UNIVERSITI PUTRA MALAYSIA

FOOT AND MOUTH DISEASE (FMD) IN CATTLE AND BUFFALOES IN TWO QUARANTINE STATIONS AND SIX SELECTED STATES OF PENINSULAR MALAYSIA BETWEEN 2010 AND 2015

NIK NUR FATIN AMIRA BINTI NIK KAMARUDIN

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NIK NUR FATIN AMIRA BINTI NIK KAMARUDIN

A project paper submitted to the
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To my parents and siblings whom I love the most, this is for all of you.

To my teachers and lecturers, this cannot be accomplished without your patience and guidance from the beginning.

“I finally did this! Yeah!”

It is hereby certified that we have read this project paper entitled "Foot and Mouth Disease (FMD) in Cattle and Buffaloes in Two Quarantine Stations and Six Selected States of Peninsular Malaysia between 2010 and 2015", by Nik Nur Fatin Amira binti Nik Kamarudin and in our opinion it is satisfactory in terms of scope, quality, and presentation as partial fulfillment of the requirement for the course VPD4999 – Project.

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Nik Nur Fatin Amira binti Nik Kamarudin
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LIST OF ABBREVIATIONS

%	Percentage
APTVM	Arahan Prosedur Tetap Veterinar Malaysia
DVS	Department of Veterinary Service
ELISA	Enzyme-Linked Immunosorbent Assay
FMD	Foot and Mouth Disease
FMDv	Foot and Mouth Disease virus
MAQIS	Malaysia Quarantine and Importation Service
MTM	Malaysia-Thailand-Myanmar
N	Number of sample tested
NSP	Non-structural Protein
OIE	World Animal Health Organization
SEA	South-East Asia
SEACFMD	South-East Asia China Foot and Mouth Disease
SKPB	Padang Besar Animal Quarantine Station
SKRP	Rantau Panjang Animal Quarantine Station
SP	Structural Protein

ABSTRAK

Abstrak daripada kertas projek yang dikemukakan kepada Fakulti Perubatan Veterinar untuk memenuhi sebahagian daripada keperluan kursus VPP4999-Projek.

PENYAKIT KUKU DAN MULUT (FMD) PADA LEMBU DAN KERBAU DI DUA STESEN KUARANTIN DAN ENAM NEGERI-NEGERI TERPILIH DI SEMENANJUNG MALAYSIA ANTARA TAHUN 2010 DAN 2015

Oleh

Nik Nur Fatin Amira bt. Nik Kamarudin

2016

Penyelia: Prof. Dr. Abdul Aziz bin Saharee

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Penyakit Kuku dan Mulut (FMD) adalah penyakit berjangkit haiwan berkuku dua. Malaysia merupakan pengimport haiwan ruminant hidup untuk keperluan domestic dan ini memberi risiko kepada industry ruminant tempatan. Pergerakan haiwan terjangkit FMD merupakan salah satu faktor utama berlakunya wabak. Objektif kajian ini adalah untuk menentukan seroprevalen

FMD pada lembu dan kerbau di Stesen Kuarantin Haiwan Padang Besar (PB) dan Rantau Panjang (RP) dari tahun 2010 sehingga 2015, dan untuk menghuraikan wabak FMD di enam negeri-negeri terpilih. Data yang wujud antara tahun 2010 dan 2015 diulas termasuk keputusan ELISA 3ABC protein non-struktur (NSP) FMD yang diperoleh dari Makmal Veterinar Kawasan Kota Bharu, dan data wabak diperoleh daripada laman sesawang Organisasi Kesihatan Haiwan Dunia- Unit Koordinasi Kawasan (OIE-RCU). Berdasarkan ujian NSP tersebut, seroprevalen FMD secara keseluruhan di stesen-stesen kuarantin adalah 36.4% (RP: 40.7%, PB: 34.9%) dan terdapat perbezaan signifikan antara kedua-dua stesen (χ^2=42.3, df=1, $p<0.05$). Seroprevalen FMD yang paling tinggi telah direkodkan pada tahun 2011 dan paling rendah pada tahun 2012. Terdapat perbezaan signifikan antara tahun ($p<0.05$). Seroprevalen yang lebih tinggi secara signifikan ($p<0.05$) ditemui pada lembu berbanding kerbau (36.6%, 30.7%, masing-masing) dan jantan berbanding betina (39.1%, 30.4%, masing-masing). Jumlah wabak FMD di negeri-negeri utara Semenanjung Malaysia dari tahun 2010 kepada 2015 adalah 69 (2010 - 19, 2015 - 4). Terengganu merekodkan jumlah wabak yang paling tinggi (26) manakala paling rendah adalah Perlis (1). Wabak FMD bulanan adalah paling tinggi pada bulan September. Vrus serotip O dan serotip O dan A merupakan penyebab utama wabak di negeri-negeri terpilih di Semenanjung Malaysia.

