UNIVERSITI PUTRA MALAYSIA

SEROPREVALENCE OF BLUETONGUE VIRUS INFECTION AMONG SMALL RUMINANT IN FOSTER FARM PROGRAMME FPV, UPM.

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It is hereby certified that we have read this project paper entitled "Seroprevalence of Bluetongue virus infection among Small Ruminant in Foster Farm Programme FPV, UPM", by Azeef Izzuddin B. Abdul Malek and in our opinion, it is satisfactory in terms of scope, quality and presentation as partial fulfilment of the requirement for the course VPD 4999 Project.

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DEDICATION

TO

FAMILY

My beloved parents,

My family members.

LECTURERS AND STAFFS

All lecturers and staffs in Faculty of Veterinary Medicine,

Universiti Putra Malaysia

FRIENDS

My wonderful DVM batch 2012 – 2017

All of my coursemates

Thank you all for the support and prayers

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Alhamdulillah, I would like to express my gratitude to Allah SWT for allowing me to finish this project without any problems. Then, I would like to thank my supervisor, Associate Prof. Dr. Faez Firdaus Jesse Abdullah, my Co-supervisors, Prof. Dato Dr. Mohd Azmi Mohd. Lila and Prof. Dr Abdul Aziz Saharee for their guidance and constructive criticism, comments and suggestions throughout this project and enabling me to complete my final year project with enough knowledge and courage. Their knowledge and patience allow me the room to work in my own way. I attribute my doctor's degree to their encouragement and effort. Without them, this final year project and including me would not be completed and lost.

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LIST OF TABLES

ABSTRAK

Abstrak daripada kertas projek yang dikemukakan kepada Fakulti Perubatan Veterinar untuk memenuhi sebahagian daripada keperluan kursus VPD 4999 - Projek.

Seroprevalens mengenai Jangkitan Bluetongue virus antara Ruminan Kecil dalam Projek Ladang Angkat FPV, UPM.

Oleh

Azeef Izzuddin B. Abdul Malek

2017

Penyelia: Prof. Madya Dr. Faez Firdaus Jesse Abdullah

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Bluetongue merupakan sejenis penyakit wajib lapor yang disenaraikan oleh World Organisation for Animal Health (OIE) yang boleh memberi impak yang besar terhdap industri ruminan terutamanya bebiri. Biasanya bebiri akan menunjukkan tanda-tanda klinikal walaubagaimanapun ruminan lain juga akan menunjukkan tanda klinikal. Ladang-ladang kambing yang terlibat dengan Program Ladang Angkat UPM tidak mempunyai informasi yang lengkap mengenai status penyakit ini. Di Malaysia, laporan terakhir yang diketahui adalah pada tahun 1995 dan terdapat outbreak baru pada tahun 2009. Oleh itu, experimen ini telah direka untuk menentukan seroprevalens bluetongue dalam ruminan kecil yang terlibat dengan Program Ladang Angkat UPM dan untuk menentukan factor risiko yang berkaitan dengan seroprevalens penyakit ini. Sampel darah diambil dari 100 ekor kambing tanpa mengira umur, baka dan jantina. Sampel serum darah telah digunakan untuk mengesan antibodi terhadap BTV melalui ELISA. Keputusannya adalah negative bagi kesemua sampel. Seroprevalens sifar bagi Bluetongue dalam kajian ini boleh disebabkan langkah-langkah pencegahan yang dilaksanakan oleh Jabatan Perkhidmatan Veterinar (DVS) berdasarkan garis panduan

yang ditetapkan oleh Organisasi bagi Kesihatan Haiwan Dunia (OIE). Kesimpulannya, kambing-kambing dari Program Ladang Angkat UPM adalah bebas daripada Bluetongue.

Kata Kunci: Bluetongue, ruminan, kambing, wabak, seroprevalens, serum darah, ELISA.

ABSTRACT

An abstract of the project paper presented to the Faculty of Veterinary Medicine in partial fulfilment of the course VPD 4999 project.

Seroprevalence of Bluetongue virus infection among Small Ruminant in Foster Farm Programme FPV, UPM.

