# SEROPREVALENCE OF BLUETONGUE INFECTION AMONG RUMINANT LIVESTOCK IN PENINSULAR MALAYSIA

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**ABSTRACT.** Bluetongue (BT) is an arthropod-borne viral disease of domestic and wild ruminants caused by Bluetongue virus (BTV). It has been reported in most tropical and subtropical regions of the world which raises significant socioeconomic concerns for international trade in animals and animal products. However, recent and updated information related to the current prevalence of BT in Malaysia is limited. The last holistic report was reported during BT outbreaks in the year of 1990s. This study aims to determine the current status of seroprevalence of BT among ruminants in Malaysia from 2013 to 2019. A total of 9,787 serum samples from buffalo, cattle, deer, goat as well as sheep were received from January 2013 until December 2019. All these serum samples were subjected to Agar Gel Immunodiffusion (AGID) test to detect the presence of antibodies towards BTV. The overall status of BTV was 20.18 % (1,975/9,787), with 56 % (14/25) in deer, 46.4 % (428/922) in cattle and 35.7 % (60/168) in buffalo. Positive BT antibodies were detected in young animals (<6 months) with 30.60 %, while 23.61 % in adult animals (>2 years). The results of this study revealed that BTV is still circulating at low level in domestic and wild ruminant livestock animals in Malaysia. It is suggested that this disease needs close monitoring to prevent possible outbreaks in the future.

*Keywords:* Bluetongue, seroprevalence, ruminants, livestock, Peninsular Malaysia

## INTRODUCTION

Bluetongue (BT) is a non-contagious vector-borne viral disease of ruminants and camelid species which is caused by the bluetongue virus (BTV) with a worldwide distribution that has great economic significance (Mertens *et al.,* 2005, Maclachlan, 2011). BTV is a member of the *Orbivirus* genus in the *Reoviridae* family and is mainly transmitted by adult female haematophagous midges in the genus *Culicoides* (Mintiens *et al.,* 2008; Coetzee *et al.,* 2012; Chatzopoulos *et al.,* 2015; Noaman & Arzani, 2017). Clinical signs usually differ among species, but generally, BT infections are characterized by fever, serous bloody nasal discharges, oedema, erosion and ulcers in the digestive and respiratory mucosae (mouth, oesophagus, stomach, intestine, pituitary mucosa, tracheal mucosa) (OIE, 2021). The clinical signs typically appear after an incubation period of about a week or sometimes longer (Mertens *et al.,* 2005; Mintiens *et al.,* 2008; Maclachlan, 2011).

BT is quite significant since its outbreaks could disrupt the trade or movement of animals and animal products between countries which leads to severe economic losses (Walton, 2004; Mintiens *et al.,* 2008). BT becomes a notifiable disease for the World Organization for Animal Health (OIE) because of its impact on economic consequences. Rushton and Lyons (2015) reported a global estimation of USD 3 billion due to abortions, death, weight loss, reduced milk yield, meat efficiency, export restrictions for live animals aside from the cost of preventive

and control measures (Blancou & Pearson, 2003; Zendulkova & Pospisil, 2007; Venter *et al.,* 2011). Overall estimates on the financial impact in France and the Netherlands were USD 1.4 billion and USD 85 million, respectively in 2007 due to the European BTV-8 outbreak in 2006 to 2008 (Velthuis *et al.,* 2010). The costs are largely attributed to the trade restrictions that were present during the outbreak period (Sinclair *et al.,* 2006; Gethmann *et al.,* 2020). Such reactions were perceived to have a greater impact than the production losses caused by the disease itself.

BT was considered originally to be an African disease which later became an emerging disease that had spread beyond its origin country due to the movement and trade of ruminant livestock starting from the mid-20th century (Maclachlan & Osburn, 2006; Maclachlan, 2011). Since sheep is not a major livestock species in many countries in South-East Asia, including Malaysia, studies of BT are given less priority compared to other diseases. However, Malaysia and her neighbouring countries such as Indonesia import European sheep breeds from time to time, therefore diagnosis of BT has been introduced for screening purposes (Chiang, 1989; Daniels *et al.,* 1995). A limited serological survey conducted in 1977 showed the exposure of BTV or a closely related virus of the BT serogroups among local animals (Hassan, 1992). Intensive diagnostic test was only introduced in 1987 following outbreaks in imported sheep from South Australia with clinical signs of head edema, erosions and ulcers in the mouth as well as lameness because of the coronitis of the feet (Hassan, 1992). Chiang (1989) reported that 159 out of 2,249 imported Australian sheep were affected with clinical BT and 82 (51.6 %) of them died after 10 weeks' post-arrival in Malaysia. Due to the outbreak, comprehensive serological surveillances involving 16,340 ruminants were conducted in 1990 using Agar Gel Immunodiffusion (AGID) where the result showed that the viral infection was higher among cattle and buffalo when compared to the latest imported Poll Dorset sheep and local goats at that time (Hassan *et al.,* 1996).