Hasil ini menunjukkan FMD adalah endemik di Malaysia dan strategi kawalan perlu dipertingkatkan.

Kata Kunci: *Penyakit Kuku dan Mulut, Lembu, Kerbau, Seroprevalen, Wabak, NSP, ELISA, Stesen Kuarantin, Semenanjung Malaysia.*

ABSTRACT

An abstract of the project paper presented to the Faculty of Veterinary Medicine in partial fulfillment of the course VPD4999 – Project.

FOOT AND MOUTH DISEASE (FMD) IN CATTLE AND BUFFALOES IN TWO QUARANTINE STATIONS AND SIX SELECTED STATES OF PENINSULAR MALAYSIA BETWEEN 2010 AND 2015

by

Nik Nur Fatin Amira bt. Nik Kamarudin

2016

Supervisor: Prof. Dr. Abdul Aziz bin Saharee

Co-supervisor: Dr. Siti Zubaidah binti Ramanoon

Foot-and-mouth disease (FMD) is a highly contagious disease of cloven-hoofed animals. Malaysia has been importing live ruminant for domestic demand and this could pose a risk to the local ruminant industry. Movement of FMD infected animals is one of the main factors for outbreaks. The objectives of this study were to determine the seroprevalence of FMD in cattle and buffaloes in Padang Besar (PB) and Rantau Panjang (RP) Animal Quarantine Stations from 2010 to 2015, and to describe the outbreaks of FMD in six selected states. The

existing data between 2010 and 2015 were reviewed including results from the Enzyme-Linked Immunosorbent Assay (ELISA) 3ABC non-structural protein (NSP) of FMD retrieved from the Regional Veterinary Laboratory Kota Bharu, and outbreaks data extracted from the World Organization for Animal Health-Regional Coordination Unit (OIE-RCU) website. Based on the NSP tests, the overall seroprevalence of FMD in quarantine stations was 36.4% (RP: 40.7%, PB: 34.9%) and there was significant difference between both stations (χ^2=42.3, df=1, $p<0.05$). The highest seroprevalence of FMD was recorded in 2011 and the lowest in 2012. There was significant difference for seroprevalence between years ($p<0.05$). Significantly higher seroprevalence ($p<0.05$) were also found in cattle than buffaloes (36.6%, 30.7%, respectively), and males than females (39.1%, 30.4%, respectively). The total number of outbreaks of FMD in the northern states of Peninsular Malaysia from 2010 to 2015 was 69 (2010 - 19, 2015 - 4). Terengganu recorded the highest (26) number of outbreaks while the lowest was in Perlis (1). Monthly FMD outbreak occurrence was the highest in September. Virus serotype O and serotype A were the main cause of outbreaks in the selected states of Peninsular Malaysia. The findings showed that FMD is endemic in Malaysia and control strategies need to be improved.

Keywords: *Foot and Mouth Disease, Cattle, Buffalo, Seroprevalence, Outbreaks, NSP, ELISA, Quarantine Station, Peninsular Malaysia.*

1.0 INTRODUCTION

Foot-and-mouth disease (FMD) is a highly contagious disease of cloven-hoofed animals including cattle, pigs, sheep and many wildlife species (Alexandersen *et al*., 2003). The FMD virus is classified within the Aphthovirus genus from the family Picornaviridae. According to the Institute for Animal Health, Pirbright Laboratory in the 1st Annual Report on 2003, the viral RNA molecule carries genetic information for viral proteins, namely structural and non-structural proteins (NSP) which are for manufacturing the structural and non-structural part of the virion, respectively.

Common clinical signs accompanying FMD infections are characterized by an acute febrile reaction with vesicular formation consistently in and around the mouth and also around the feet. Vesicles may also be seen on snouts and muzzle, mammary glands and teats, prepuce, vulva and other parts of skin. Lesions will cause pain and lameness to the animal (Alexandersen *et al*., 2003).

From the study by Hasaballa and Abbo on 2010, the overall prevalence rate of FMD in cattle within the period of 12 years (1996 to 2007) was 11.1% with a case-fatality rate of 0.62%. This disease is considered an important disease in a tropical country such as Peninsular Malaysia and strategic

vaccination has been done at the area close to Thailand to control the disease. However, FMD is now widespread throughout Peninsular Malaysia.

Cattle and buffaloes traded are usually tested serologically to detect the presence of antibodies against FMD virus using the Nonstructural Protein (NSP) Enzyme Linked Immunosorbent Assay (ELISA). This test is useful to differentiate infected from vaccinated animals by detecting the types of antibodies formed. This reflects the circulation of FMD virus in the source country or area.

