Prevalence and Associated Risk Factors of Bovine Viral Diarrhoea (BVD) in Ruminants in Selangor

Nur Husna Abdul Rahman[1], Wan-Nor Fitri[1]*, Noor Asyikin Abu[2], Vijayakumar Suntharam[1], Nur Sakinah Ahmat[1], Nur Aisyah[1,3], Mohd Fahmi Mahsuri[4], Zulkhairi Azizi Zainal Abidin[5], Faez Firdaus Abdullah Jesse[4]

[1]Department of Farm And Exotic Animal Medicine and Surgery, Faculty of Veterinary Medicine, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor Darul Ehsan, Malaysia, [2]Department of Veterinary Services, Selangor State, Lot 28, Jalan Utas 15/7, Seksyen 15, 40200 Shah Alam, Selangor, Malaysia, [3]MARDI Kluang Address MARDI Kluang, KM15, Jalan Kluang-Kota Tinggi, 86009, Kluang, Johor, Malaysia, [4]Department of Veterinary Clinical Studies, Faculty of Veterinary Medicine, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor Darul Ehsan, Malaysia, [5]Department of Recreation and Ecotourism, Faculty of Forestry and Environment, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor Darul Ehsan, Malaysia.
*Corresponding author: wannorfitri@upm.edu.my

Abstract

Bovine Viral Diarrhoea (BVD) is caused by pestivirus, which has an economic impact on the ruminant industry. Most study focuses on cattle as the most affected species having detrimental effects on the reproductive soundness. However, the role of small ruminants in BVD transmission requires further understanding as they can also be affected by BVD. Thus, a cross-sectional study was carried out in Selangor with an objective (1) to determine the seroprevalence of BVD in cattle, deer, sheep, and goats and (2) to identify the associated risk factors of BVD. A total of 596 healthy animals i.e., 176 cattle, 212 goats, 100 sheep and 108 deers were randomly selected and sampled between 2021 to 2024 in 19 selected farms in Selangor. Blood samples were collected from all of the animals and the serum samples were tested against the detection of antibodies against p80-125 protein (NSP2-3), a non-structural protein (NS3), highly conserved, and common to all strains of pestiviruses such as BVD, Border Disease (BD), and BVD-Antigen using a specific monoclonal antibody (E^{rns}). The risk factors were analysed by running a univariate and multivariate logistic regression model compiled using a backward-selection procedure analysis to obtain the odds ratio (OR). This study found that the herds seroprevalence of BVD among the farms was 57.89% (n = 19). Cattle seroprevalence is 29.54% (n = 176), goats 11.3% (n = 212), sheep at 50% (n = 100), and deer at 0% (n = 108). Only one breeding ram was tested positive for the BVD-Antigen test. The key risk factors for BVD in cattle included being dairy cattle (OR = 12.60, $p < 0.001$), lactating (OR = 31.2, $p < 0.001$), raised in semi-intensive systems (OR = 106.08, $p < 0.001$), kept in cattle-only herds (OR = 26.32, $p < 0.002$), and being located in urban areas (OR = 191.95, $p < 0.001$). For small ruminants, significant risk factors included goats raised in intensive systems (OR = 6.73, $p < 0.001$) and female sheep (OR = 2.25, $p = 0.047$). The findings highlights that BVD seroprevalence in sheep and goats in Selangor, identifying a positive BVD antigen result in a breeding ram, emphasizing the sheep's role in BVD transmission. In short, the multi-species ruminant farming in Malaysia should be cautioned for the risk of BVD transmission.

Keywords: c-ELISA, logistic regression model, odds ratio, pestivirus

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INTRODUCTION

Bovine Viral Diarrhoea (BVD) is an important reproductive disease in cattle caused by herpesvirus, mainly the BVDV-1 (1a and 1b predominant in America, Asia, and Europe; 1c predominant in Australia) and BVDV-2 (Yeşilbağ *et al*., 2017) strains. BVD is a single-stranded RNA virus that belongs to the Pestivirus genus of the Flaviviridae family and primarily affects cattle (Pecora *et al*., 2009; Lanyon *et al*., 2014; Daves *et al*., 2016). BVDV-2 genotype causes more severe and acute clinical signs in susceptible animals (Houe, 2003; Daves *et al*., 2016). In Malaysia, BVDV-1a was detected in a clinically healthy bull in Selangor that was imported from Australia (Khalid *et al*., 2024). The source of BVD infection is from persistently

infected (PI) cattle to free-diseased cattle and other species of animals such as sheep, goats, buffalo, and deer (Nugroho *et al*., 2022). Two types of transmission in BVD are vertical transmission, normally from PI cattle to the foetus during the first or second trimester, and horizontal transmission through contaminated fomite, aerosol, milk, or semen (Ames, 2008; Nelson *et al*., 2016). While young animals can be exposed to BVD after birth through milk, fomites, or aerosols, their unique risk lies in in utero exposure from infected cows. This distinguishes their vulnerability from adults, as calves can be born either persistently infected (PI) or as normal calves that later suffer from BVD (Ames, 2008; Nelson *et al*., 2016).

