian Universiti (TPU) di negeri Selangor. Sampel serum atau plasma telah diasingkan daripada sampel darah dan disimpan pada suhu -20°C sehingga analisis dijalankan. ELISA langsung (PrioCHECK ® BVDV antibodi) telah digunakan untuk mengesan antibody terhadap BVDV. Keputusan menunjukkan bahawa secara keseluruhannya prevalen jangkitan BVDV adalah 33.2% (135/407). Terdapat perbezaan signifikan dalam prevalen antara setiap individu ladang; 75.9% (66/87), 26.0% (66/254), 13.3% (2/15), 2.8% (1/36), 0% (0/15), masing-masing bagi ladang A, E, B, C dan D. Prevalen jangkitan BVDV pada kumpulan haiwan mengikut baka, umur, jantina, status laktasi dan juga bunting menunjukkan variasi yang signifikan. Didapati signifikan yang ketara pada lembu yang terjejas adalah lebih kepada lembu betina

35.5% (127/358) daripada jantan 16.3% (8/41); haiwan dewasa (36.7%) daripada muda (15.2%),bunting (42.9%) berbanding tidak bunting (31.1%); laktasi (51.1%) daripada tiada laktasi (25.8%). Mengikut baka, lembu tenusu Friesian-Sahiwal dan Jersey-Friesian merupakan yang paling terjejas manakala lembu pedaging seperti Kedah-Kelantan adalah kurang terjejas. Kesimpulannya, kajian ini mendedahkan bahawa jangkitan BVDV adalah sangat lazim padal embu di Selangor dan berbeza-beza antara ladang, baka, jantina, umur, laktasi dan status kebuntingan. Kajian ini adalah yang pertama di Malaysia untuk meneroka status jangkitan BVDV dalam kalangan lembu dan menjadi asas dalam penyiasatan lanjut BVDV di masa hadapan.

Katakunci: BVDV, lembu, factor risiko, ELISA langsung, prevalen

ABSTRACT

Prevalence Of Bovine Viral Diarrhea Virus (BVDV) Infection In Cattle In The State Of Selangor

By

Larry Daves

2015

Supervisor: Dr. Nurhusien Yimer

The aims of this study were to investigate prevalence of BVDV infection in cattle population based on selected farms in Selangor and its interaction with possible risk factors. A total of 407 blood samples were collected from five selected Ladang Angkat of UPM and Taman Pertanian Universiti (TPU) within Selangor. Serum or plasma samples were separated from the collected blood samples and stored at -20 °C until analyses. A direct ELISA (PrioCHECK®BVDV antibody) was used to detect antibody against BVDV following the protocol given by the manufacturer. Results demonstrated that the overall prevalence of BVDV infection was 33.2% (135/407). There was a significant difference in the prevalence among individual farms; 75.9% (66/87), 26.0% (66/254), 13.3% (2/15), 2.8% (1/36), 0% (0/15), for A, E, B, C and D farm respectively. Animals grouped according to breed, age, sex, lactation and pregnancy status showed significant variation in BVDV prevalence. 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%); more lactating (51.1%) than non-lactating (25.8%) cows affected. According to breed, dairy Friesian-Sahiwal and Jersey crosses were the most affected while beef cattle breeds such as Kedah-Kelantan were least affected. In conclusion, the study revealed that BVDV infection are highly prevalent in cattle in Selangor and varies with farms, breed, sex, age, lactation, and pregnancy status. This study is pioneer to explore the status of BVDV infection in cattle populations in Malaysia and would help as a foundation for further investigations on BVDV.

Keywords: BVDV, cattle, risk factor, direct ELISA, prevalence

1. INTRODUCTION

Bovine viral diarrhea (BVD) disease is caused by BVD virus (BVDV) which is a small, enveloped, single-stranded RNA virus (Pecora et al., 2009) that belongs to the genus Pestivirus (Flaviviridae Family) and primarily infects cattle (Lanyon et al., 2014). There are two known genotypes of BVDV (BVDV-1 and BVDV-2) which differ in their antigenic and genetic properties. According to Houe (2003), 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 cytopathicity, 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 that includes 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 animals. Pregnant cattle which are exposed to NCP BVDV between 42 and 125 days of gestation may produce a persistently infected calf if it is born alive (Fulton et al., 2008). Persistently infected calves basically are immunotolerant to the virus strain and perfect carriers that keep shedding the virus for their entire lives (Polak & Zmudzinski, 1999). Infection to BVDV during the pregnancy period also may lead to early embryonic death where the animal will return to estrus cycle, calf born with congenital diseases, or born weakened. If naïve 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 animals do (Lanyon et al., 2014).