Preventive measures have been taken by the Department of Veterinary Services Malaysia (DVS) based on the guideline prepared by the OIE to control outbreaks of BT. BT is a notifiable disease by the OIE and for this reason, it is listed in the National Animal Disease Surveillance Program (DVS, 2011; DVS, 2014). The program is one of the key components in animal health activities in Malaysia and helps in determining the control and eradication of significant and notifiable animal diseases to protect the livestock industry and animal welfare in the country (DVS, 2011). Since Veterinary Research Institute (VRI) in Ipoh serves as the national reference laboratory under DVS, VRI has been receiving samples to test for BTV antibodies in livestock animals in Peninsular Malaysia. This paper describes the seroprevalence of bluetongue infection among ruminant livestock in Peninsular Malaysia based on the samples received for antibody detections by AGID test in VRI from 2013 to 2019.

## MATERIALS AND METHOD

### Samples And Data Collection

Records from January 2013 to December 2019 were retrieved from the Mammalian Virology Laboratory in VRI. The data consisted of serological results of tests performed on animal samples (blood) sent by regional laboratories and state veterinary departments in Malaysia. A total of 9,787 blood samples of ruminants with various backgrounds were received for testing in the Mammalian Virology Laboratory in VRI.

### Serological Test

Blood samples were centrifuged at 3,000 rpm for 5 minutes, after which the harvested serum was transferred into a sterile 1.5 ml microcentrifuge tube. The sera were then inactivated at 56 °C for 20 minutes prior to storage at -20 °C until testing. The BTV antibody test kit (VMRD, USA) was used to detect antibodies by AGID assay, as recommended by the OIE. The test was performed according to the instructions of the manufacturer. Soluble BTV antigen and positive reference serum were included in the assay.

### Data Analysis

The serological results and other information such as locality (state), type of work, age and species of the sampled animals were tabulated and analyzed using a statistical package (SPSS version 22).

## RESULTS AND DISCUSSION

A total of 1,975 (20.18 %) from 9,787 serum samples were tested positive for BTV infection using the AGID test as shown in Figure 1. Results showed that Pahang has the highest cumulative total samples in Peninsular Malaysia with a total of 4,567 samples throughout the years (Table 1). However, only 371 (8.12 %) samples had positive antibodies towards BTV. Based on the numbers, 41 out of 93 samples from Negeri Sembilan and 875 out of 2,198 samples from Johore had been tested positive, equivalent to 44.09 % and 39.81 %, respectively. These outcomes showed that both states have the highest percentage of BT seropositive compared to other parts in Peninsular Malaysia. Based on the sample distributions by type of work (Table 2), it is known that monitoring (87.21 %) is the main purpose for serum testing for BTV antibody detection followed by research (3.97 %), surveillance (3.49 %) and animal movement (2.84 %).

