

Serological Surveillance of Akabane Disease, Bluetongue, Epizootic Haemorrhagic Disease and Q Fever in Dairy Cattle from Selected States in Malaysia

Intan Noor Aina Kamaruzaman^{1,2*}, Nurul Fatihah Zulkifli¹,
Nur Anis Mastura Zainal Abidin¹, Muhammad Azam Majnon¹,
Satishkaran Balachandran¹, Soon Heng Goh¹, Mohd Farhan Hanif Reduan³,
Mohammad Sabri Abdul Rahman^{2,4}, Norhidayu Sahimin⁵, Sazaly AbuBakar⁵
and Shih Keng Loong^{5*}

¹Public Health and Zoonotic Research Group, Faculty of Veterinary Medicine, Universiti Malaysia Kelantan, 16100 Pengkalan Chepa, Kelantan, Malaysia.

²Department of Veterinary Preclinical Sciences, Faculty of Veterinary Medicine, Universiti Malaysia Kelantan, 16100 Pengkalan Chepa, Kelantan, Malaysia.

³Department of Veterinary Paraclinical Studies, Faculty of Veterinary Medicine, Universiti Malaysia Kelantan, 16100 Pengkalan Chepa, Kelantan, Malaysia.

⁴Department of Veterinary Diagnostic, Faculty of Veterinary Medicine, Universiti Malaysia Kelantan, 16100 Pengkalan Chepa, Kelantan, Malaysia.

⁵Tropical Infectious Diseases Research and Education Centre, Higher Institution Centre of Excellence, Universiti Malaya, 50603 Kuala Lumpur, Malaysia

ABSTRACT

Vector-borne diseases are one of the significant threats to the livestock industry worldwide. Biting midges, mosquitoes, and ticks serve as vectors, transmitting pathogens during blood feeding. This leads to clinical manifestations in livestock and economic losses. Moreover, these animals are prone to retaining pathogens and becoming carriers for life. In this study, the seroprevalence of four vector-borne diseases was evaluated in selected farms across four major dairy-producing states in Malaysia. A total of 120 blood samples were collected, and serum extraction was performed. Serological testing was conducted using commercial ELISA kits to detect antibodies against *Coxiella burnetii* (Q fever), bluetongue virus, epizootic haemorrhagic disease (EHD) virus and Akabane virus. The results showed that antibodies against bluetongue virus are the most detected in dairy cattle across four states (86.7%), followed by EHD virus (69.2%), Akabane virus (35.8%) and lastly *C. burnetii* (7.5%). Among all states, Johor and Perak recorded the highest prevalence rate for all four diseases, followed by Pahang and Sabah. Findings from this study suggest evidence for the presence of vector-borne diseases in the country and imply the risk to the livestock industry, human health, and food security.

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Authors' Contribution

INAK and SKL conceptualized and designed the study. NFZ, NAMZA, MAM and SB performed the experiments. INAK, SHG, MFHR, MSAR, NS, SA and SKL analyzed the data and interpreted the results. INAK, SHG, MFHR and MSAR supervised the study. NS, SA and SKL provided resources for the study. INAK and SKL prepared the original draft. INAK, SHG, MFHR, MSAR, NS, SA and SKL revised and edited the subsequent drafts. All authors have read and agreed to the final version of the manuscript.

Key words

Akabane, Bluetongue, Epizootic haemorrhagic disease, Seroprevalence, Q fever, Infectious disease

INTRODUCTION

Vector-borne diseases pose significant threats to ruminants, including cattle, sheep, and goats, impacting

their health, productivity, and welfare. Vectors such as ticks, mosquitoes, flies, fleas, and lice serve as significant carriers and reservoirs for numerous pathogens, including bacteria, viruses, and protozoa. These pathogens can be transmitted on to human hosts highlighting their zoonotic potential (Chakraborty *et al.*, 2023). To date, some vector-borne diseases are listed as notifiable diseases by the World Organisation for Animal Health (WOAH) and WOAH plays a significant role in imposing international trade restrictions (WOAH, 2014).