The virus causes significant economic losses to the farming industry due to reduced reproductive performance of the infected animal and immunosuppression that leads to secondary infection (Diéguez et al., 2009). The farmer also struck with a severe economical impact in term of repeat breeding, abortion, increased neonatal mortality, and increased death among young stock (Thobokwe, 2003 ; Mahmoud & Allam, 2013).

Since 60 years back, BVDV still continues to cause significant economic losses to the cattle industry worldwide. In many countries, the prevalence of the sero-positive BVDV can reach up to 90% (Niskanen, 1995). For example, Thailand and Argentina have reported a BVDV prevalence of 73% and 70% respectively (Kampa et al., 2004; Pecora et al., 2009). In Malaysia, despite the unrestricted movement of cattle and importation from BVDV endemic countries such as Thailand and Australia, the disease seems to be overlooked with no investigation and report on BVDV infection in domestic animals so far.

Thus, the purposes of this study were to investigate the sero-prevalence of the BVDV infection and possible associated risk factors in cattle in the state of Selangor, Malaysia, based on selected farms from Ladang Angkat of UPM and Taman Pertanian Universiti (TPU) cattle farm.

2. LITERATURE REVIEW

2.1 Aetiological Agent

Bovine viral diarrhea (BVD) disease is caused by BVDV which is classified under the genus of Pestivirus in the family of Flaviviridae and it primarily infects cattle (Lanyon et al., 2014). BVDV is an enveloped single-stranded RNA virus about 12,500 nucleotides in length and with particle size only up to 40 to 50 nm (Niskanen, 1995). According to Houe (2003), there are two genotypes of BVDV—BVDV-1 and BVDV-2. The two genotypes differ in their antigenic and genetic properties, yet both are able to cause acute and persistence infection.

Based on cytopathicity effect (CPE) in cell culture, BVDV can be further divided into two different biotypes; cytopathic BVDV (CP) and non-cytopathic (NCP) BVDV. When CP BVDV inoculated into a cell culture, it is able to cause cell damages that include vacuolization and cell lyses but these CPEs are observed with NCP BVDV. The NCP BVDV is much more predominant than CP BVDV, and responsible for most of the disease manifestation and the production of persistently infected (PI) calves (Fulton et al., 2009).

2.2 Epidemiology of BVDV

BVDV is known to infect cattle worldwide (Hans Houe, 2003) and able to cross infect other ruminant animals such as buffalo, sheep, goats, pigs, deer, camels and alpacas (Diéguez et al., 2009; Lanyon & Reichel, 2014). Meanwhile, BVDV infection to

the naïve pregnant animal can affect the foetus differently depending on the stage of gestation when infection occurred.