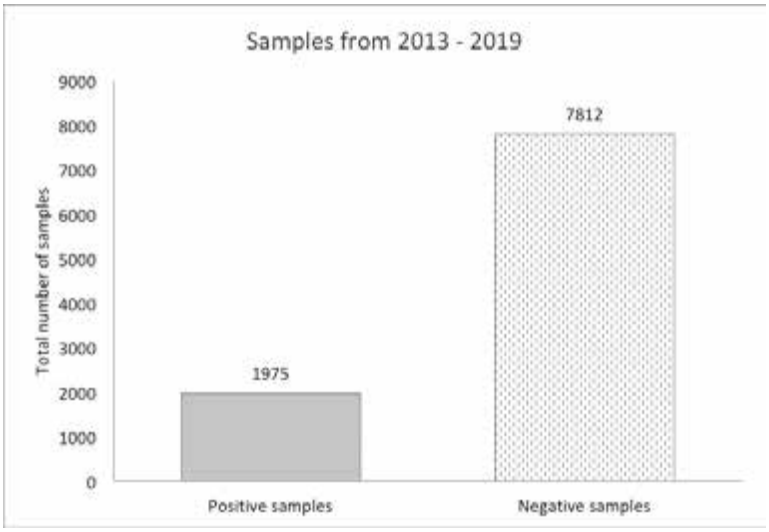


**Figure 1.** Total number of samples received for Bluetongue serology test in VRI from 2013 to 2019.

The result of this study provides evidence on the serological prevalence of BTV in Peninsular Malaysia from the year 2013 to 2019. Concisely, with regard to livestock and domestic ruminants in Asian countries, the seroprevalence rate of BTV obtained in this study is still considered low compared to the previous reports reported in India and Nepal, but the current result is comparable to the previously reported cases from China and Kazakhstan. Overall, BT seropositivity among ruminants tested for six consecutive years was 20.18 %. This seropositivity level corresponds to the 17.1 % seroprevalence rate among sentinel ruminants, such as cattle, buffalo, and goats in China (Kirkland *et al.,* 2002) and 23.2 % seroprevalence reported among livestock in Kazakhstan (Lundervold *et al.,* 2003). The findings described in study by Tigga *et al*. (2015) and Khanal *et al.* (2016) stated less than 43.68 % in ruminants in Jharkhand, India and 45.20 % among domestic ruminants in the highlands of Nepal respectively.

**Table 1.** Samples Distributions by States in Peninsular Malaysia from 2013 to 2019.

| States | Total number of samples | Positive samples | Seropositive level (%) |
|---|---|---|---|
| Negeri Sembilan | 93 | 41 | 44.09 |
| Johore | 2198 | 875 | 39.81 |
| Kelantan | 326 | 125 | 38.34 |
| Perak | 2084 | 519 | 24.90 |
| Kedah | 221 | 36 | 16.29 |
| Pahang | 4567 | 371 | 8.12 |
| Terengganu | 206 | 7 | 3.40 |
| Perlis | 35 | 1 | 2.86 |
| Malacca | 45 | 0 | 0.00 |
| Sarawak | 10 | 0 | 0.00 |
| W.P. K. Lumpur | 2 | 0 | 0.00 |

**Table 2.** Total Percentage of Sample Distributions by Type of Work from 2013 to 2019.

| Type of work | Total number of samples | Percentage (%) |
|---|---|---|
| Monitoring | 8535 | 87.21 |
| Research | 389 | 3.97 |
| Surveillance | 342 | 3.49 |
| Animal movement | 278 | 2.84 |
| Import | 145 | 1.48 |
| Diagnostic | 72 | 0.74 |
| Export | 16 | 0.16 |
| References | 10 | 0.10 |

BT was previously declared as an endemic disease among Malaysian livestock during the 1990s (Saharee & Fatimah, 1993; Hassan *et al.,* 1996; Mintiens *et al.,* 2008). This disease was monitored and controlled through massive serological surveys as well as animal movement restrictions (Hassan *et al.,* 1996). Subsequently, there has been hardly any report regarding the presence of BT disease in Malaysia since the year of 2000s. However, in 2009, BT antibody was found in a group of imported sheep in Peninsular Malaysia (DVS, 2014). Since then, active surveillance programs have been executed yearly by the authorities in Peninsular Malaysia causing more sample distributions in these areas compared to Sabah and Sarawak. This study result reveals that a high number of seropositive samples from Pahang were identified in 2013. Consequently, close monitoring was carried out intensively to immediately control, eradicate, and prevent the possible spread of this disease where this led to higher number of serum samples from Pahang in comparison with other states in this study. BT is a vector-borne viral disease of ruminants. Climatic conditions play an essential role in the existence of the vector for BTV since Culicoides require warmth and moisture for both breeding and feeding (Mellor *et al.,* 2000; Jiménez-Clavero, 2012; Mozaffari *et al.,* 2014). The climate in Malaysia is equatorial; hot, humid and rainy throughout the year, similar to Indonesia, Singapore, Brazil, Colombia, Kenya, Nigeria and Central African Republic. Temperatures in Malaysia are high and stable between 21 °C to 32 °C with a rainfall between 2,000 mm to 2,500 mm annually (Department of Irrigation & Drainage, 2017). Briefly, with respect to BT occurrence in ruminants, infection is still present in Peninsular Malaysia, and it probably persists due to the hot and humid climate which promotes the proliferation of *Culicoides* spp.