In dairy cattle, vector-borne diseases cause major economic losses worldwide due to reduced milk yield, expensive treatment costs and leather damage (Singh *et al.*, 2022). Apart from common pathogens affecting the

* Corresponding author: intanaina@umk.edu.my, loongsk@um.edu.my
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animals, other neglected pathogens such as bluetongue virus, *Coxiella burnetii*, Akabane virus and epizootic haemorrhagic disease (EHD) virus may also become silent threats in the farms, affecting livestock productivity. Given their potential impact on livestock productivity, economic losses, and zoonotic risk, this study focuses on four vector-borne diseases; Q fever, bluetongue, EHD and Akabane. These diseases have been reported in various regions globally but remain underexplored in Malaysia, necessitating further investigation.

Query fever is a bacterial zoonotic disease caused by the intracellular bacterium, *C. burnetii*. The disease affects ruminant's reproductive systems, causing reproductive failures (Cantas *et al.*, 2011; De Oliveira *et al.*, 2018). In Malaysia, Q fever has been reported in small ruminants such as goats and sheep (Jeese *et al.*, 2020; Ahmad *et al.*, 2024) and occasionally in humans (Khor *et al.*, 2018). *C. burnetii* is primarily transmitted by both direct (via reproductive discharges) and indirect methods (via tick bites) (Berri *et al.*, 2001; Duron *et al.*, 2015). Diagnosis of Q fever can be rather difficult due to the carrier status of the animals or when the animals are subclinically infected. The clinical signs are only apparent during pregnancy by which the condition is irreversible, resulting in abortion and stillbirth (Berri *et al.*, 2007).

Bluetongue is an infectious viral disease of ruminants caused by an Orbivirus. Due to the impact of economic losses, WOAHA has imposed a ban on the movement of animals from the affected countries to bluetongue-free countries. The last outbreak of bluetongue in Malaysia was reported in 1995 (Sharifah *et al.*, 1995). The disease causes severe hemorrhages in various organs, and animals may die due to multiorgan failures (Machlachan *et al.*, 2015). Bluetongue virus is transmitted by *Culicoides* biting midges that serve as the biological vector for the virus (Duan *et al.*, 2021; Kar *et al.*, 2022). To date, bluetongue has been reported in small ruminants worldwide, with some evidence of resistance in cattle (Coetzee *et al.*, 2012). Another ruminant vector-borne pathogen closely related to bluetongue virus; the EHD virus can also cause microhemorrhage in animals. Similar to bluetongue, the EHD virus is transmitted via biting midges, and deer are most susceptible to the infection (Allen *et al.*, 2019). EHD is present in many parts of the world despite never being reported among the livestock population in Malaysia.

Lastly, Akabane disease is a viral disease of ruminants transmitted by an arthropod-vector. The disease, which causes congenital formations of animal fetuses, is caused by the Akabane virus from the genus *Orthobunyavirus* (Gao *et al.*, 2022). The occurrence of the disease is not known and possibly never reported among the livestock population in Malaysia. This study aims to investigate the

seroprevalence of these neglected vector-borne diseases in Malaysian dairy cattle, providing essential data for disease surveillance and control.

MATERIALS AND METHODS

Sample collection

A group of dairy cattle, regardless of age, breed, and group, were collected randomly from high-producing dairy cow farms in Malaysia, including from Sabah, Perak, Pahang, and Johor (Fig. 1). The sampling size was determined based on the availability of lactating cows on the farms during the sampling period. Samples were collected from a representative farm in each state: 34 samples from Johor, 34 from Pahang, 29 from Perak, and 23 from Sabah, totaling 120 samples. Sampling was conducted over a period of 6 months from March 2022 until September 2022 during the dry season. Prior to blood collection, the dairy cattle were visually inspected for signs of infection, and only healthy animals were included in the present study. The blood samples were collected via the coccygeal vein method and transferred to serum separator tubes (SST) to allow serum separation without centrifugation. The samples were kept chilled in a polystyrene box packed with ice packs during transportation and then stored in a chiller at -20 °C upon arrival at the laboratory until further testing.

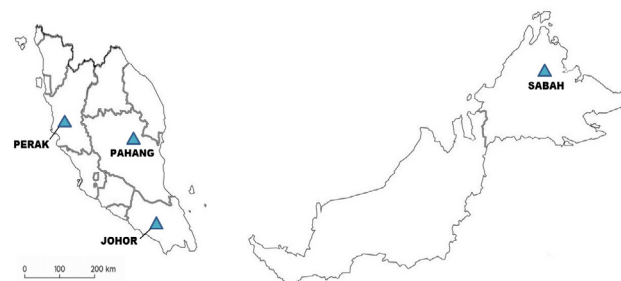


Fig. 1. Location of the sample collection sites for this study.