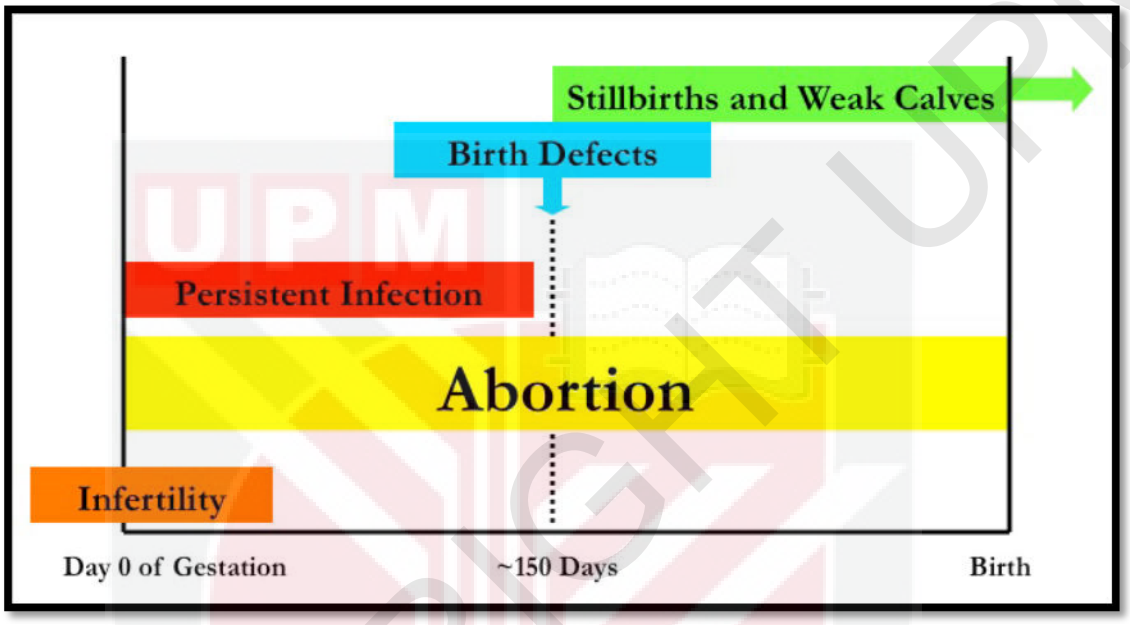

Figure 1 Consequences of fetal BVDV infections, subsequent to a BVDV infection in a pregnant cow or heifer during different times of gestation. (Source: www.aces.edu)

Based on a study done by Lanyon and Reichel (2014), acute infection by NCP BVDV biotype in cows and heifers around the time of mating or during earlier pregnancy can cause failure in conception and early embryonic death which lead to repeated breeding and also low pregnancy rate of the herds. Infection between 42-125 days (Fulton et al., 2009) or 60 days to 120 days (Thobokwe, 2003) of gestation can either lead to abortion or the calf become immunotolerant to BVDV which also known as persistently infected animals that act as a primary carrier of the virus.

Meanwhile, the BVDV infection that occurred via transplacental route during mid-gestation may result in malformation of ocular and central nervous system of the calves such as hydrocephalus, hydroencephaly and cerebellar hypoplasia, as well as ocular degeneration, arthrogryposis, and brachygnathism (Lanyon & Reichel, 2014). Deformity that occurs on the foetus is due to teratogenic effect of the BVDV infection (Lanyon et al., 2014) that might lead to abortion or congenital defect upon birth.

The BVDV infection that takes place at the last trimester of pregnancy whereby the foetus at this stage is immunocompetent, might result in birth of a calf with sero-positive to BVDV. Some infection at this late gestation might result in stillbirth or calves that appear weak at birth. Other study claimed that the calves might have lower fertility where the heifer's first breeding might be delayed compared to normal heifer without BVDV complication before calving (Lanyon & Reichel, 2014).

2.3 Transmission

Persistently infected (PI) animals are known as the primary carrier of the virus and shed it for entire life (Larson, 2002; Kelling, 2004; Lanyon et al., 2014). Usually, nose-to-nose or sexual contact of healthy animal with the PI animals are the most common route of transmission of the virus. However, the spread of virus can be through any secretion of bodily fluid of the carrier which also includes semen (Lindberg et al., 2006). PI animals basically spread NCP biotype of the virus to other naïve cattle while CP biotype has shown to be capable of inducing the infection only under experimental condition (Lanyon et al., 2014).

Other than PI animals, immunocompetent animals with active BVDV infection can also become source of infection (Goyal, 2003). Upon infection, transient viremia occurred up to 14 days and the cattle might recover completely within 3 weeks post infection if there is no other superimposed infection. Even though they might recover completely from the acute infection, but the virus can persist within the peripheral mononuclear cells for more than 3 months where they might be capable to spread the virus at low concentration via nasal discharge, urine, milk, semen, saliva secretion, and also fetal fluids (Lanyon et al., 2014)

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2.4 Impact of BVDV

BVDV infection is known to cause a considerable damage in infected herd due to various impact of the disease in term of health and production which includes decrease in milk production, reduced in reproductive rate due to early embryonic death and abortion, growth retardation, and increase mortality among young stock (Lindberg,

2002). Due to the effect of the virus that is able to cause immunosuppression in infected animals might increase the incidence of other diseases within the affected herd (Lindberg, 2002; Lanyon et al., 2014). Based on the study by Lanyon et al. (2014), the immunosuppressive effect could be due to the direct effect of BVDV on circulating T and B lymphocytes while causing apoptosis of lymphocyte in gut associated lymphoid tissue (GALT) which leads to lymphopenia.

