Journal of Advanced Veterinary Research

https://advetresearch.com

Schmallenberg Virus (SBV) Infection Among Small Ruminants in Selected States of Peninsular Malaysia

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ARTICLE INFO

Original Research

Received:
01 October 2021

Accepted:
02 January 2022

Keywords:

Small ruminant, Schmallenberg virus (SBV), Seroprevalence, Risk factors, Peninsular Malaysia

ABSTRACT

Schmallenberg virus (SBV) is an emerging arthropod-borne pathogen of domestic and wild ruminants responsible for devasting economic losses in European countries with a potential for spreading across boundaries. Three hundred sixty-two (362) serum samples were randomly collected from 7 selected sheep and goat farms from January to October 2019 to investigate the seroprevalence of SBV among small ruminants in Negeri Sembilan and Terengganu states. Seroprevalence of SBV was determined using commercial ID vet® SBV multispecies cELISA test. The farmers completed a structured questionnaire to furnish farm management data to evaluate the risk factors associated with the seroprevalence of SBV among individual animals. A Chi-square test was performed to determine the association between seroprevalence of SBV and putative risk factors of individual animals. Based on the ELISA test, a total of 78 individual animals were seropositive with an overall prevalence of 21.5% and the true prevalence of 22.1% (95% CI:18.06-26.71). Multivariable logistic regression analysis further revealed that Negeri Sembilan state ($p<0.001$; OR=14.290; 95% CI=5.384-37.930), Breed: Malin sheep ($p<0.001$; OR=20.453; 95% CI=4.807-87.022), Barbados Black Belly sheep ($p<0.01$; OR=69.947; 95% CI=16.819-290.901), Boer goat ($p<0.001$; OR=9.108; 95% CI=3.191-25.997) and crossbred goat ($p=0.001$; OR=4.964; 95% CI=1.905-12.934) are risk factors of SBV among individual animals. This study highlights the prevalence of a new transboundary disease among small ruminants in Malaysia. The result obtained here provides preliminary data that could guide livestock producers and policy makers in designing suitable prevention and control measures.

J. Adv. Vet. Res. (2022), 12 (1), 62-67

Introduction

Livestock production occupies a central position in the economy and ecosystem of developing countries (Devendra and Thomas, 2002). The livestock sector is currently one of the rapidly growing agriculture components in developing countries of Asia (Thornton, 2010). Most of the 368,080 goats and 133,478 sheep in Malaysia (DVS, 2017) are owned by smallholder farmers who practice a semi-intensive system in which individual flocks between 5-50 heads reared in raised pens with access to limited grazing in the day and some feed supplementation at night (Chandrawathani, 1998). Due to the high local demand for mutton and goats' milk in Malaysia, the small ruminant production is fast becoming an attractive enterprise (Melissa *et al.*, 2016). The industry plays a vital role in food security by providing meat, milk, and manure (DVS, 2008). It also contributes to the Agricultural gross domestic products (Shanmugavelu and Azizan, 2006) through cash income and employment generation (DVS, 2014). The prevalence of various diseases is responsible for morbidity, mortality, and the increased cost of production due to additional costs of drugs and veterinary care (Jesse *et al.*, 2019). Pneumonic pasteurellosis, endoparasites, mange, contagious ecthyma, melioidosis, blue tongue and caseous lymphadenitis have a long-term recognition as significant economic diseases of small ruminants in Malaysia (Chandrawathani, 1998). Additionally, Q-fever, caprine arthritis, encephalitis virus, and Brucellosis are emerging threats (Noordin *et al.*, 2010; Jesse *et al.*, 2018, 2020; Paul *et al.*, 2021a). Moreover, parasitic gastroenteritis and hemotropic mycoplasmosis of small ruminants have emerged as serious threats in recent times (Paul *et al.*, 2020; Paul *et al.*, 2021b)

Schmallenberg virus (SBV) is an emerging arthropod-borne pathogen of ruminants in Europe that is spreading to other continents through livestock trade (Hoffmann *et al.*, 2012). Schmallenberg is an enveloped, negative-sense, segmented, single-stranded RNA virus of the family Bunyaviridae (Lievaart-Peterson *et al.*, 2012). Like other arthropod-borne Simbu viruses, biting midges and mosquitoes are responsible for transmitting SBV (Saeed *et al.*, 2001; Conraths *et al.*, 2013). Placental transmission occurs in sheep (van den Brom *et al.*, 2012), but there is no evidence of direct spread from animal to animal (Conraths *et al.*, 2013). Previously, the SBV genome and antibodies were detected more commonly in sheep flocks