By

Azeef Izzuddin B. Abdul Malek

2017

Supervisor: Associate Prof. Dr. Faez Firdaus Jesse Abdullah

Co-Supervisor: Prof. Dato Dr. Mohd Azmi Mohd. Lila and Prof. Dr Abdul Aziz Saharee

Bluetongue disease is a notifiable disease listed by the World Organisation for Animal Health (OIE) where it can causes significant problem to the ruminant industry especially in sheep. Sheep usually develops clinical signs, however occasionally other ruminants also acquire the same clinical signs. There is lack of information related to this disease status in UPM's foster farm goats. In Malaysia, the last known report of this disease was in 1995 and recent outbreak was in 2009. Therefore, this experiment was designed to determine the seroprevalence of bluetongue infection among small ruminants in UPM's foster farms and to determine the risk factors associated with the seroprevalence of the disease. Blood samples were collected from 100 goats regardless of their age, breed and gender. The blood serum samples were used to detect the antibody towards Bluetongue virus (BTV) by ELISA. The results were negative for all samples. The zero seroprevalence of bluetongue in this study could be due to the prevention measures implemented by the Department of Veterinary Services (DVS)

based on the OIE guidelines. Thus, the goats from UPM's foster farms are free from Bluetongue.

Keywords: Bluetongue, ruminant, goat, outbreak, seroprevalence, blood serum, ELISA.

1.0 GENERAL INTRODUCTION

Bluetongue virus (BTV) belongs to the virus family of Reoviridae and genus of Orbivirus. BTV is an arthropod-borne virus transmitted via the bites of midges from the Culicoides spp. genus among its ruminant hosts (Darpel *et al.*, 2009; Kameke, Kampen, & Walther, 2017). There are twenty-six BTV serotypes currently recognised in the world but there are only six in Peninsular Malaysia; BLU1, 2, 3, 9, 16 and 23 (Hassan, 1992; Sharifah *et al.*, 1995). Sheep are the major hosts for Bluetongue where clinical symptoms can be observed while other ruminant hosts are affected subclinically (Joardar *et al.*, 2016). The affected sheep typically have fever, nasal discharges which range from serous to mucopurulent, facial oedema where sometimes may involve the submandibular region and the axillae. Cyanotic tongue, dyspnoea and inflamed coronary band can be observed in severe cases (Darpel *et al.*, 2007). Pregnant ewes that are infected will induce abortions, stillbirths, embryonic death and congenital malformations in lambs while in rams, degeneration of the testis was seen with epididymitis (Osburn, 1994; Bürstel *et al.*, 2009; Belbis *et al.*, 2013). Infections in cattle and goats are usually subclinical but according to Scott, cattle that are infected with BTV will usually have pyrexia, coronary band inflammation, nasal discharges and conjunctivitis (2011). Goats usually have the same signs as the cattle however in some goats, they will only show signs of fever and decrease in milk production (Anonymous, 2015).

The Culicoides are small flies or known as ‘biting midges' and the female Culicoides feed on the blood of mammals for reproduction (Mellor *et al.*, 2000). During this

activity, the BTV are transmitted among ruminants. The BTV replicates in the digestive system of the midges and it will be released into the salivary glands. It takes ten to fifteen days for the cycle from infection to transmission takes place and most vectors become infected throughout their life (Eaton *et al.*, 1990; Mellor, 1990, 2000). Other types of arthropods had BTV isolated in them such as sheep ked (Luedke *et al.*, 1965), ticks (Bouwknegt *et al.*, 2010) and mosquitoes (Brown *et al.*, 1992). The disease also can be transmitted via semen from a viraemic bull that contains blood and by placental transmission (Osburn, 1994; Wilson *et al.*, 2008; Saegerman *et al.*, 2011)

Bluetongue cause significant impact to the sheep industry compared to other ruminants as the morbidity rate is 100% while the mortality rate ranges from 2-70% (Anonymous, 2013). The diagnosis of this disease is based on clinical signs, post-mortem findings and epidemiological assessments with laboratory examination (Afshar, 1994). Samples that are examined by the laboratory are non-coagulated blood, serum, tissue samples and also brain tissue from foetuses (Afshar, 1994; Tweedle and Mellor, 2002). BTV isolation in cell lines of insect origin, mammalian BHK-21 or CPAE can be used (Anonymous, 2004; Mecham, 2006). Reverse transcription-polymerase chain reaction (RT-PCR) allows the serotyping of the BTV RNA six months after the infection (MacLachlan *et al.*, 1994). Serological tests for detecting specific antibodies by competitive ELISA allows rapid detection on the 6th post-infection day in the serum or plasma antibody of the infected animals (Koumbati *et al.*, 1999).