While a study by Houe (2003) which focuses on economical impact of BVDV infection in dairies reported that BVDV infection are endemic that occurred worldwide and result in major economic losses. Most of the economical losses were due to the high prevalence of the disease with negative effects toward reproduction and health condition in affected herds.

PI animals that are immunotolerant to the BVDV contribute to the continuous introduction and the cycle of active infection in affected herds by shedding the virus. Control and eradication program usually focus on elimination of this PI animals to prevent the spread of the virus to the entire animal within the herd. For example like Switzerland, the country had implemented the compulsory programme which aims on identification and elimination of PI animals through antigen testing of all newborn calves. This was carried out between 2008 and of 2011 at a cost of up to 60 million CHF or equivalent to RM 230 million throughout the program (Stahl & Alenius, 2012).

2.5 Clinical signs and symptoms

Naïve cattle affected by acute infection of BVDV show several non-specific clinical signs and symptoms such as depression, inappetance, fever, decrease milk production and diarrhea. Growth performance and fertility of affected cattle also decline following acute infection. Immunosuppressive effect of the virus lead to more complicated clinical signs especially when superimposed by other diseases (Niskanen, 1995)

Meanwhile for persistently infected (PI) animals, they usually develop a fatal disease called mucosal disease that occurs due to mutation of the NCP biotype and become CP biotype of BVDV (Peterhans et al., 2010). Some researchers suggest that superinfection by CP BVDV can induce the development of mucosal disease in the PI animals (Brownlie, 1990). But a study by Niskenen (1995) showed that mutation of NCP BVDV is much more plausible than superinfection by exogenous BVDV since the NCP and CP biotypes isolated and indentified in mucosal disease animal have very identical genetic coding which supports the hypotheses.

According to Presi et al. (2011), only about 10% of the PI animals live up to 2 years old as most of the PI animals will develop the mucosal disease after 6 months old (Brownlie, 1990). The mucosal disease can be either acute or chronic form. The acute form of the disease usually develops pyrexia, leucopenia, dysenteric diarrhea, and erosive lesions of the mouth and nares. Animals with acute mucosal disease might die within few days after the onset of the disease. Meanwhile for chronic form, animals

might develop intermittent diarrhea and wasting syndrome, lameness due to coronitis and eruptive lesion on skin of interdigital cleft, and also a characteristic lesion of mucosal disease which is a focal ulceration of mucosa of cecum, proximal colon or rectum. The chronic form of this disease might last from weeks to months (Lindberg, 2002; OIE, 2008).

3. MATERIALS AND METHODS

3.1 Animals and management

The target animals in this study were cattle within the State of Selangor. The selected farms are basically from four adopted cattle farms under 'Ladang Angkat' UPM program and also one from Ladang 16 Taman Pertaninan Universiti (TPU) cattle farm. Each of the farms under Ladang Angkat UPM program are run by private owners and while Ladang 16 TPU is owned by the Universiti Putra Malaysia (UPM).

Each of the farms is denoted as farm A, B, C, D, and E according to the description given below;

- A represents the farm owned by En. Razlan which is found located at Lenggeng area within the border of Selangor and Negeri Sembilan. It is a dairy cattle farm with total population up to 250 animals and increasing as the owner newly imported animals during the period of study.

- B represents the farm owned by En, Jaswant Singh which is located at Rawang, Selangor. It is a dairy cattle farm with total population of 70 animals which includes female, male, and young calf. This farm is newly relocated to new land area.

- C represents the farm owned by En. Baghwan Singh which is located at Teras Jernang, Bangi within the State of Selangor. It is a dairy cattle-buffalo integration farm with total population up to 80 animals.

- D represents the farm owned by En. Jaweer Singh which is located at Sungai Tangkas, Bangi. It is also a dairy cattle-buffalo integration farm with total population up to 80 excluding the buffalo.

- E represents the Taman Pertanian Universiti (TPU) farm which is owned by UPM, located within Serdang, Selangor. TPU farm consists of both dairy and beef cattle with estimated population about 55 and 160, respectively where the cattle are grazing on separated paddock or grazing area. The total populations of cattle are more than 300 animals during 2012, but reduced significantly in current population.