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ISSN: 2090-6277/2090-6269/

followed by cattle and goats in Europe (Conraths *et al*., 2013). SBV antibodies also obtain in wild ruminants such as the fallow deer, roe deer, reindeer and camelids are documented in the United Kingdom (Jack *et al*., 2012). Additionally, the domestic dog also plays host to SBV (Wensman *et al*., 2013), but to date, there is no record of human infection (Ducomble *et al*., 2012). The symptoms of acute SBV infection in ruminants include fever, anorexia, poor condition, decreased milk production and diarrhoea, which resolve spontaneously within a few weeks (Gibbens, 2012). However, a wide range of developmental abnormalities, stillbirths and abortions occur in pregnant sheep (van den Brom *et al*., 2012), while some infections are cryptic (Garigliany *et al*., 2012).

The clinical diagnosis of SBV poses a severe challenge to veterinarians due to frequent subclinical presentation and marked similarity with the bovine-herpes virus type 1, bluetongue, and foot-and-mouth disease of ruminants (Hoffmann *et al*., 2012). Virus neutralisation test, indirect immunofluorescent antibody test, or ELISA could be used to detect specific antibodies against SBV in test sera (Conraths *et al*., 2013). The PCR technique is also useful in detecting the SBV genome from the blood of viraemic animals or the brain tissues of malformed neonates (Hoffmann *et al*., 2012; Wernike *et al*., 2015). SBV is isolated from blood, brain, and serum samples by the inoculation of African green monkey kidney epithelial (Vero cells), baby hamster kidney fibroblast (BHK-21) and Culicoides variipennis larvae (KC) cells (Doceul *et al*., 2013).

Small ruminant production in Malaysia is affected by the high morbidity, mortality, and cost of treatment due to the prevalence of diseases. Infectious agents, toxic agents, environmental stressors, and genetic abnormalities are the leading causes of reproductive inefficiency among ruminants (Chung *et al*., 2019). In Malaysia, Jesse *et al*. (2019) reported that reproductive dysfunction is one of the most significant sequelae of diseases in the smallholder ruminant production system. The global emergence of SBV broadens the spectrum of our current understanding of diseases causing reproductive failure in ruminant livestock production, which should be considered as a differential in the clinical diagnosis of infertility. Despite the emergence and spread of SBV as a significant economic disease in European countries, its potential to spread across boundaries and the heavy dependence of the Malaysian livestock industry on the importation of breeding stock. Knowledge specifically related to SBV infection is very limited in the tropics, particularly in Malaysia, where suspected clinical cases involving the reproductive system of ruminants often presents, there has been no report on its prevalence in the country. Therefore, this study conducted a pioneer serological survey to elucidate the seroprevalence and contributing factors of SBV among small ruminant flocks in the states of Negri Sembilan and Terengganu, Malaysia.

Materials and methods

Ethics statement

The Institutional Animal Care and Use Committee of Universiti Putra Malaysia (UPM/IACUC/AUP-013/2018) and the Department of Veterinary Services (DVS) approved the sample and data for this study. Trained Veterinarians assisted with collecting blood samples, and we observed controlled conditions in performing assays in the Clinical Research Laboratory, University Veterinary Hospital, Universiti Putra Malaysia.

Study Area

Terengganu state is on the east coast of Peninsular Malaysia between 5.3117 ^{0}N and 103.1324 ^{0}E of the equator and has an estimated 13,035 km^2. The state of Negeri Sembilan is on the southwest coast of Peninsular Malaysia between 2.8 2.7258° N and 101.9424° E with an estimated total area of 6,686 km^2. Terengganu has 37000 small ruminants, while Negeri Sembilan has 63673 small ruminants kept in individual smallholder or government farms within the rural communities (DVS, 2017). All the farms included in this study were respective smallholder outlets that practice intensive management, which confines animals to pens in elevated roofed houses without grazing or semi-intensive management, which allowed limited grazing with feed supplementation. The animals were aged by examining their dentition and grouped as young (<1 year) or adult (one year and above). The various breed of small ruminants was identified using phenotypic characteristics and grouped as exotic and indigenous/local breeds (DVS 2013).