Detection of antibodies against *C. burnetii*, EHD, Akabane and bluetongue viruses

All samples were subjected to screening of the four vector-borne diseases using commercial ELISA-test kits; ID Screen® Bluetongue Competition, ID Screen® Q Fever Indirect Multi-species, ID Screen® Akabane Competition and ID Screen® EHDV Competition (Innovative Diagnostic, France), respectively. All steps were performed as per the manufacturer's recommendation. The control sera (positive and negative) provided in the respective kits and the collected serum samples were diluted accordingly

before the procedure. Following that, the respective ELISA plates were read using an ELISA plate reader fitted with a 450 nm filter, and an average of three readings were taken for each sample and for each test. The interpretation and validation of the results are detailed in Table I.

For competitive ELISAs (bluetongue, EHD and Akabane), the results were calculated via competition percentage (S/N %) using the following formula:

$$\frac{S}{N} \% = \frac{OD_{\text{sample}}}{OD_{\text{NC}}} \times 100$$

Where; OD, optical density; NC, negative control. Meanwhile for Q fever, the results were calculated via sample to positive ratio (S/P %) using the following formula:

$$\frac{S}{P} \% = \frac{OD_{\text{sample}} - OD_{\text{NC}}}{OD_{\text{PC}} - OD_{\text{NC}}} \times 100$$

Where; OD, optical density; NC, negative control, PC, positive control. The result for each test is interpreted in Table I.

RESULTS AND DISCUSSION

The commercially available ELISA kits used in the present study detected the exposure of dairy cattle to bluetongue virus, *C. burnetii*, EHD virus, and Akabane virus in Malaysia. All states were sero-reactive to at least three vector-borne pathogens. The distribution of the vector-borne diseases across the four states is presented

in Figure 1. From the results, bluetongue has the highest seropositivity among the animals (86.7%), followed by EHD (70%), Akabane (35.8%) and lastly Q fever (7.5%). In terms of disease-state distribution, Johor recorded the highest number of multi-affected animals, represented by bluetongue (100%), Akabane (41.2%), EHD (67.6%), and Q fever (8.82%). The seroprevalence distribution of other diseases in all states is tabulated in Table II.

The present study was primarily conducted due to the scarcity of information on the exposure of Malaysian dairy cattle to vector-borne diseases capable of causing economic damage and risking food security. Additionally, the present study also aimed to establish a baseline for these selected vector-borne diseases in Malaysia. To our knowledge, this is the first study to describe the serological evidence of exposure to bluetongue virus, Akabane virus, EHD virus, and, to a lesser extent, *C. burnetii* among the dairy cattle population in Malaysia. Our findings strongly suggest that these animals are continuously exposed to these pathogens. Among them, bluetongue (86.7%) and EHD (70%) have the highest detection rate across all states, followed by Akabane (35.8%) and Q fever (7.5%), respectively. Bluetongue and EHD viruses are closely related but genetically diverse due to the geographic distribution of virus serotypes and differences in the distribution of ruminant hosts and *Culicoides* spp. (Rivera et al., 2021). Currently, there are seven serotypes of EHD virus and 27 bluetongue virus serotypes worldwide.

Table I. ELISA interpretation for each test.

ELISA test	Competition percentage (S/N%) or sample to positive ratio (S/P%)			Validation ^b
	Positive	Negative	Doubtful	
Bluetongue	<40	≥40	-	OD _{NC} >0.7
EHD	≤30	≥40	30 < S/N < 40	OD _{NC} >0.7
Akabane	<30	≥30	-	OD _{PC} >0.35
Q Fever	50 < S/P > 80 ^a	≤40	40 < S/P ≤ 50	OD _{NC} >0.6

^a For Q Fever ELISA, S/P% more than 80 is considered as strong positive. ^b Validation of each test can also be interpreted using the ratio of OD mean values. For Bluetongue and EHD, the test is valid if the mean value of OD_{PC} to OD_{NC} is <0.3. For Akabane, the test is valid if the mean value of OD_{PC} to OD_{NC} is <0.5. For Q fever, the test is valid if the mean value of OD_{PC} to OD_{NC} is >3.0.