Management in term feeding management, watering, as well as vaccination program between the farms is slightly different. Farm C and E is managed extensively and let the cattle grazing. Extra feed supplement like pellets, soy bean, PKE and PKC which only given during milking, as well as giving a cut grass such as Napier. For semi-intensive farm such as farm A, B, and D, the farmers usually practice the cut-and-carry of pasture such as Napier grass and mixture of concentrate like soy bean meal. Water is also available where they fill up the feeders or feeding parlor after the feed provided finish. The entire farm was following the standard vaccination protocol such as vaccination for Foot and Mouth Disease (FMD) and Hemorrhagic septicemia (HS) but there is no history of vaccination for BVDV in the entire farm and most of the owners did not know about the disease.

The production types of the farms are either for dairy or beef cattle and also there is no age restriction in selection for sampling. The animal is considered as young calves with age less than 9 months old, and as adult if older. The predominant breeds of cattle found in the selected farms include Friesian-Sahiwal, Kedah-Kelantan, Brangus, Braford, Jersey, Friesian, and Brahman. Pregnancy and lactation status are recorded for each of the female animals. The management of the majority of the farms selected is an extensive system while some had semi-intensive systems. While the calves were held in cattle pens separated from their dams which is commonly practiced by dairy cattle farmers. The total number of animals used for sampling from each farm in this study is shown in the Table 1 below.

Table 1 Total number of animals used for sampling and farm management

FARM	Animal Number	Farm management
A	87	Semi-intensive
B	36	Semi-intensive
C	15	Extensive
D	15	Semi-intensive
E	254	Extensive
TOTAL	407	

The Friesian-Sahiwal contributed about one-third of the total samples collected (133/409) followed by Kedah-Kelantan which is the local beef breed, Friesian cross,

Jersey, Brangus, Braford, and Simmental breed. Friesian-Sahiwal breed is considered as different group from Friesian cross due to the breed composition of Friesian-Sahiwal alone is higher. While other breeds such as Kedah-Kelantan, Friesian cross and Jersey consist of the pure-breed as well as some crosses with Braford, Simmental, Brahman, Charolais, and Brangus.

3.2 Sampling and storage

Blood samples were taken 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 most of the farms where jugular vein was not an option, whereas most of the blood samplings in calves were done via the jugular vein. The fresh blood samples were transported in icebox with temperature of less than 5°C to the laboratory and on the same day, centrifuged at 1800 rpm for 10 minutes (Kubota, Japan) to separate the serum or plasma from the whole blood. The collected serum/plasma portion was stored in -20°C refrigerator (Acson, Malaysia) in labeled microtubes (Eppendorf®, Malaysia) with animals' ID. From the total 254 samples obtained from farm E (TPU), 241 of the serum samples were archived samples collected during the previous year.

3.3 Direct ELISA

The ELISA procedure to detect antibody against BVDV was done according to the protocol provided by the manufacturer of the test kit (PrioCHECK®BVDV antibodies, Switzerland). The principle of the test is for detecting the presence of sero-positive BVDV infection. The test kit is an inhibition ELISA giving a signal that is reciprocal to the sample antibody concentration. The test employs two monoclonal antibodies (mAb) that recognize 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 ul of serum or plasma samples were incubated with the inactivated BVDV antigen in the wells that came coated with the first mAb. After 1 hour incubation and washing, the second mAb which is conjugated with an enzyme was added that generates a color signal then followed by second incubation for one hour and washing. Subsequently, the chromogen 3,3', 5,5;-tetramethylbenzidine (TMB) substrate is dispensed to all wells and after 15 minutes incubation, colour development was stopped by addition of a sulfuric acid that act as a stop solution for chromogen TMB reaction. The colour signal was measured by using ELISA microplate reader (TECAN, Switzerland) at 450nm using data analysis software Magellan^{TM}.

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 and the animals are either free from the agent or persistently infected animals, while positive results with Pi ≥ 50% was considered to indicate that the animals are currently or were previously infected by the BVDV. The sensitivity and specificity of the test kit are 98% and 99% respectively.