Sampling and data collection

The sample size for estimating the overall prevalence of SBV at 95% confidence level, an expected prevalence of 98% (Méroc *et al*., 2014), a 5% desired absolute precision and an estimated 496,558 small ruminants in Malaysia (DVS, 2017), was calculated according to Thrusfield (2005). We found that a minimum of 30 samples per farm was required for estimating the prevalence of SBV, but we collected a minimum of 40 samples to increase precision. In total, we included 7 farms and 362 individual sheep (n=132) and goats (230) in the study. The individual farmers were contacted by telephone for consent to take part in the survey. We visited participating farms and randomly collected blood samples for the serological study between March and September 2019. An assistant physically restrained each animal, and approximately 5ml of blood was collected from the jugular vein and kept in tubes. Also, we collected data on the age, sex, and breed of each animal on sampling a sampling form. The farmers also completed a structured questionnaire to furnish management data for risk analysis.

Separation and storage of Serum

Serum was separated from coagulated blood by centrifugation at 3000 rpm for 15 minutes (Eppendorf® AG 22331, Hamburg Germany) and kept deeply frozen at -20℃ before ELISA.

Detection of anti-Schmallenberg virus antibodies

SBV detection from sheep and goat sera were done using commercial ID vet® SBV multispecies ELISA test kit produced by ID vet, Montpellier, France (Specificity: 100%, Sensitivity: 97.6%) as described in previous studies (Helmer *et al*., 2016; Pejaković *et al*., 2018). Preparation of assay, incubation steps (Memmert®, Germany) and washing were according to manufacturer instructions, and the optical densities at 450nm were measured using ELISA Microplate Reader (Tecan Sunrise, Switzerland). The S/P value of each sample was calculated according to manufacturer instructions.

Statistical analysis

Summary of data was in Microsoft Excel® Spreadsheet Software version 2016 and statistical analyses in SPSS version 22.0 (SPSS, Chicago, IL, USA). The apparent prevalence was estimated as p=d/n, where; d=number positive and n=number examined (Thrusfield, 2005). Estimation of the true prevalence and respective 95% confidence intervals was according to Rogan and Gladen (1978) and Brown *et al*. (2001). Univariable

analysis using a chi-square test to determine the association between SBV seropositive status and putative risk factors of individual animals. The significant ($p<0.05$) variables were included in a multivariable logistic regression analysis model using a backward stepwise (conditional) method to determine the risk factors while controlling for other covariates at $p>0.10$. p-values < 0.05 considered significant in all analyses.

Results

As presented in Table 1, out of 362 small ruminants tested, a total of 78 were seropositive for SBV with 21.5% overall and 22.1% (95% CI: 18.06-26.71) true prevalence. The state, flock, breed, production, and management showed significant associations ($p<0.05$) with SBV seropositive status of individual small ruminants (Table 2). We further analysed the risk factors associated with SBV seropositivity among individual animals using a multivariable logistic regression model. The multivariable analysis revealed that Negeri Sembilan state ($p<0.001$; OR=14.290; 95% CI=5.384-37.930) and Breed; Malin sheep ($p<0.001$; OR=20.453; 95% CI=4.807-87.022), Barbados Black Belly sheep ($p<0.01$; OR=69.947; 95% CI=16.819-290.901), Boer goat ($p<0.001$; OR=9.108; 95% CI=3.191-25.997) and crossbred goat ($p=0.001$; OR=4.964; 95% CI=1.905-12.934) are the risk factors associated with SBV among individual animals (Table 3).