Persistently infected animals do not show any clinical signs, as they continuously shed the virus throughout their lives. On the other hand, naïve infected cattle will show clinical signs including diarrhoea, hypersalivation, reproductive signs (abortion, early embryonic death, stillbirth), respiratory disease, and sudden death (Patel *et al*., 2019; Passler *et al*., 2016). The disease affects the reproductive performance and milk yield of dairy herds in addition to an increase in calving interval, culling of calves, infected cattle and calf mortality (Arnaiz *et al*., 2021; Yue *et al*., 2021; Sa'bani *et al*., 2024). Similar to cattle – sheep and goats may exhibit reproductive failure, neonatal death, and abortion when infected with BVD (Diao *et al*., 2021). In pregnant does, BVD infection causes 100% abortion, or kids will die less than two hours after delivery (Bachofen *et al*., 2013; Broaddus *et al*., 2009). The BVDV-2 caused an abortion storm in the sheep population in Spain in 2017, while BVDV-1 caused the same problem in the Turkey sheep population in 2018 (Partida *et al*., 2017; Bulut *et al*., 2018). In these species, BVD is closely related to Border Disease (BD) under the pestivirus family, which may also cause the same clinical manifestation as BVD, further complicating disease investigation. However, BD, also known as Hairy Shaker Disease (HSD), can cause small, weak lambs that are often hairy and occasionally exhibit shaking which were symptoms that are not typically associated with BVD infection in sheep and goats (Moeller, 2012). In an acute infection, lethargy, pyrexia, and cough were reported in BVD-infected deer (Ridpath *et al*., 2007). In 2008, two white-tailed deer suffered from a multiorgan infection due to BVD (Chase *et al*., 2008; Ridpath *et al*., 2008).

The most common and reliable method to test for BVD is the detection of virus BVDV-specific antigen and antibodies (Lanyon *et al*., 2014). Virus isolation is the gold standard for BVD diagnosis, but due to its extensive methods, RT-PCR is more commonly applied to screen or diagnose BVD infections (Lanyon *et al*., 2014). A serological test is also useful to measure the seroprevalence level of the herds toward BVD, like the ELISA method and agarose gel immunodiffusion (AGID) test (Lanyon *et al*., 2014; Lanyon *et al*., 2013). Another test that can be used is the serum neutralising test (SNT), as it is a highly specific test but less popular because it is expensive and time-consuming (Lanyon *et al*., 2014). However, PI cattle will not develop antibodies, thus resulting in a negative sample in antibody-detection serological methods. The BVD serology detection methods that have been used in Selangor are ELISA antibody (Daves *et al*., 2016), ELISA competitive ELISA assay (Khalid *et al*., 2024), and SNT (DVS, 2020; DVS, 2021). Khalid *et al*. (2024) also did RT-PCR and detected BVD-Ag from a seronegative bull. Wernike and Beer (2019) report that BVD E^{rns} Antigen ELISA is reliable in detecting the presence of BVD antigens in the serum. Therefore, this study used Antibody ELISA (cattle, goat, sheep, and deer) and Antigen ELISA (cattle and Ab-positive goat sheep, and deer) to determine the prevalence of BVD in Selangor due to its high specificity, high sensitivity, cost, and time effectiveness for the large sample size.

MATERIALS AND METHODS

Ethical Approval

Ethical considerations by the Institutional Animal Care and Use Committee (Ref: UPM/IACUC/AUP-R080/2022) of Universiti Putra Malaysia were obtained. This study has also received an approval from the Department of

Veterinary Services Malaysia (Ref. No. JPV. BPI.600-1/7/1 (2023-11).

Study Period and Location

This study is a cross-sectional study, located in the state of Selangor on the west coast of Peninsular Malaysia approximately 3°20′N and 101°30′E. It is divided into nine districts including Sabak Bernam, Kuala Selangor, Hulu Selangor, Gombak, Petaling, Klang, Hulu Langat, Kuala Langat, and Sepang. Data collection was carried out from 2021 to 2024 by random sampling. This study focuses on large (cattle) and small ruminants (goat, sheep, and deer).