3.4 Statistical data analysis

Data obtained were analyzed using descriptive statistics to determine the sero-prevalence of the virus infection in cattle at herd and general sample population level in the State of Selangor using the formula below (Bonita, 2006).

$$\text{BVDV Disease Prevalence} = \frac{\text{Animal with positive ELISA result for BVDV in specified time period}}{\text{Total number of animal in same time period}} \times 100\ \%$$

The chi-square test was done using a software known as Statistical Package for the Social Sciences (SPSS) v.20 (IBM Inc, USA) to demonstrate the interaction between the BVDV status of the animal and risk factors which include age group, production

types of animals, sex, pregnancy status, and lactation status, at a confidence level of 95% ($P<0.05$). The overall differences in prevalence of BVDV infection among the breeds and pair-wise test between any of the two breeds were determined by using the Kruskal-Wallis test and Mann-Whitney U test, respectively to identify where the significant difference lies.

4. RESULT

Table 2 shows the overall sero-prevalence of BVDV infection in the total sample population as well as within the individual farms investigated. The overall prevalence of BVDV found is 33.2% which can be used as representative for the prevalence of the BVDV infection in the State of Selangor. Farm A showed the highest prevalence with 75.9% of the animals tested were sero-positive against BVDV infection. Farm A contributed almost half of the BVDV seropositive samples out of the total 407 animals investigated; it represented 66 samples out of 136 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 from samples collected in farm D (0%).

Table 2 Prevalence of BVDV infection in cattle

Farm	Number of Animals Tested	BVDV Sero-positive	BVDV disease Prevalence
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%

During sampling animals were identified according to their sex, age, breed, pregnancy status, lactation status, and production type, considered as possible risk factors to be investigated for their effect on prevalence. A chi-square test at P-value <0.05 showed presence of significant association between the sero-prevalence of BVDV found with all the above listed risk factors (Table 3 & Table 4).

It was found that significantly higher 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 cows (42.9%) than non-pregnant (31.1%); lactating (51.1%) than non-lactating (25.8%) cows were affected and sero-positive to antibody against BVDV (Table 3).

Table 3 Prevalence of BVDV infection and association with risk factors

Risk Factors	Status	Total	Test Result		Percentage (%)	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	

Moreover, as shown in Table 4 and Figure 2, breed was also found to affect significanty (P< 0.05) the sero-prevalence of BVDV 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 cross (51.9%), Friesian-Sahiwal (45.1%), Braford (13.9%), Brangus (11.9%), Simmental (6.1%), while the lowest prevalence was recorded in KK cross (3.0%). The graphic distribution of the prevalence among the breeds is shown in Figure 2.

Table 4 Distribution of prevalence of BVDV according to breeds of cattle

Risk Factor	Breeds	Total	Tested Positive	Tested Negative	Percentage (%)
Breed	Braford cross	36	5	31	13.9 [a]
	Friesian-Sahiwal	133	60	73	45.1 [b]
	Simmental cross	33	2	31	6.1 [ac]
	Brangus	42	5	37	11.9 [ac]
	KK cross	66	2	64	3.0 [c]
	Friesian cross	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)