Discussion

This study recorded 21.5% overall and 22.1% true prevalence of SBV among individual small ruminants, revealing potential economic impacts on smallholder sheep and goat production in Peninsula Malaysia. Earlier reports in Europe show that SBV infection in small ruminants causes substantial financial losses due to decreased milk production in dairy animals, infertility, congenital malformations, increased incidence of dystocia and neonatal death (Garigliany *et al*., 2012; Wernike *et al*., 2013). Even though this is the first report on SBV infection in Peninsula Malaysia, the overall seroprevalence is lower than earlier reports in Europe, where Méroc *et al*. (2014) reported a 98.03% rate in Belgium at the end of the 2012 epidemic. Similarly, Helmer *et al*. (2016) reported a 53.3% seroprevalence of SBV among small ruminant flocks in Germany. Azkur *et al*. (2013) also reported a prevalence of 39.8% and 1.6% among sheep and goats in Turkey. The higher rates of SBV detection in European countries is because the virus originated in Europe and spread gradually to other places where prevalence rates are generally much lower. On the other hand, the overall prevalence of SBV recorded in this study is higher than the 16.26% reported among sheep flocks in Lebanon (Abi-Rizk *et al*., 2017). Following the emergence of SBV as a significant disease of domestic ruminants in Europe (Conraths *et al*., 2013), the host range and geographic spread have expanded. So far, SBV occurs in wild camelids in the UK (Jack *et al*., 2012), dogs in Sweden (Wensman *et al*., 2013) and equine species in far Iraq (Rasekh *et al*., 2018). SBV infection in sheep and goats is usually subclinical and transplacental transmission commonly leads to abortions and stillbirths with severe malformations in the nervous and skeletal tissues of the foetus (Maclachlan and Dubovi, 2010; Gibbens, 2012). The associated high rate of neonatal mortality, dystocia, ewe death, poor welfare and financial burden raises serious concerns for farmers (Stokes *et al*., 2018).

We have seen from this study that the seroprevalence of SBV was associated with the state of sample collection such that prevalence was higher among small ruminants in Negeri Sembilan ($p=0.000$; OR=14.290; 95% CI=5.384-37.930) than those from Terengganu state. This finding is similar to a previous report where different seroprevalence rates of SBV occurred in northeast, northwest, southwest and southeast regions of Germany (Helmer *et al*., 2016). All the seven flocks

Table 1. The overall seroprevalence of SBV among goats in two states of Peninsula Malaysia.

Variables	Categories	Apparent prevalence	True prevalence	95% CI
States	Terengganu	19/180 (10.6%)	10.80%	7.03-16.29
	Negeri Sembilan	59/182 (32.4%)	33.20%	26.68-40.49
Flocks	A	14/40 (35.0%)	35.90%	22.68-51.74
	B	02/40 (5.0%)	5.10%	1.42-16.91
	C	02/55 (3.6%)	3.70%	1.03-12.63
	D	01/45 (2.2%)	2.30%	0.12-11.85
	E	26/74 (35.2%)	35.10%	25.24-46.50
	F	27/49 (55.1%)	56.50%	42.33-69.82
	G	06/59 (10.2%)	10.40%	4.86-20.97
Species	Goats	49/230 (21.3%)	21.80%	16.91-27.71
	Sheep	29/132 (22.0%)	22.50%	16.14-30.50
Breed	SH-White Dorper	06/79 (7.6%)	7.80%	3.61-15.98
	SH-Malin	09/13 (69.2%)	70.90%	43.41-89.47
	SH-Barbados Black Belly	14/40 (35.0%)	35.90%	22.68-51.74
	GT-Boer	19/51 (37.3%)	38.20%	25.94-52.23
	GT-Crossed breeds	30/179 (16.8%)	17.20%	12.29-23.48
Gender	Male	29/107 (27.1%)	27.80%	20.07-37.10
	Female	49/255 (19.2%)	19.70%	15.22-25.10
Age	Young	19/102 (18.6%)	19.10%	12.56-27.94
	Adult	59/260 (22.7%)	23.30%	18.46-28.85
Production	Meat	76/297 (25.6%)	26.20%	21.48-31.60
	Dairy	01/55 (1.8%)	1.9	0.10-9.84
	Mixed	01/10 (10%)	10.30%	0.53-41.41
Management	Semi intensive	70/218 (32.1%)	32.90%	26.91-39.52
	Intensive	8/144 (5.6%)	5.70%	2.91-10.84
Overall	All small ruminants	78/362 (21.5%)	22.10%	18.06-26.71

CI: Confidence Interval, SH: sheep, GT: goat, alphabets (A, B, C….) represent different farms.

Table 2. Univariable association between seroprevalence of SBV and exposure factors.