Sample Size Calculation

Sample sizes are calculated using the Scalex SP calculator for each species (Naing *et al*., 2022). According to the most recent reports, the expected BVD seroprevalence in Selangor is 7.6% (DVS, 2021). The required sample size was a minimum of 108 cattle with a margin of error of ± 5% and 95% confidence (5%, 15%) and an estimated total population of 35,860 cattle. A total of 176 cattle were sampled from 10 farms for this study. Meanwhile, prevalence studies for goats are scarce in Malaysia, Thailand, and Australia. The prevalence of BVD in sheep in Australia is absent (Evans *et al*., 2018), and only 3% (Huaman *et al*., 2020) was reported in wild deer for BVD. The expected prevalence is 3% with a margin of error of ±5% and 95% confidence (5%, 15%) and an estimated total population of 33,222 goats, 10,844 sheep and 125 deer (DVS, 2023). The minimum sample size for each species was 45 individuals, but the total sample exceeded the minimum sample size of 212 goats (12 farms), 100 (4 farms) sheep, and 108 (3 farms) deer. Farmers' lists and contact numbers were obtained from DVS and were contacted for sampling one month prior.

Animals

Convenience sampling was used to select farms, depending on the permission and willingness of farmers to cooperate in the study. Animals were selected randomly upon farmers' permission, and only clinically healthy animals were selected based on physical assessment and examination of vital parameters such as respiratory rate, rectal temperature, and pulse rate (Khalid *et al*., 2024). The number of samples for each species was 176 cattle, 212 goats, 100 sheep and 108 deer**.** Host-level risk factor was noted for each individual, including age (adult > 1 year old or young < 1 year old), sex, pregnancy, and lactation status. Meanwhile, the herd-level risk factor was noted for each farm as a production system (intensive, semi-intensive, or extensive), number of species in the herd (multi-species or single species), farm type (commercial or smallholder), and farm demography (urban, suburban, or rural). The farm demographic was determined by The National Rural PPP 2030 (Town and Country Planning Department, 2024), Azari *et al*. (2022), and the Department of Statistics Malaysia (2024).

Blood Sampling and Processing

Blood samples were collected from cattle, goats, sheep, and deer from the jugular veins of clinically healthy animals. Blood collection was done using a vacutainer needle, an 18 G needle for cattle, and a 21G needle for goat, sheep, and deer, drained into a 5 mL plain blood tube (BD Vacutainer, UK). After collection, the blood tube was labelled accordingly, put into the icebox for transport at 4°C, and sent to the Theriogenology and Cytogenetics Laboratory, Faculty of Veterinary Medicine, Universiti Putra Malaysia immediately. The serum was separated from the whole blood by centrifugation at 5,000 × g for 5 minutes (Fitri *et al*., 2017). The serum is transferred to a 1.5 ml microcentrifuge tube labelled and stored at -20°C before being analysed in batches using a micropipette.

Enzyme-Linked Immunosorbent Assay

The competitive enzyme-linked immunosorbent assay (C-ELISA) was used to detect the presence of antibodies against p80-125 protein (NSP2-3) for BVD and BD (Pestivirus) (ID Screen® BVD p80 Antibody Competition, France) (Wernike and Beer, 2019; Hanon *et al*., 2017). A total of 596 (176 cattle, 212 goats, 100 sheep, 108 deers) samples in this study were

included in the antibody detection against BVD. All reagents and samples were left at room temperature 21 ± 5°C and homogenised by vortexing before use. The samples were processed based on manufacturer guidelines. In brief, samples were diluted in buffer, incubated at 37°C and underwent a series of washing before adding substrates and finally a stop solution. The test was validated when the mean negative control OD was greater than 0.7, and the positive control OD was less than 0.3. The competition percentage was calculated as (S/N% = OD sample/ODNC × 100), and a positive result was obtained when the S/N% ≤ 40%, doubtful result at the reading of 40% ≤ S/N ≤ 50%, and negative at S/N > 50%. Detection of antigen was performed using the ELISA BVDV Ag/Serum Plus test (IDEXX, Liebefeld, Switzerland) using specific monoclonal antibodies for BVDV (E^{rns}) on the plates (Wernike and Beer, 2019). Positive samples of goats (n = 24), sheep (n = 50), cattle (n = 52) and the negative (n = 123) and doubtful cattle sample (n = 1) were included in the antigen detection against BVD (Khalid *et al*., 2024). The manufacturer's guideline to conduct ELISA is routine, involving sample detection solution, a series of incubation at 37°C, washing, and the addition of tetramethylbenzidine and TMB substrates. The test was validated when the mean of negative control was ≤ 0.250, and the difference between positive and negative control was ≥0.150. Then, the corrected OD is calculated at the sample minus the mean of the negative control. The corrected OD value was interpreted when the S-N ≤ 0.300 and positive at > 0.300. Both antigen and antibody ELISA were scored by optical density (OD) reading at 450 nm absorbance. Colour reactions developed were measured using Magellan v7.1, 1998–2001 software, with Elisa M. Reader (Sunrise, Austria).