Table II. The seroprevalence distribution of selected vector-borne diseases in all states.

State	No. of samples	No of seropositive samples with %			
		Q fever ^b	Bluetongue	EHD ^b	Akabane
Sabah	23	0 (0.0%)	23 (100%)	18 (78.3%)	9 (39.1%)
Pahang	34	5 (14.7%)	21 (61.8%)	24 (70.6%)	8 (23.5%)
Perak	29	1 (3.45%)	26 (89.7%)	19 (65.5%)	12 (41.4%)
Johor	34	3 (8.82%)	34 (100%)	23 (67.6%)	14 (41.2%)
^a Overall positive with %		9 (7.5%)	104 (86.7%)	84 (70%)	43 (35.8%)

^aThe total number of seropositive samples is calculated based on the number of positive samples in all states. ^b Doubtful results are treated as weak

positive, thus included in number of seropositivity.

Outbreaks caused by bluetongue virus have been reported worldwide, mainly driven by animal movement and climate change (Maclachlan *et al.*, 2015; Brand and Keeling, 2017). In Malaysia, bluetongue outbreaks were first described in the early 1990s, with 16 serotypes of bluetongue viruses isolated from cattle and sheep (Sharifah *et al.*, 1995). However, this study contradicted recent findings by the Veterinary Research Institute (VRI) Malaysia, which analyzed archived samples from 2013-2019 and found a low overall prevalence (20.2%) of bluetongue among ruminant livestock in Peninsular Malaysia (Pauzi *et al.*, 2021). Notably, both studies pointed out that the state of Johor has the highest number of seropositive animals, similar to the present study. In comparison to EHD, unfortunately, there is a lack of data in Malaysia although EHD outbreaks have been reported in ungulates worldwide (Maclachlan *et al.*, 2015).

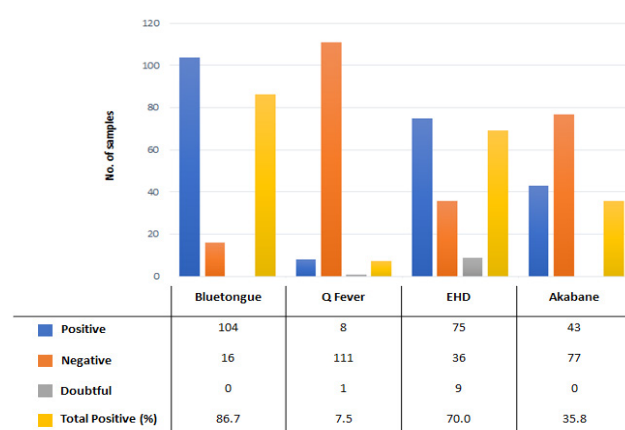


Fig. 2. ELISA results showing the seroprevalence of selected vector-borne diseases in Malaysian dairy cattle.

When comparing EHD and bluetongue data, all states show variation between 0-36% (data not shown). In this study, particularly for EHD, nine samples showed doubtful results (Fig. 2). It was plausible that this was due to the cross-reactivity of antibodies. In some studies, doubtful results are treated as positive (Valas *et al.*, 2023), which could be attributed to the low antibodies detected in the serum sample. Further confirmation via re-testing or alternative diagnostic methods would strengthen these findings. Nevertheless, the competitive ELISA used in this study is recommended by WOAH, and is known for its high sensitivity and specificity, and is highly convenient for laboratory use (Roja *et al.*, 2019). Furthermore, both viruses can be differentiated using advanced laboratory methods such as the fluorescent microsphere immunoassay

(FMIA), which can distinguish serogroups within a single serum sample (Drolet and Reister-Hendricks, 2021), but this method is not available at our laboratory.

Although our study indicated that a large proportion of Malaysian dairy cattle (86.7%) had been exposed to bluetongue, the disease caused by the virus does not critically affect the animals. The disease is mostly seen in sheep, occasionally in goats, and less frequently in cattle. This is because cattle are the natural reservoir of bluetongue and play a crucial role in the epidemiology of the disease (NAHS, 2019; Alkhamis *et al.*, 2020). In sheep and goats, where the disease is more pronounced, the animals tend to have significant clinical signs: fever, conjunctivitis, lacrimation, congestion of nasal and oral mucosa, and edema of the face and lips. Problems arise when the bluetongue virus is transmitted to sheep/goats since most smallholder farmers in Malaysia raise both ruminants in the same compound. Similar to bluetongue, cattle are also a mammalian reservoir of EHD virus, though the disease is more prominent in wild ruminants such as deer. As such, monitoring for EHD is warranted as Malaysia is home to several vulnerable deer species (Khodri *et al.*, 2023), such as the sambar deer (*Cervus unicolor*) and red Muntjac (*Muntiacus muntjak*).