Variables	Categories	Examined	Positive	Negative	*P*-value
States	Terengganu	180	19 (10.6)	161 (89.4)	0.000*
	Negeri Sembilan	182	59 (32.4)	123 (67.6)	
Flocks	A	40	14 (35.0)	26 (65.0)	0.000*
	B	40	2 (5.0)	38 (95.0)	
	C	55	2 (3.6)	53 (96.4)	
	D	45	1 (2.2)	44 (97.8)	
	E	74	26 (35.2)	48 (64.9)	
	F	49	27 (55.1)	22 (44.9)	
	G	59	6 (10.2)	53 (89.8)	
Species	Goats	230	49 (21.3)	181 (78.7)	0.882
	Sheep	132	29 (22.0)	103 (78.0)	
Breed	SH-White Dorper	79	6 (7.6)	73 (92.4)	0.000*
	SH-Malin	13	9 (69.2)	4 (30.9)	
	SH-Barbados Black Belly	40	14 (35.0)	26 (65.0)	
	GT-Boer	51	19 (37.3)	32 (62.7)	
	GT-Crossed breeds	179	30 (16.8)	149 (83.2)	
Gender	Male	107	29 (27.1)	78 (72.9)	0.096
	Female	255	49 (19.2)	206 (80.8)	
Age	Young	102	19 (18.6)	83 (81.4)	0.397
	Adult	260	59 (22.7)	201 (77.3)	
Production	Meat	297	76 (25.6)	221 (74.4)	0.000*
	Dairy	55	1 (1.8)	54 (98.2)	
	Mixed	10	1 (10)	9 (90.0)	
Management	Semi intensive	218	70 (32.1)	148 (67.9)	0.000*
	Intensive	144	8 (5.6)	136 (94.4)	

*Significant difference (p<0.05), SH: sheep, GT: goat, alphabets (A, B, C….) represent different farms.

Table 3. Results of multivariable logistic regression showing risk factors associated with seroprevalence of SBV antibodies in small ruminants

Risk factor	β	SE	Wald	95% CI	Odds	*P*-value
State (Negeri Sembilan)	2.66	0.498	28.514	5.384-37.930	14.29	<0.01
Breed						
Sheep (White Dorper)					1	
Sheep (Barbados Black Belly)	4.248	0.727	34.122	16.819-290.901	69.947	<0.01
Sheep (Malin)	3.018	0.739	16.689	4.807-87.022	20.453	<0.01
Goat (Boer)	2.209	0.535	17.041	3.191-25.997	9.108	<0.01
Goat (Crossed breeds)	1.602	0.489	10.75	1.905-12.934	4.964	<0.01

β: Regression coefficient, SE: Standard error, Wald: Wald's statistical value

included in this survey were seropositive with an intra-flock seroprevalence of 2.2-35% in Terengganu and 10.2-55.1% in Negeri Sembilan. The observed 100% flock prevalence of SBV in our study agrees with Helmer *et al.* (2016), who observed that small ruminant flocks surrounded by wet, or woodland were mostly affected by SBV. The herds of small ruminants examined in the present study also inhabit dense forest environments, which may account for the observed prevalence of SBV. As seen from the questionnaire results, herds surveyed in Negri Sembilan practised a semi-intensive management system that allows limited grazing within the forests and thereby increase their exposure to Culicoides biting midges and mosquitoes. Also, variations in local ecological conditions and management practices that influence the bionomics and distribution of Culicoides vectors may account for the observed differences in the prevalence of SBV between the two states. While the management of small ruminants is not a risk factor in this study, the univariable analysis showed that it was associated with SBV seropositivity, such that individual animals raised on the semi-intensive management system had a higher prevalence than those on the intensive system. This finding implies that the intensive management system had a protective effect by minimising exposure of small ruminants to the vectors of SBV. In contrast, grazing in semi-intensive management increased their exposure to Culicoides and Mosquito on the field (Helmer *et al.*, 2016). Incidentally, Culicoides and mosquitoes are abundant in the Malay Peninsula due to heavy rainfall, high humidity, high temperature and dense forest vegetation cover, which are suitable for their bionomics (Willis and Alexander, 1989).