Data Analysis

Statistical analysis was done using IBM SPSS Statistics for Windows, Version 29.0. Individual and herd prevalence for antibody detection and antigen detection were calculated by dividing the number of positive animals/herds by the total number of animals/herds tested, which was multiplied by 100%. The risk factors of BVD exposure and logistic regression models were built separately for individual seroprevalence of cattle, goat, sheep, and herd seroprevalence data to test significant associations with BVD seropositivity. The risk factor analysis does not include the deer species as there is no positive result of BVD-antibody in this study. Only positive antibody samples for sheep and goats, while all cattle samples were included in the Antigen detection test. This is due to the natural carrier state of cattle (PI cattle), able to show negative antibodies yet positive antigens towards BVD (Khalid *et al*., 2024). Chi-square was used to measure the association between the variables i.e., production type, sex, pregnancy status, lactation status, age, production system, number of species, farm type, farm demography, and cattle herd in farm and the seropositivity towards BVD. Then, the univariable association between the binary outcome of significantly associated variables was analysed using binary logistic regression. Only the explanatory variables that were statistically significant at the 5% level were considered for multivariable logistic regression. This model was built in a stepwise backward manner, resulting in a model in which only significant risk factors ($p < 0.05$) were retained. Odds ratios, including 95% CI, are reported for all significant variables (Sarrazin *et al*., 2013; Ahasan *et al*., 2016). The steps were repeated to test the association between herd seroprevalence and potential risk factors (production system, number of species, farm type, farm demography, and cattle herd).

RESULTS AND DISCUSSION

A total of 126 samples were positive, 447 samples were negative, and 25 samples were doubtful for BVD-Antibody detection (Table 1). Doubtful results are classified as not-positive in this study, thus they are not included in the seroprevalence calculation. It is worth noting that the doubtful results mainly arose due to the manufacturer's protocol and different ELISA kits and were not due to any scientific technical stand point. Meanwhile, all of the Ab-positive sheep (n

= 50), goats (n = 24) and all cattle samples (n = 176 cattle) were tested for BVD-antigen which shows only one sheep sample that was positive. The seroprevalence result by species and farm is shown in Table 2. Farm A, B, C, D, E, F, G, H, I, and N (n = 10) was considered as a herd with cattle farms, while Farm J, K, M, N, O, P, Q, R, and (n = 9) was considered herds without cattle farms. The total farm seroprevalence was 57.89% (n = 19) for BVD-Antibody, and farm prevalence was 5.26% (n = 19) for BVD-Antigen. Nine (81.8%) of the seropositive farms have a presence of a cattle population.

Table 1. Result of BVD-antibody and BVD-antigen of each species

Species	ELISA BVD-Antibody			ELISA BVD-Antigen	
	Positive (S/N ≤ 40%)	**Negative (S/N > 50%)**	**Doubtful (40% ≤ S/N ≤ 50%)**	**Positive (S/N ≤ 0.300)**	**Negative (S/N > 0.300)**
Cattle (n = 176)	52	124	2	0	176
Goat (n = 212)	24	174	14	0	24
Sheep (n = 100)	50	41	9	1	49
Deer (n = 108)	0	108	0	-	-
Total	126	447	25	1	249

Cross-species transmission could occur on these farms. However, Farm G and Farm I cattle populations were seronegative, but the goat and sheep populations, respectively, were positive. The cattle population from these two farms were beef cattle, suggesting a short rearing period in the herd before slaughtered or traded. Meanwhile, the goat and sheep populations were bred and raised in the same place (longer rearing period), suggesting that the exposure could originate from previous batches of cattle before the sampling period. The goat population in Farm N was found to be positive. However, the cattle population was not sampled, though it is highly likely that the exposure was from cattle. Meanwhile, the other three farms that were not mixed with cattle were also positive for BVD-antibody. The three farms were dairy goat farms with sheep (Farm J), dairy goat farm (Farm K) and meat goat farm (Farm Q). The likely source of exposure for Farm K was from a cattle and buffalo farm located within a 5km distance. However, the source of exposure for Farm J and Farm Q was unknown.