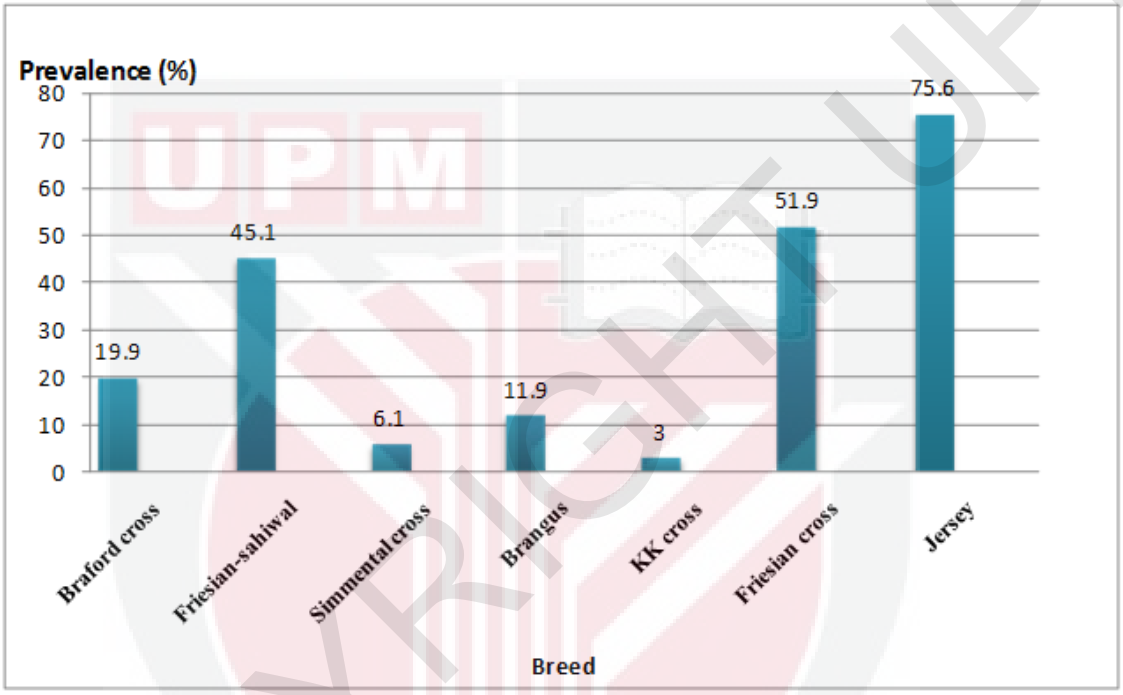

Figure 2 Prevalence distribution of BVDV according to the breeds of cattle tested

5. DISCUSSION

The overall prevalence obtained from the present study is 33.2% which is comparable with a report from Saudi Arabia (26%) (Mohmoud, 2013) but less compared to other places such as Thailand, 73% (Kampa et al., 2004), Australia, 80% and up to 100% in some of the states (Lanyon & Reichel, 2014). The infection is most probably due to the fact that Malaysia imports live animals particularly cattle from above BVDV endemic countries such as Australia and Thailand (Johari & Jasmi, 2009; Salina, 2015). The finding is also supported by previous reports of Sudharshana (1999) and Walz (2015) that stated worldwide BVDV antibody prevalence ranging from 13% to 90%.

Breed of cattle investigated has been shown to have a significant association with BVDV prevalence in the current study. Based on the data obtained, dairy breeds showed higher prevalence rate as shown primarily in the Jersey breed (75.6%), Friesian crosses (51.9%), including Friesian-Sahiwal (45.1%). Moreover, most of these dairy breeds were imported which might indicate that importation acted as a contributing factor to the introduction of BVDV in the State of Selangor. Meanwhile, for beef cattle which comprises mainly the local Kedah-Kelantan (KK) breed and their crosses showed the least prevalence (3%). The possible explanation for the low prevalence in local KK breeds could be, the animal might be free from the disease in the first place but get infected once crossed or kept with other imported cattle that probably carried the virus. Yet, the real scenario only can be explained with further study of the prevalence of this virus in the local breed found throughout Malaysia. This also might explain why farms with dairy production have higher prevalence which is up 75.9% that consists of Jersey

and Friesian crosses that have the highest prevalence observed in this study compared to the other breeds of cattle.

All of the risk factors that considered as contributing factors to BVDV infection have shown significant association to the prevalence in the current study. The 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%); more lactating (51.1%) than non-lactating (25.8%) cows affected. The data obtained might indicate that these risk factors have important role in BVDV occurrence. Risk factors to BVDV occurrence might vary from place to place. For example, a study by Almeida (2013) in southern Brazil had hypothesized that 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 (2012), 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 sero-positive 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 depends on imported breeds of cattle such as Jersey and Friesian crosses as the most common breeds of choice for milk production in Malaysia from disease endemic countries such as India

and Brazil with BVDV prevalence of 15.3% and 56% respectively (Sudharshana et al., 1999), as well as importing from neighboring country like Thailand (DVS, 2011). Australia with high sero-prevalence of BVDV as documented by Lanyon (2014) is one of the contributing factors of high prevalence rate especially in farm A where the dairy animal source mostly imports from Australia as mentioned by the owner. In production of dairy cattle, male calves is not usually kept longer within the farm where the farmer usually sell it as a veal (EFSA, 2012).