The neighbouring countries of Malaysia have various status of Bluetongue for each respective countries. In Iran, the BT seroprevalence would be 89% (Najarnezhad &

Rajae, 2013), Lao People's Democratic Republic have a 96% seroprevalence (Douangngeun *et al.*, 2016), Thailand have 75% in indigenous sheeps (Apiwatnakorn *et al.*, 1996) and 1.11% was reported in Hokkaido, Japan (Giangaspero, 2012). In India, which is a big country will have a wider range of seroprevalence between 5 - 48.7% (Sodhi *et al.*, 1981; Harbola *et al.*, 1982; Janakiraman *et al.*, 1991; Chandel *et al.*, 2004; Walton, 2004; Ravishankar *et al.*, 2005; De *et al.*, 2009; Joardar *et al.*, 2014). New Zealand has not have Bluetongue in its country until now while Australia did have an outbreak of this disease before 2000 but the status is not reported at all. China reported to have also a range between 38.6 - 74.4% of seroprevalence for bluetongue (Xu *et al.*, 2016).

In our country, the last reported case were in 1992 by Hassan *et al.* and also 1995 by Sharifah *et al.* Till date, there is no prevalence study being carried out in the Peninsular region. In 2009, there was a positive case confirmed by the laboratory about a small bluetongue outbreak. The number of ruminant farmers are also increasing where they could have a agriculture background or not, making the potential of this disease to be re-introduce is quite high due to the lack of information about diseases and farm management. The Foster farms also have no record about this disease since it started operation. In Europe, around 128 million dollars were used for the implementation of BTV control programmes and surveillances after their latest bluetongue outbreak in 2006, which can increase a nation’s budget to maintain the food security (Pinior *et al.*, 2015).

1.1 THE STUDY RATIONALE

The rationale behind this study based on several factors that are:

1) The ruminant industry is increasing in Malaysia among the public with more people venture into agriculture. They obtain their animals from anywhere either the local or the international market that may re-introduce diseases that are considered to be eradicated in Malaysia. Bluetongue can cause significant loss to the sheep industry compared to other ruminant livestock.
2) To obtain extra information related to the exposure of BTV among small ruminants in the Foster Farm Programme of FPV, UPM.
3) Bluetongue is also understudied in Malaysia as the last research related to this disease was carried out in 1995 and no follow up on the seroprevalence of this disease after that in Malaysia.

1.2 STUDY OBJECTIVES

The objectives of this study are:

1. To determine the seroprevalence of bluetongue among small ruminant farms involved in the Foster Farm Programme of FPV, UPM.
2. To determine the risk factors and its association towards the seroprevalence of bluetongue from the selected farms in Foster Farm Programme of FPV, UPM.

1.3 HYPOTHESES

The hypothesis for this study would be the seroprevalence of Bluetongue is low or zero in farms involving Foster Farm Programme of FPV, UPM.

2.0 LITERATURE REVIEW

2.1 AETIOLOGICAL AGENT

Bluetongue virus (BTV) belongs to the genus Orbivirus and family of Reoviridae that consist 27 recognized serotypes (Mertens *et al.*, 2005; Zientara *et al.*, 2014). BTV is a 90nm in diameter non-enveloped virus with a triple layered icosahedral protein capsid (Huismans and Erasmus, 1981; Nason *et al.*, 2004; Roy and Noad, 2006). It consists of ten linear double-stranded RNA genome segments, Seg-1–10 with encoding structural proteins VP1 until VP7. The BTV has non-structural proteins that are NS1, NS2, NS3/NS3a, NS4 and a newly discovered S10-ORF2 (Belhouchet *et al*., 2011; Ratinier *et al*., 2016). VP2 and VP5 are the two major proteins at the outer layer where VP2 protein determines the serotype of the virus. It is responsible for the receptor binding, haemagglutination and eliciting the host specific immunity (Verwoerd *et al*., 1972; Mertens *et al*., 1989; Hassan and Roy, 1999; Roy and Noad, 2006).