The current seroprevalence of cattle is 29.54% (n = 176). It was slightly lower than the study in 2016 at 33.2%. Screening reports from DVS were 21.1% in 2020 and 7.6% in 2021, which was lower than the current study. The prevalence of BVD-antigen was absent in this study, and Khalid *et al*. (2024) reported 0.04% of BVD prevalence in cattle. The trend of high seropositive value of BVD and low antigen value is common for BVD. In China, the seroprevalence of BVD in four bovine species (dairy cattle, beef cattle, yaks, and water buffalo) was reported at 58.09%, while the antigen prevalence is only 1.39% (Deng *et al*., 2015). Specifically, for cattle herds, the seroprevalence that was calculated in this study was 63.6% (n = 11), and it was mostly contributed by dairy cattle herds by 80% (n = 5), similar to previous seroprevalence report in cattle in Selangor at 80% (Daves *et al*., 2016).

In our perspective, this is the first study of BVD seroprevalence in goats in Malaysia at 11.3% (n = 24). Globally, the prevalence of BVD was not well documented in goats, but it was recorded at 31.3% in Austria (Krametter-Froestscher *et al*., 2006) and 54% in India (Mishra *et al*., 2009). The prevalence of BVD-Antigen in goats is absent (n = 24) in this study. In Indonesia, 10% (n = 20) of goats were found to be positive for BVD-Ag in 2021 (Hidayat *et al*., 2021) and 13% in 2022 (n = 39) (Retno *et al*., 2022). Since the ELISA kit detects p80, a protein shared by BD and BVD, the source of exposure between BVD and BD cannot be established.

Table 2. Farm characteristics and animals were sampled using the BVD-Antibody and BVD-Antigen positive samples

Farm	Type	Area	Production system	Species	Animals sampled	BVD-Ab (BVD-Ag) Positive
A	Commercial	Urban	Extensive	Cattle	82	7 (0)
				Deer	81	0 (NA)
B	Commercial	Rural	Intensive	Cattle	32	17 (0)
C	Commercial	Rural	Intensive	Cattle	8	6 (0)
				Goat	NS	NA
D	Commercial	Rural	Intensive	Cattle	6	0 (0)
				Goat	NS	NA
E	Commercial	Sub-urban	Semi-intensive	Cattle	19	17 (0)
				Goat	NS	NA
F	Smallholder	Sub-urban	Intensive	Cattle	1	0 (0)
				Goat	5	0 (NA)
G	Commercial	Urban	Intensive	Cattle	5	0 (0)
				Goat	10	2 (0)
H	Commercial	Sub-urban	Intensive	Cattle	6	6 (0)
				Sheep	92	49 (1)
				Goat	NS	NA
I	Commercial	Sub-urban	Intensive	Cattle	17	0 (0)
				Sheep	3	1 (0)
				Goat	33	8 (0)
J	Smallholder	Urban	Intensive	Sheep	NS	NA
				Goat	32	4 (0)
K	Commercial	Sub-urban	Intensive	Goat	35	1 (0)
L	Smallholder	Urban	Intensive	Cattle	NS	NA
				Goat	7	0 (NA)
M	Smallholder	Urban	Intensive	Goat	5	0 (NA)
				Sheep	3	0 (NA)
N	Smallholder	Urban	Intensive	Cattle	NS	NA
				Goat	35	1 (0)
				Sheep	2	0 (NA)
O	Smallholder	Urban	Intensive	Cattle	NS	NA
				Goat	8	0 (NA)
P	Smallholder	Urban	Intensive	Goat	22	0 (NA)
Q	Smallholder	Urban	Intensive	Goat	20	8 (0)
R	Commercial	Sub-urban	Intensive	Deer	23	0 (NA)
S	Smallholder	Urban	Intensive	Deer	4	0 (NA)

BVD-Ag = BVD-Antigen, BVD-Ab = BVD-Antibody, NS = not sampled, NA = not available.