**Table 3.** Multivariable Analysis of the Animal Seropositivity Outcome.

| Variables | Total number of samples | Total Positive samples | Total Seropositive level (%) |
|---|---|---|---|
| **Age** | | | |
| < 6 month | 134 | 41 | 30.60 |
| < 1 year | 66 | 4 | 6.06 |
| ≥ 1 year | 1809 | 79 | 4.37 |
| ≥ 2 year | 7574 | 1788 | 23.61 |
| Unspecified | 204 | 63 | 30.88 |
| Total number of samples | 9787 | 1975 | 20.18 |
| **Species** | | | |
| Deer | 25 | 14 | 56.00 |
| Cattle | 922 | 428 | 46.42 |
| Buffalo | 168 | 60 | 35.71 |
| Goat | 2008 | 616 | 30.68 |
| Sheep | 6664 | 857 | 12.86 |
| Total number of samples | 9787 | 1975 | 20.18 |

Multivariable analysis of the animal seropositivity outcome based on age group and species are summarized in Table 3. These numbers may not reflect the whole age group of all species, as the records of certain samples provided unspecified and incomplete information. However, the analysed data will be discussed to provide an overview of the BT scenario in Malaysia.

The results of this study show that 30.60 % of positive BT antibodies were from the younger age group which are from the age group of less than 6 months and 23.61 % were from the adult age group which are from the age group over the age of 2 years old. Deer species had the highest percentage of seropositive detection (56 %) among other ruminant species followed by cattle at 46.42 % seropositive detection. In this study, sheep had the lowest seropositive of BTV detection at 12.86 % only although they had the largest number of serum samples analyzed.

The findings of this study reveal that there is an association between the BTV infection rate and the age of the animal. It was observed that younger animals have the highest rate of seropositivity compared to other age groups. According to a study by Gaydos et al. (2002), it was discovered that maternal antibodies to epizootic hemorrhagic disease (EHD) and BT viruses in white-tailed deer remained for up to about 6 months of age which may explain the possible reason the younger animals were tested positive for BT antibodies. The present study also shows that calves have higher possibility to get infected with BTV after the age of 2 years old. Animals at this age are usually released into the pasture for grazing which probably increases the probabilities of exposure to the infected vectors and subsequent BTV infection. This can be prevented by limiting the frequency and duration of outdoor grazing to reduce the exposure to *Culicoides* vectors. Besides, the control of vectors by using insecticides or protection from vectors may lower the possibilities of *Culicoides* bites and consequently the risk of exposure to BTV infection (Eisen & Eisen, 2011; Manual Merck Veterinary, 2014).

Deer had shown the highest seropositive level among species which may be due to the sample size bias originating from the availability of the animals. It was found that 46.42 % of cattle have BT antibodies which shows that the species is more susceptible to BT than other local animals. When the first outbreak of BT occurred in Malaysia in October 1987 in a batch of imported sheep from South Australia, local goats and sheep which had close contact with those infected did not suffer from the disease (Hassan *et al.,* 1996). Later in 1990, another surveillance was conducted using AGID and the findings demonstrated that the cattle had the highest infection among 16,340 ruminants that were involved in the study (Hassan *et al.,* 1996). Several previous studies have observed similar results indicating that the percentage of BTV antibody levels are higher in cattle which ranges between 49 % to 60 % compared to 13.7 % to 20 % in sheep or goats (Nevill, 1978; Behymer *et al.,* 1991; Ward *et al.,* 1994; Singer *et al.,* 1998; Bonneau *et al.,* 2002; Lelli *et al.,* 2004). These findings are in accord with the present study where the cattle samples showed higher percentage of seropositivity among other ruminants' samples. The herd immunity against this disease among cattle livestock is known to be higher compared to small ruminant livestock particularly in sheep population. Therefore, the clinical signs and symptoms are rarely exhibited in cattle compared to those in sheep. Thus, this has led to a minor preventive and control measure of the disease for cattle species. This scenario may cause the infected cattle to become a source of carrier of this disease via Culicoides to other ruminants (Williamson *et al.,* 2008).

## CONCLUSION

As for conclusion, the data obtained and observed in this study provide a baseline information on the current seroprevalence of Bluetongue infection among ruminant livestock in Peninsular Malaysia. This study has concluded that BTV still exists and is circulating among ruminant livestock in Peninsular Malaysia. Therefore, it is suggested that the current existing monitoring and surveillance programs of BTV should be carried out consistently and continuously to prevent outbreak of this disease in the future among ruminant livestock in Malaysia. In future, holistic study of seroprevalence is needed by adopting a bigger sample size involving deer, buffalo, sheep, goat and cattle in order to predict the overall status of BT in Malaysia.

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