The VP7 protein forms the inner capsid that is the main determinant of serotype specificity and provides the epitope in enzyme-linked immunosorbent assay test (ELISA) for detection of antibodies against BTV (Oldfield *et al*., 1990; Prasad *et al*., 1992; Roy, 1992; Anthony *et al*., 2007). The innermost layer consists of the VP3, VP1, VP4 and VP6 proteins that are required in the viral RNA transcription and replication (Grimes *et al*., 1998; Schwartz-Cornil *et al*., 2008)

2.2 VIRULENCE FACTORS

Research has focused on the non-structural proteins that are the virulence factors for this disease. The NS2 protein is the major component for the viral inclusion bodies and involved in the recruitment of BTV mRNA for replication (Brookes *et al*., 1993; Owens *et al*., 2004; Lymperopoulos *et al*., 2006; Kar, 2007). The NS3 protein enhances the permeability of the cytoplasmic membrane that facilitates virus release from mammalian or insect cells or also known as viroporin (Hyat *et al*., 1991; Roy, 2008; Han and Harty, 2004). Furthermore, it also allows the BTV particles to leave host cells through a budding mechanism, which operates in insect cells where no cytopathic effect is induced by the virus (Wirblich *et al*., 2006).

The reassortment of the ds-RNA segments are due to the segmented nature of the BTV genome if the host cell has a concurrent infection by several, different serotypes that allows the development of viral diversity (Oberst *et al*., 1987; Stott *et al*., 1987; Belyaev and Roy, 1993; Batten *et al*., 2008; Carpi *et al*., 2010). This gives rise to changes in virulence and serological characteristics of the virus (Cowley and Gorman, 1989; Nuttall *et al*., 1992; O'Hara *et al*., 1998; Batten *et al*., 2008). NS4 too have been demonstrated to facilitate BTV replication in vitro in IFN-competent cells and in vivo in sheep. The virus requires the NS4 to overcome the cellular innate antiviral responses (Ratinier *et al*., 2016).

2.3 TRANSMISSION

Bluetongue are transmit mostly by insect vector through the biting midges from the genus Culicoides, Diptera of Ceratopogonidae. There are 1300 to 1400 species from the genus Culicoides but only 30 of them are BTV vectors (Mellor *et al*., 2000; Meiswinkel, 2004; Borkent, 2005). The Culicoides are present in warm, damp and muddy areas where they are most active before sunset until sunrise (Mellor *et al*., 2000). Midges have two to six weeks of a direct life cycle that involves an egg, larvae, pupae and an adult stage (Mellor *et al*., 2000; Veronesi *et al*., 2009). Only some female midge species will take in blood meal three to four days prior to laying eggs (Birley and Boorman, 1982; Mellor *et al*., 2000). They acquire BTV when feeding on blood of infected ruminants where it replicates in their digestive tract. Later on, it is released into the haemocoel and into the salivary glands and individual vectors remains infectious for life (Chandler *et al*., 1985; Mellor, 2004)

Midge's activity are markedly influenced by ambient temperature, air humidity and total seasonal rainfall (Mullens *et al*., 1995; Wellby *et al*., 1996; Mellor, 2000). Global warming allows longer activity of biting midges and which allows longer periods of BTV transmission (Tweedle and Mellor, 2002). Biting midges can fly over a distance of two km uninterrupted from other factors (Ducheyne *et al*., 2007). Some other arthropod vectors can be involved such as sheep ked (Melophagus ovinus) (Luedke *et al*., 1965), ticks (Bouwknegt *et al*., 2010) and mosquitoes (Brown *et al*., 1992). Bull semen can transfer the virus if the bull is viraemic when there is blood or white blood cell that have BTV (Kirkland *et al*., 2004; Wilson *et al*., 2008). Cattles have been recorded to shed BTV across the placenta (Thomas *et al*., 1986; Menzies *et al*., 2008;

Backx *et al*., 2009), sheep (Saegerman *et al*., 2011) and in dogs (Wilbur *et al*., 1994). Recently, Menzies *et al*. (2008) stated that ingestion of the infected bovine foetus placenta have cause transmission among ruminants. In an experimental study stated that colostrum can also infect a newborn calf with BTV (Backx *et al*., 2009).