Table 3. Cattle, goat, sheep, and deer seroprevalence and association to host and herd risk factors

Risk factors variable	Category	Freq.	Positive no. (%)	χ^2 (p-value)
Herd				
Production system	Intensive	17	9 (52.9)	1.626 (0.444)
	Semi-intensive	1	1 (100.0)	
	Extensive	1	1 (100.0)	
Number of species	Single species	7	4 (57.1)	0.003 (0.960)
	Multi-species	12	7 (58.3)	
Farm type	Smallholder	8	2 (25.0)	6.134 (**0.013**)
	Commercial	11	9 (81.8)	
Farm demography	Urban	10	5 (50.0)	0.540 (0.76)
	Rural	3	2 (66.7)	
	Suburban	6	4 (66.7)	
Cattle				
Production type	Dairy	73	42 (57.53)	46.943 (< **0.001)**
	Beef	103	10 (9.71)	
Sex	Male	57	15 (26.32)	0.422 (0.516)
	Female	119	37 (31.09)	
Pregnancy status	Yes	12	4 (33.33)	0.031 (0.860)
	No	107	33 (30.84)	
Lactation status	Yes	56	31 (55.36)	29.069 **(< 0.001)**
	No	37	6 (16.22)	
Age	Adult (>1 y/o)	162	46 (28.40)	1.295 (0.255)
	Young (<1 y/o)	14	6 (42.86)	
Production system	Intensive	43	12 (27.91)	56.520 **(< 0.001)**
	Semi-intensive	64	39 (60.94)	
	Extensive	69	1 (1.45)	
Number of species	Single species	32	17 (53.13)	10.447 **(0.001)**
	Multi-species	144	35 (24.31)	
Farm type	Smallholder	7	0 (0.00)	3.057 (0.08)
	Commercial	169	52 (30.77)	
Farm demography	Urban	88	7 (7.95)	39.474 **(< 0.001)**
	Rural	46	23 (50.00)	
	Suburban	42	22 (52.38)	
Goat				
Production type	Dairy	100	13 (13.0)	0.53 (0.466)
	Meat	112	11 (9.8)	
Sex	Male	59	4 (6.78)	1.68 (0.195)
	Female	153	20 (13.1)	
Pregnancy status	Yes	17	4 (23.53)	1.841 (0.175)
	No	136	16 (11.8)	
Lactation status	Yes	52	10 (19.2)	2.630 (0.105)
	No	101	10 (9.9)	
Age	Adult (>1 y/o)	192	23 (12.0)	0.879 (0.349)
	Young (<1 y/o)	20	1 (5.0)	
Production system	Intensive	191	16 (8.4)	16.644 (< **0.001**)
	Semi-intensive	21	8 (38.1)	
Farm type	Smallholder	99	12 (12.10)	0.119 (0.731)

	Commercial	113	12 (10.62)	
Demography	Urban	124	15 (12.1)	0.6 (0.741)
	Rural	83	9 (10.84)	
	Suburban	5	0 (0.00)	
Cattle herd	Herd with cattle	91	11 (12.08)	0.732 (0.690)
	Herd without cattle	121	13 (10.7)	
Sheep				
Sex	Male	50	20 (40.00)	4.000 (**0.046**)
	Female	50	30 (60.00)	
Pregnancy status	Yes	23	15 (65.22)	0.483 (0.487)
	No	27	15 (55.56)	
Lactation status	Yes	1	1 (100.00)	0.680 (0.409)
	No	49	29 (59.18)	
Age	Adult (>1 y/o)	94	47 (50.00)	0.000 (1.000)
	Young (<1 y/o)	6	3 (50.00)	
Production system	Intensive	8	1 (12.50)	4.891 **(0.027)**
	Semi-intensive	92	49 (53.26)	
Farm type	Smallholder	3	0 (0.00)	3.093 (0.079)
	Commercial	97	50 (51.55)	
Demography	Urban	3	0 (0.00)	3.093 (0.079)
	Suburban	97	50 (51.55)	
Cattle herd	Herd with cattle	97	0 (0.00)	3.093 (0.079)
	Herd without cattle	3	50 (51.55)	
Deer				
Sex	Male	49	0 (0.00)	-
	Female	59	0 (0.00)	-
Pregnancy status	Yes	5	0 (0.00)	-
	No	54	0 (0.00)	-
Lactation status	Yes	2	0 (0.00)	-
	No	57	0 (0.00)	-
Age	Adult (>1 y/o)	94	0 (0.00)	-
	Young (<1 y/o)	14	0 (0.00)	-
Production system	Intensive	23	0 (0.00)	-
	Semi-intensive	23	0 (0.00)	-
	Extensive	62	0 (0.00)	-
Farm type	Smallholder	4	0 (0.00)	-
	Commercial	108	0 (0.00)	-
Demography	Urban	85	0 (0.00)	-
	Rural	23	0 (0.00)	-
Cattle herd	Herd with cattle	81	0 (0.00)	-
	Herd without cattle	27	0 (0.00)	-

Freq: Frequency.