In term of age, sero-prevalence of BVDV was found to be higher in adult animals compared to young calves with less than 9 months old. This could be probably due to some of the calves investigated might be PI animals who are known to be immunotolerant to the virus and do not produce antibody against the virus to be detected by the ELISA test. According to Fulton (2009), the prevalence of PI animals in south central United States was revealed to be 0.55%, while Houe (1995) recorded the prevalence of PI animals in Michigan (USA) to be 0.13%. The prevalence of PI animals worldwide (Larson, 2004) has been reported between 0.13% and 2%. Further systematic investigation based on the samples collected from the present study 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 due to peripartum immunosuppression effect (Patra, 2013). This is due to the stress hormone changes in the body like the increase of cortisol hormone which is well known as stress hormone 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 sero-positivity and lactation might be due to the higher risk of getting infection from the workers that milked the cows. The virus can easily be transmitted through fomites such as contaminated cloth or equipment (Niskanen & Lindberg, 2003; Almeida et al., 2013) 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 types of production. For example, farm A has the highest prevalence of sero-positive BVDV (75.9%) because the owner mostly imports cattle from endemic countries such as Thailand and Australia (Salina, 2015). According to the Department of Veterinary Services (DVS), Malaysia, 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 reported variable degree of BVDV prevalence in their cattle population. Therefore, if the farm imports more animals from Thailand with prevalence of 73%, it is likely to have higher sero-positive BVDV animals in the farm. One the other hand, if importation of cattle was from India with

BVDV prevalence is lower 15.3%, it might result in low prevalence of BVDV (Sudharshana et al., 1999).

While farm D, with no detected sero-positive 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. In addition, farm size could be also another factor for the difference in sero-prevalence found as the larger the number of animals tested, the higher might be the probability of finding positive animals (Humphry et al., 2012).

The use of PrioCHECK®BVDV antibody testing in this study revealed the first evidence that the BVDV infection is prevalent in the state of Selangor in particular and in Malaysia in general. However, based on the current study it is not possible to tell the genotype of BVDV that might be predominant, either BVDV-1 or BVDV-2. Knowing the genotype of BVDV is very important in term of control of the infection via vaccination approaches. For example the study by Lanyon (2014) stated that BVDV type 1 is predominant in Australia with subtype 1c being the most prevalent in that country, while in study by Fulton (2009) 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.

Some countries such as Australia only allowed the use of killed vaccine, while other countries such as Austria and Scandinavia (Sweden, Finland, and Norway) do not allow usage of any BVDV vaccine but instead the countries implemented the control and eradication method to eliminate the PI animals from the population which significantly reduced the incidence of the BVDV infection (Lindberg, 2002; OIE, 2008).

6. CONCLUSION

The study revealed that BVDV exists in the cattle population of state of Selangor, Malaysia at a significant prevalence level (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 open up a window to see 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 the possible risk factors such as sex, age, breed, production type (dairy or beef), lactation status, and pregnancy status, as contributing factors to the prevalence of the BVDV. Despite some of the explanations provided, full understanding of the association between these risk factors and BVDV prevalence requires more detail investigations. Breed differences in the sero-positivity of BVDV demonstrated by higher prevalence among the dairy cattle that mainly comprises imported Friesian crosses and Jersey breeds compared to the beef cattle that were mainly composed of the local Kedah-Kelantan, 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.

As of today, there is no documented study about the prevalence of BVDV in Malaysia and to our understanding; this study is the first of its kind to report the prevalence of BVDV in the state of Selangor in particular and in the country in general. Hence, the finding can serve as a foundation for further detail investigation of the BVDV infection in Malaysia and to take necessary evidence-based national measures to combat the disease.

7. RECOMMENDATION

In general, this study revealed that the BVDV is prevalent in Malaysia especially in the State of Selangor. However, it is beyond the scope of the study to identify the predominant genotype of the virus responsible for sero-positivity of BVDV infection in Malaysia. Therefore, further study to identify the BVDV's genotype, whether BVDV-1, BVDV-2, or both are predominant in Malaysia, is highly recommended. Furthermore, future prevalence study to identify PI animals at herd, state as well as country level is also recommended as they are main sources of infection to other animals and main targets in a control and eradication programs. Since the current study was limited to the state of Selangor, extending the investigation on prevalence of BVDV infection to other states of Peninsular and East Malaysia would be very useful to know the overall status of BVDV in Malaysia. To minimize further more introduction of the disease, it is highly recommended for Malaysia and Malaysian farmers to target those countries which are less endemic for BVDV or if possible free of the disease for cattle importation.

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