Concerning species, this study did not detect any association between SBV seropositive status and the species of small ruminants. This finding agrees with an earlier study by Wernike *et al.* (2015), who reported a within-herd seroprevalence of 24.7% in sheep and 26.4% in goats. However, our result is contrary to an earlier study that reported differences in the prevalence of SBV antibodies between sheep and goats (Conraths *et al.*, 2013). Moreover, Helmer *et al.* (2016) also reported a noticeably higher seroprevalence of SBV among sheep than goats. Still, they could not show further evidence for a differential risk of seropositivity between the two species. In this study, we also observed that four leading breeds of small ruminants in Malaysia were associated with the risk of SBV seropositive status. Among the sheep breed, exotic White Dorper was the least affected while the indigenous Malin was the most affected. However, the exotic Boer goat had a higher prevalence of SBV compared to crossed breeds. In general, the risk of SBV infection was highest in Barbados Black Belly sheep

(p<0.01; OR=69.947), followed by Malin sheep (p<0.01; OR=20.453), Boer goat (p<0.01; OR=9.108), and crossed bred goats (p<0.01; OR=4.964). The species, breed and sex of animals are genetically controlled intrinsic or endogenous factors which serve as primary sources of variation in resistance or susceptibility to disease occurrence in each population (Thrusfield, 2005). Therefore, the high seroprevalence of SBV observed among exotic Barbados Black Belly sheep and Boer goats may be linked to the importation of livestock for breeding improvement in the small ruminant industry.

There was no association between gender and SBV seropositive status of individual animals in this study, but the seroconversion rate was noticeably higher among males. This finding suggests higher exposure to the arthropod vectors on the field due to prolonged contact with infection as most male animals stay longer on the farm for breeding purposes. Moreover, male animals are usually restless and wander extensively, searching for breeding partners during grazing and may acquire greater exposure to the arthropod vectors on pasture. Also, there is evidence for the presence of SBV in the female genital tract of sheep and goats, suggesting the possibility of genital transmission (Wernike *et al*., 2013), which may infect several mating partners during a single heat period. Although not a risk factor, the seroprevalence of SBV was noticeably higher in adults than young individual animals. This finding is like earlier studies that reported increased SBV seropositivity with age in cattle, sheep, goats (Méroc *et al*., 2014; Jiménez-Ruiz *et al*., 2019) and wild animals (Linden *et al*., 2012; García-Bocanegra *et al*., 2017). Therefore, older animals have a higher possibility of exposure to risk factors and are more likely to be at risk of being infected and remain so for life. Even though production is not a risk factor, the univariable analysis revealed its association with SBV seropositive status of small ruminants such that we observed a significantly lower prevalence in dairy animals. This finding is because dairy animals in the study areas are kept permanently indoors and therefore better protected from Culicoides and mosquitoes than the semi-intensively managed meat and mixed production animals.

Conclusion

High prevalence of anti-SBV antibodies and a significant association (p<0.05) with the state, flock, breed, production, and management was detected among small ruminants in this study. The risk of SBV infection was higher among animals from Negeri Sembilan state, especially the breeds of Malin sheep, Barbados Black Belly sheep, Boer goat. The serological evidence of an emerging and new transboundary disease among small ruminants in this study shows a potential threat to livestock productivity and the agricultural economy of Malaysia.

The authors recommend a large-scale serosurvey of small ruminant flocks to determine the overall seroprevalence and risk factors associated with the newly emerging SBV infection. The control of Culicoides and Mosquitoes vectors through careful timing of grazing to avoid peak periods of fly activity, the application of suitable repellents and destruction of fly breeding habitats in grazing environments. Also, the vaccination of all young animals before six months with commercially available vaccines will minimise spread. Future studies to determine the countrywide prevalence and economic impacts, including the characterisation of the SBV genome among ruminants, is a prerequisite for formulating a policy framework for adequate control.

Acknowledgement

The authors are grateful to the Director-General Veterinary Services Malaysia (DVS) and the Director of Veterinary Service Negeri Sembilan and Terengganu state for approval to conduct this study. We are also grateful to the staff of the Clinical Research Laboratory, Faculty Veterinary Medicine, Universiti Putra staff involved in the sampling and laboratory aspect of this study.

Conflict of interest

The authors have no conflict of interests to declare.

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