Table 4. Univariate and multivariate analysis of host and risk factors of herd, cattle, goat, and sheep

Category	Univariate analysis			Multivariate analysis		
	OR	**p-value**	**95% CI**	**OR**	**p-value**	**95% CI**
Herd Overall						
Farm type						
Smallholder	1	-	-			
Commercial	13.500	**0.021**	1.47–123.74			
Cattle Species						
Production type						
Dairy	12.60	< **0.001**	5.66–28.06			
Beef	1	-	-			
Lactation status						
Yes	11.78	< **0.001**	2.44–31.79	31.2	**0.002**	3.66–265.93
No	1	-	-	1	-	-
Production system						
Intensive	26.32	**0.002**	3.28–211.49			
Semi-intensive	106.08	< **0.001**	13.83–813.52			
Extensive	1	-	-			
Number of species						
Single species	3.53	< **0.001**	1.60–7.79			
Multi-species	1	-	-			
Farm demography	0.79	< **0.001**	0.03–0.21	191.95	< **0.001**	16.31–2259.42
Urban						
Rural	0.90	0.82	0.34–2.10	76.41	**0.001**	5.65–1033.92
Suburban	1	-	-	1	-	-
Goat						
Production system						
Intensive	6.73	< **0.001**	2.43–18.64			
Semi-intensive	1	-				
Sheep						
Sex						
Male	1	-	-			
Female	2.25	**0.047**	1.01–5.01			
Production system						
Intensive	1	-	-			
Semi-intensive	7.98	0.057	0.94–67.46			

OR = odds ratio.

The seroprevalence of BVD-antibody of sheep in this study was recorded as 50% (n = 100). It was higher than in other countries, such as Tanzania, at 3.3% (Torsson *et al*., 2017), and in the US, at 5.6% (Silveira *et al*., 2018). In Indonesia, there was zero prevalence from both ELISA-Antigen and ELISA-Antibody (Hidayat *et al*., 2021) in sheep. It is similar to Australia, where there was an absence of BVD prevalence from 875 breeding ewes (Evans *et al*., 2018). In the current study, 1% (n = 50) BVD-Antigen was prevalence in ram against which was slightly higher than New Zealand at 0.05% (n = 270) which the sheep were co-grazing with cattle (Evans *et al*., 2019). Most of the sheep in this study were sampled from farm H, in which there were 46 sheep and six cattle were BVD-Antibody positive, and one sheep was BVD-Antigen

positive. The animals were raised intensively, and the enclosure between species was <1km. Cattle-to-sheep BVD transmission could occur even though the BVD-Antigen was negative in the cattle population. It may have originated from the cattle herd that was reared on the farm however was traded or slaughtered before the sampling period. The adult ram might be a PI animal for BVD as it was used as a breeder on the farm, causing a high BVD seroprevalence in the sheep population. Similarly, 10 PI sheep were detected at a prevalence of 0.32% (n = 3112) in West Austria using the RT-PCR method. It was noted that seroprevalence of BVD in sheep was higher in farms with a cattle population (Krametter Froetscher *et al*., 2010; Krametter Froetscher *et al*., 2007), highlighting the importance of cattle in disease transmission.

This study reports the prevalence of deer species in Selangor (n = 108) is absent; thus, the disease is not significant in the deer species. The prevalence of BVD in deer was reported to be low and only ranges from 1.3% to 4.5% (Huaman *et al*., 2020). In this study, only one farm was raised together with cattle species. However, they did not share the same grazing area, and the enclosure was separated. The white-tailed deer population in Austria was most likely in contact with the same grazing area as cattle. Thus, the BVD prevalence was recorded as high as 63.4% (Cantu *et al*., 2008). It shows the importance of separating cattle from other ruminant species to avoid disease transmission. Seroprevalence for the overall herd, cattle, goat, and deer species was identified in Table 3.

In Table 4, a univariate and multivariate analysis was performed on all risk factors that show a significant association. Only 'farm type' (X2 = 6.134, p = 0.013) was significantly associated with BVD seropositive herds. The univariate analysis shows that commercial farms (OR = 13.50, p = 0.021) are more likely to be seropositive against BVD than smallholder farms. Commercial farms have more frequent visitors and vehicles, leading to increased BVD transmission (Kumar *et al*., 2018). Other herd-risk factors such as the production system (intensive, semi-intensive, and extensive), number of species (single-species, multi-species), and farm demography (urban, rural, and suburban) were not significant in the overall herd seroprevalence risk factor. This finding is aligned with Jokar *et al*. (2021) in multi-species ruminant farms, which found insignificant BVD risk factors in Iran (Jokar *et al*., 2021).