2.4 HOST RANGE

This disease affects all ruminants but sheep often develop clinical signs. In cattle, it may have a prolonged viraemia that may act as carriers for this disease but in the BTV-8 epidemic in Europe showed that cattles also develop clinical signs. (Tweedle and Mellor, 2002; Thiry *et al*., 2006; Darpel *et al*., 2007; Elbers *et al*.,2008a). This disease also may affect camelids, elephants, deers, dogs and even bears (Rivera *et al*., 1987; Erasmus, 1990; Wilbur *et al*., 1994; Howerth *et al*., 1995; Ruiz-Fons *et al*., 2008; Evermann, 2008; Garcia *et al*., 2009).

2.5 PATHOGENESIS

After introduction of the virus into the animal by the midges, it is transported to the local lymph nodes from the skin by the animal's dendritic cells where the initial virus replication will take place (MacLachlan, 2004; Hemati *et al*., 2009). Then, it spreads into the blood circulation causing a primary viraemia that seeds most of the secondary organs, i.e., lymph nodes, spleen and lungs (Barratt-Boyes and MacLachlan, 1995; Sanchez-Cordon *et al*., 2010). The virus later on replicates in the vascular endothelial cells, macrophages and lymphocytes (Barratt-Boyes and MacLachlan, 1995; Drew *et*

al., 2010a). The virus particles appear to be sequestered invaginatiing the erythrocyte membrane (Brewer and MacLachlan, 1994; MacLachlan, 2004) that prolongs viraemia in the presence of neutralizing antibodies (Richards *et al*., 1988; Brewer and MacLachlan, 1994). It causes injury to small blood vessels in target tissue, resulting in vascular occlusion and tissue infarction. The thrombocytes, dendritic cells, macrophages and BTV-infected endothelial cells will produce vasoactive mediators that increase damage to the endothelium, increasing the vascular permeability and will leads to the development of oedema and effusions (MacLachlan *et al*., 2009; Drew *et al*., 2010a)

2.6 CLINICAL SIGNS

Bluetongue have an incubation period of four to eight days (Tweedle and Mellor, 2002) where it is followed by fever, tachypnea, and hyperaemia of the lips and nostrils with excessive salivation. There would be also presence of nasal discharge that is initially serous, and then becomes mucopurulent and dried that forms a crust around the nostrils. Facial oedema appears with petechiae develop on the conjunctiva and ulcers on the oral mucosa. In rare instances, the tongues are cyanotic. Some cases have dyspnoe, profuse haemorrhagic diarrhoea or vomiting that leads to aspiration pneumonia. At the end of the pyrexia stage, sheep may have coronitis, laminitis or paresis and muscle necrosis that cause an arched back and are reluctant to move.

Torticollis, dermatitis and breaks in the wool have been recorded (Brewer and MacLachlan, 1994; Tweedle and Mellor, 2002; Elbers *et al*., 2009; Kirschvink *et al*., 2009). Pregnant ewes that are infected may have abortion, foetal mummification and

the birth of weak calves with potential congenital defects (Osburn *et al*., 1971; Osburn, 1994; MacLachlan *et al*., 2000; Tweedle and Mellor, 2002).