Meanwhile, antibodies against the Akabane virus were detected in animals across all states, with the highest prevalence in Perak (41.4%) and Johor (41.2%), respectively. Although Akabane disease has never been reported in Malaysia, it is present worldwide, particularly in East Asia (Kono *et al.*, 2008; Jun *et al.*, 2012; Tzeng *et al.*, 2022). The disease causes congenital abnormalities during fetal development, which are often characterized by arthrogryposis, hydranencephaly, or microanencephaly in ruminant livestock. Infections may result in sporadic abortions and premature or stillbirths (Manavian *et al.*, 2023). It is thought that older female dairy cattle may have high seropositivity to the Akabane virus due to them being kept on the farm for a long time for milk production. Interestingly, none of the farms sampled in this study reported any history of teratogenic abnormalities among their herds despite the high seropositivity observed in the animals. This could be because the infected cattle are generally asymptomatic, with only transient fever noted and no other prominent clinical signs (Metwally *et al.*, 2023).

Lastly, Q fever had the lowest seroprevalence among dairy cattle in most states (7.5%) and it was not detected in Sabah. Previously, studies on Q fever in Malaysia were mostly described in small ruminants (Jesse *et al.*, 2020; Ahmad *et al.*, 2024) and none in cattle. The low seroprevalence of Q fever in this study is consistent with

other studies elsewhere (Ferrara *et al.*, 2022; Sadiki *et al.*, 2023). The exact reason for this remains unclear; however, we speculate that the low serotitre may be related to the age of the animals at the time of sampling. It is believed that younger animals (less than 1-2 years old) exhibit lower infection rates compared to older animals (Ahmad *et al.*, 2024). Although low prevalence was noted, the results should not be underestimated. Our finding suggests that these animals may potentially spread Q fever within the farm, which poses a zoonotic risk to people working in the farms. Besides that, Q fever may also be transmitted to people by drinking unpasteurized milk from infected cattle.

The seropositivity for multiple diseases observed in this study suggests that these animals are constantly exposed to these pathogens. Factors such as inadequate farm biosecurity, climate changes, and insufficient ectoparasitic/vector control may increase the likelihood of these animals getting infections, allowing the diseases to persist within the population. However, serological detection alone cannot differentiate between active and passive immunity (e.g., maternal antibodies or vaccination). Therefore, further investigation, including pathogen isolation and identification from appropriate samples (e.g., bodily fluid, reproductive tissues, and ectoparasites) and downstream molecular methods, is necessary to confirm active infections within the population. Additionally, the analysis of associated risk factors such as age, breed, housing condition, and contact with other animals may provide valuable information to give an overview of the disease distribution in the farms (Ferrara *et al.*, 2022). Moreover, it would be interesting to test other ruminants near the studied cattle farms and to compare the antibody titers to understand the correlation of the immune responses of each species.

CONCLUSION

The detection of antibodies against bluetongue virus, Akabane virus, EHD virus, and *C. burnetii* among dairy cattle in several states in Malaysia suggests continuous exposure to or possible circulation of the respective diseases in the farms. This study provides baseline information regarding the current seroprevalence, which would serve as part of a surveillance program for the herd health status of dairy cattle in Malaysia to prevent potential outbreaks in the future. A deeper understanding of vector-borne disease transmission in ruminants is essential for developing evidence-based policies, improving farm biosecurity, and implementing effective control measures to safeguard both animal and public health

DECLARATIONS

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Ethical statement

Ethics approval was obtained via the Institutional Animal Care and Use Committee (IACUC) Universiti Malaysia Kelantan with a code: UMK/FPV/ACUE/RES/002/2021. Approval for dairy cattle blood sampling was obtained from the respective farm owners/operators prior to the commencement of the study.

Statement of conflict of interest

The authors have declared no conflict of interest.

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