The current study found that dairy cattle have higher odds of contracting BVD (OR = 12.6, p < 0.001), which is supported by Daves *et al*. (2016) study. Dairy animals were kept in the facilities longer, increasing BVD exposure in the herd, compared to beef cattle kept for slaughter and trade. More importantly, dairy cattle were mostly imported from BVD-endemic countries such as Australia and New Zealand. Apart from dairy animals, lactating cattle (OR = 31.2, p < 0.001) also have higher odds of BVD exposure than non-lactating cattle. This result supports the high stress in dairy animals during milking due to high energy demand (Daves *et al*., 2016; Purnama *et al*., 2019; Demil *et al*., 2021). For herd risk factors, we found that cattle reared in a semi-intensive system (OR = 106.08, p < 0.001) and intensive system (OR = 26.32, p = 0.02) of contracting BVD than the extensive system. Extensive system farms have larger land areas, which commonly have a lower stocking density than semi-intensive and intensive systems. A high stocking density facilitates disease transmission more rapidly Demil *et al*. (2021). Other than that, cattle reared in urban areas (OR = 191.95, p < 0.001) have higher odds of BVD exposure than in suburban areas. Urban areas are smaller, leading to high stocking density, which facilitates disease transmission. The result is reinforced by a study in Tamil Nadu urban areas, which shows that shortage of space and higher stocking density lead to active disease transmission (Kumar *et al*., 2018). More interestingly, this study found that the cattle population in rural areas have an even higher exposure (OR = 76.41, p = 0.001) to BVD contraction than in suburban areas. Elucidating locational factors is challenging and poorly understood. Consideration of the disease transmission includes location, environment and demographics of the population (Sohel *et al*., 2019). There is a lack of deeper understanding,

which highlights further research in the area of the cause of locational differences in BVD contraction in this study. However, the potential explanations that arrived in this study were a combination of farmers' level of awareness, education, and veterinary support for BVD. Moreover, cattle-only farm was shown to have higher odds (OR = 3.53, $p < 0.001$) than cattle farms mixed with other ruminant species. Cattle-only farms have a larger cattle population than cattle farms with other species. Since PI animals are cattle, having a larger number of cattle increases the odds of BVD transmission. In contrast, having more ruminant species will not increase the odds of BVD transmission.

The univariate analysis shows that goats reared in an intensive system are significantly more exposed to BVD than goats that are reared in a semi-intensive system (OR = 6.73, $p < 0.001$). The goats in intensive systems have a higher stocking density and are kept together in a small area, causing stress. Goat farms that practice intensive systems are mostly commercial farms due to the ability to keep a high stock of animals. Commercial farms have a higher frequency of animal movement, visitors, and vehicles, leading to an increase in disease transmission (Kumar *et al*., 2018). No other risk factors were significant for goats in this study. Female sheep were found to have higher odds for BVD infection at (OR = 2.25, $p = 0.047$) than male sheep due to the higher stress of pregnancy, as half of the positive female sheep were pregnant. Pregnant animals are attributed to the peripartum immunosuppression effect, which makes them vulnerable to diseases (Daves *et al*., 2016). Other individual and herd risk factors for sheep were not significant.

CONCLUSION

This study explores BVD seroprevalence in sheep and goats in Selangor. Positive BVD antigen (BVD-Ag) result was found in a breeding ram that was reared in proximity to cattle. It highlights the importance of sheep in BVD transmission. Several significant herd risk factors, including farm demography, farm type, farming system, and multi-species farming, were identified. Multi-species ruminant farming is a more common rearing system of ruminants in Malaysia; however, consideration should be given to focusing farming on a single species in regard to BVD transmission.

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AUTHORS' CONTRIBUTIONS

NHAR and WNF: planned the study design and planning. WNF and FFAJ: Acquired the funding for the project and its supervision. NHAR, NAA, NA, WNF, VS, NSA, MFM: contributed to sample preparation, farm sampling, laboratory, and result analysis. NHAR: drafted the manuscript. ZAZA: Study design, coherency of data and overall content of the manuscript. All authors discussed the results and contributed to the final manuscript.

COMPETING INTERESTS

The authors declare that they have no competing interests.

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