Goats are less frequently infected with BTV but if do, the signs are similar to but less severe than in sheep. In some cases, goats have shown a sudden drop in milk production, pyrexia, facial oedema, nasal discharge and scabs on the nose and lips, erythema of the skin of the udder and small subcutaneous haemorrhagic lesions (Dercksen *et al*., 2007). In cattle clinical disease is rare but BTV-8 infection cause clinical signs (Tweedle and Mellor, 2002; Elbers *et al*., 2008b). The early stages are characterised by fever and depression followed by erosion and necrosis of the oral and nasal musocae, nasal discharge, excessive salivation, conjunctivitis, lameness, ulcerative dermatitis, coronitis,, oedema and hyperaemia. The skin of teats is often inflamed and may crack and peel (Tweedle and Mellor, 2002; Thiry *et al*., 2006) with reduced milk production (Thiry *et al*., 2006; Mehlhorn *et al*., 2008). Infection of pregnant dams can result in early death and resorption of the embryo, abortion, malformed and weak calves (MacLachlan *et al*., 2000; Tweedle and Mellor, 2002; Elbers *et al*., 2008a).

In white-tailed deer, this disease is similarly to epizootic haemorrhagic disease of deer with an acute course manifested as haemorrhagic diathesis as a sequela to disseminated intravascular coagulation where it is a widespread haemorrhages throughout the body (Waldvogel *et al*., 1992; MacLachlan *et al*., 2000). It also have swelling of the head and neck, hypersalivation, blood-stained nasal discharge and bloody diarrhoea (Radostits *et al*., 1994; Howerth *et al*., 2001)

2.7 PATHOLOGY

Post-mortem lesions will reveal subcutaneous tissues infiltrated with gelatinous fluid in the head, haemorrhages in the pulmonary artery or aorta, hyperaemia, or occasionally cyanosis of the oral mucosa with petechiae and ecchymoses. Erosions may be present in the lips, tongue and cheeks. There could be hyperaemia of the ruminal pillars and reticular folds. The spleen, lymphatic nodes and tonsils are enlarged and have petechial haemorrhage. The tongue root, pericardial sac, kidney, gut and subcutaneous tissues have petechial haemorrhage. In addition, inflammation of the upper respiratory tract, pulmonary oedema, pleuritis, pericarditis or enteritis may be present (Tweedle and Mellor, 2002; Darpel *et al*., 2007; Mauroy *et al*., 2008; MacLachlan *et al*., 2009).

Histological findings include hypertrophy of endothelial capillaries, perivascular oedema and infiltration of macrophages and lymphocytes into the skeletal and cardiac muscles, vascular congestion and tissue infarction. In acute cases, heart and skeletal muscles have haemorrhage while in chronic cases, it will have fibrosis and infiltration with mononuclear cells (Brodie *et al*., 1998; Tweedle and Mellor, 2002; MacLachlan *et al*., 2009).

2.8 DIAGNOSIS

Samples that usually collected for laboratory examination are non-coagulated blood, blood serum, tissue samples of spleen, lymph nodes, lungs, liver, bone marrow and foetus brain tissue (Stott *et al*., 1983; Parsonson, 1990). For transportation of samples, serum should be at –20 °C and the other samples should be kept on ice (Tweedle and Mellor, 2002). Bluetongue virus can propagate in embryonated chicken eggs, cell cultures or in sheep for virus isolation. Reverse transcription-polymerase chain reaction (RT-PCR) allows serotyping and detection of BTV RNA as late as six months while ELISA and immunofluorescence tests can be use too (MacLachlan *et al*., 1994). Competitive ELISA can detect serogroup-specific antibodies against BTV by targeting the VP7 protein while the serum neutralisation test has the highest specificity and sensitivity of all the tests but it is the most expensive and time-consuming (Reddington *et al*., 1991; Hamblin, 2004).

3.0 METHODOLOGY

3.1 SERUM SAMPLE COLLECTION

3.1.1 Location

Sampling was done on goats from the farms that are involved with Foster farm Programme FPV, UPM.

3.1.2 Animals

One hundred goats regardless of age, sex and breed were controlled for this study.

3.1.3 Samples

Blood samples were collected from the jugular vein into a plain tube, kept in ice and transported in the ice box to the laboratory for analysis.

3.1.4 Serum preparation

The plain tube undergoes centrifugation at 3000 rpm for 5 minutes. The serum is then transferred into the Eppendorf tubes for storage purpose at temperature of -20^{o}C.

3.2 SEROLOGICAL TEST

3.2.1 Test kit Preparation

A Bluetongue Virus Antibody Test Kit via competitive ELISA (cELISA) was purchased from the company of Veterinary Medical Research and Development. The kit contains 2 Antigen-coated plates, Positive control, Negative Control, Antibody-Peroxidase Conjugate, Wash solution concentrate, Substrate solution and Stop solution. The reagents were taken out from storage that are 2-7ºC into room temperature. All of the controls are ready to use and requires duplicates for each plate while the serum samples used are tested undiluted. The plates are removed from the foil pouch container. The conjugate are also ready to use without any dilution. Lastly, the wash solution is diluted 1 part of it to 49 parts of distilled water with each well need approximately 1ml of the wash solution during washing.

3.2.2 ELISA test procedure

The positive and negative controls were transferred in duplicates for each plates while 90 serum samples were transferred into each wells with replicates by using a pipette set at 25ul for each well. After transferred, firmly tapped each of the long sides of the loaded assay plate 7 times for a total of 14 taps to make sure the samples coat the bottom of the wells and the plates are incubated at room temperature for 15 minutes. Then, 25ul of Antibody-Peroxidase Conjugate was added to each well and the plate contents were mixed by firmly tapping each of the long sides of the plate 7 times for a total of 14 taps. It was then incubated for 15 minutes at room temperature. The plates were then washed manually by dumping the well contents and striking the

inverted plates 4 times on a clean paper towel. The wash solution was filled into each well using a multichannel pipette and then dumps the well contents again before striking it inverted on a clean paper towel. It was repeated another 2 additional times for a total of 3 washes. After washing the plates, 50ul of Substrate solution was added into each well and firmly tap each of the long sides of the plates 7 times for a total of 14 times and incubate it for 10 minutes at room temperature with avoid leaving it to direct sunlight. Then, 50ul of Stop solution was added into each well and mix the contents by firmly tapping each of the long sides of the well 7 times for a total of 14 taps. Without emptying the wells, the plates were read on a microplate absorbance spectrophotometer with the optical density (O.D) reading wavelength to 630nm.

3.3 VALIDATION & RESULTS INTERPRETATION

Results	Optical densities (O.D)
Negative Control	between 0.300 to 2.000
Positive Control	1/2 of the mean of the Negative control
Positive Samples	< ½ of the Negative controls
Negative Samples	> ½ of the Negative controls

Table 1. Optical densities representation for Positive Control, Negative Control and samples

4.0 RESULTS

Total of 100 goat serum samples were collected randomly from goat population in the farms that are involved with the Foster farm Programme FPV, UPM and were tested for the detection of BTV antibody using competitive ELISA test. The test result revealed negative for all tested serum samples.

5.0 DISCUSSION

This study shows that the goats in UPM's Foster farm are free from this disease. It could be that the virus is not present in these farms because we cannot dismiss that there is absence of Culicoides spp. in the farms involved but the virus itself has not yet establish in the Culicoides population at these farms. Based on Mellor (2004), BTV will remain in the infected Culicoides throughout its life where there is slight possibility of this virus to be present in the goat population of the UPM's Foster farm. This virus also cannot survive in the environment without a living host making the host and the vectors present in the UPM's Foster farm area are free from BTV.

Department of Veterinary Service of Malaysia practices preventive measures based on the guidelines prepared by the World Organisation for Animal Health (OIE) for Bluetongue. In Malaysia, this disease is consider as eradicated thus making the prevalence status in our country should be zero since 1995 and it had been highlighted that the outbreak occurred in 2009 was due to importation. Until date, there is no any reported cases by DVS related to this disease. The control measures that Malaysia practise for importation from countries that have bluetongue vectors are vaccination of the imported animals 60 days prior to importation that would be stated in the Health certificate of those animals but in cases of unvaccinated animals, they are tested for the presence of antibodies for the virus serotype of the original country. For countries that are not free from Bluetongue are consider risky for importation so extra measures are done by vaccination of these animals, quarantine and also practising vector control at the intended farms. The quarantine duration are the same for all ruminants, which

are 30 days which is enough to cover its incubation period that is 5-10 days (Sperlova, 2011).

DVS did prepare actions needed to be done if there would be a positive case in our country that is also based on OIE guidelines (Department of Veterinary Service, 2014). There would be both trace back and trace forward of the disease to control the further spread of the disease by controlling animal movement. Three types of zoning would be practiced in case of an outbreak; first would be infectious zone that is 20km radius from the initial case. The animals that are consider as high risk will be examined for the clinical signs of this disease and serological testing will be carried out. Positive animals are restricted from moving out from this zone but only for slaughtering purpose and vector controls are carried out. Then, the control zone would be between 21-100km from the initial case where active surveillance for clinical signs among ruminants are done while high risk host are tested prior moving out from this zone. The vectors are also kept on surveillance for this disease. The last zone would be 101-150km from the initial case where surveillance are done on livestock and vectors based on the National Surveillance Programme. Livestock are not allowed to be vaccinated for this disease to maintain our country's free status while animals with clinical signs are usually treated symptomatically. European Union (EU) recommended vector control action rather than stamping out because in recent studies, there are high rate of subclinical infections in wild and domestic animals with the vectors continue on harbouring this virus even after the mass culling (Caporale & Giovannini, 2010).

Vector control and surveillance would be the best control in an outbreak for this disease as this is an arthropod-borne virus mainly by Culicoides spp. (Darpel *et al*.,

2007). Culicoides breed in moist conditions such damp, muddy areas and organic matter and they can live on for 2-6 weeks (Veronesi *et al*., 2009). Modification of these areas can remove the potential breeding sites of this vector reducing the vector's population. For the larva stage, by applying larvicide Abate that contains 5% temephos granulated with gypsum on the midge breeding ground will kill the larvae. A research done by Schmahl *et. al* (2009a) mentioned that insecticides can be for environmental control for adult midges. Repellents such as fron diethyl toluamide (DEET) can be used for direct protection of the potential host where it can be effective for four hours. The usage of synthetic pyrethroid such as deltemethrin, cyfluthrin and permethrin allows protection against adult midges for 3-5 weeks can be in form of spray-on or the impregnated ear tags (Mehlhorn *et al*., 2008). Surveillance will be carry out yearly on the vectors of this disease which can include sheep keds, ticks and even mosquitoes (Luedke *et al*., 1965; Brown *et al*., 1992, Bouwknegt *et al.,* 2010).

The Bluetongue virus will remain infectious for its entire life based on Mellor (2004). These virus have a common antigenic determinant for all its serotypes which would be the antigen protein P7 (Afshar *et al*., 1992). Competitive Elisa would the best serologic test for BTV antibody detection because it reduces the chance of cross-reaction with any other Orbivirus by using monoclonal antibody (Afshar *et al*., 1995).The ELISA kit used for the experiment would be from the VMRD® Bluetongue kit which has a sensitivity of 100% and specificity of 99% making it very reliable.

The farms involved in this project practiced a good Herd Health Programme in terms of waste management, environmental management and biosecurity (Abdullah *et al*., 2015). This can reduce the potential exposure of this disease to the UPM's foster

farms. The University Veterinary Hospital (UVH) team of UPM have a very quick response to any complaints of the farm owners regarding about their abnormal animals in the herd. This allows treatment and diagnosing the disease faster which can prevent further transmission of this disease. The farmers are also kept on being educated and gain more knowledge everyday with the help of the UVH staffs and veterinarians that are involved with their farm management.

6.0 CONCLUSION & RECOMMENDATION

CONCLUSION

In this study, the goat farms involved with the Foster Farm Programme are free from Bluetongue.

RECOMMENDATION

Further studies can be done in the future by using larger sample size for more conclusive results. Besides that, we can also include other farms that are closed with UPM but they are not involved with the Foster Farm Programme of FPV, UPM to determine the prevalence of this disease for farms that have not been consulted with staffs of FPV. Cattles can be used as target samples where they have the potential to be the carriers of this disease too. Lastly, it would be to sample the farms from other states to get the overall prevalence for each state and also our country.

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