1.1 Rationale of the study

Malaysia has been importing live animals to meet the domestic demand of red meat (Gleeson, 2002). Since ruminant industry in Malaysia mostly involves smallholder farmers, there is low self-sufficiency level of ruminant products, especially beef, and importation has to occur to allow more ruminants to come in to support the production of milk and meat in Malaysia. However, increase in the intensity of the population, will favor the spread of diseases.

This disease is one of the major Transboundary Animal Diseases (TADs) and can give negative effect to the trade of livestock and their products (Kibore *et al*., 2013). Therefore, the control and eradication of FMD virus is very important in Malaysia as the presence of this virus and manifestation of

clinical symptoms in susceptible animals can strictly cut the exportation of animals, including animal by-products to FMD free-zone country which are high value-markets (Abila and Foreman, 2006).

In this study, the focused areas for seroprevalence study are two government quarantine stations at the northern border of Peninsular Malaysia, which are Padang Besar Animal Quarantine Station in Perlis, and Rantau Panjang Animal Quarantine Station in Kelantan. This study also includes description of outbreak in selected states in Peninsular Malaysia which usually increase during festive seasons. During those times, movements of animal were found to be active and spreads of disease occur, resulting in high number of outbreaks in those selected states.

1.2 Objectives and hypothesis

This study is conducted with the objectives:

1) To determine the seroprevalence of FMD in cattle and buffaloes in two animal quarantine stations of Peninsular Malaysia 2010 and 2015.

2) To describe the outbreak of FMD in selected northern border states of Peninsular Malaysia between 2010 and 2015.

2.0 LITERATURE REVIEW

2.1 History of FMD

The probable foot and mouth disease (FMD) was earliest described in cattle by an Italian monk in Venice in 1514. The animal that was infected refused their feed and showed reddening of oral mucosa and vesicular formation on the feet and also in the oral cavity itself. However, according to descriptions reported 500 years ago, most of them recovered. This has a strong resemblance to the cases that we encounter nowadays (Jamal and Belsham, 2013).

In Malaysia, the disease was first reported in Peninsular Malaysia on 1973 and is still being reported till today. However, the East Malaysia states which are Sabah and Sarawak were announced as FMD free zone by World Organization of Animal Health (OIE) on 2004 as stated in Protokol Veterinar Malaysia 2011.

2.2 Epidemiology of FMD

2.2.1 Agent of FMD

The culprit of FMD is from the genus Aphthovirus from the family Picornaviridae (Hasaballa and Abbo, 2010). It is a non-enveloped icosahedral

virus, with the diameter of 26nm and having RNA of around 8.4kb. The RNA is translated into 12 structural and non-structural proteins after cleaved proteolytically from polyprotein during intracellular cytoplasmic replication (Alexandersen *et al.*, 2003).

There are seven (7) different serotypes of FMD virus with indifferent clinical manifestation from each other, namely, O, A, C, Asia 1, Southern Africa Territories (SAT) 1, SAT 2, and SAT 3 (Alexandersen *et al*., 2003). Among outbreak cases from 1996 to 2007 that occured in Malaysia, 83.4% were due to serotype O FMDv, type A (11.9%) and type Asia 1 is 4.7% (Hasaballa and Abbo, 2010). According to Kitching (2005), serotype O is known as the aggressive type. In Malaysia, source of FMD infection was via the entrance of infected animals or products (Abdul-Hamid, *et al.*, 2013).

2.2.2 Susceptible Host and Mode of transmissions

Susceptible animals for FMD include all domesticated and wild cloven hoofed animals for instance, cattle, buffalo, pigs, sheep and goats for domesticated animal and usually occur in deer for wild animal (Sarma, 2004). According to Nawaz *et al*., 2015, seroprevalence in cattle is higher than in buffalo, in his study conducted at Faisalabad. Extensive exotic blood and cross breeding among cattle in the region can lead to high susceptibility of cattle

towards FMD (Zulfiqar, 2003). In contrast to another study conducted by Awan *et al.*, on 2009, in Punjab, there were 10.3% susceptibility in buffaloes and 9.9% which was lower in cattle.

Megersa *et al.*, (2009) found that males had higher seroprevalence compared to females and the finding was similar with studies conducted by Awan *et al*., 2009. However, Nawaz *et al.* in 2014, found that females had higher seroprevalence of FMD due to exposure to stress. Animal under frequent exposure to stress is also susceptible to disease.

Infection starts when susceptible animals are exposed to infected animals or environment including infected fomites. The main source of virus is diseased animals and transmission occurs via direct contact with such animals. The virus of FMD can be found in all excretions and secretions of an acutely infected animal including urine, feces, semen, milk, expired air and saliva. Fluid from FMD-associated vesicles such as amniotic fluids and aborted fetus in sheep can also contain the virus (Aftosa, 2015).

While in another study by Alexandersen *et al.*, on 2003, the most common way of FMD virus transmission is by direct contact which is from mechanical transfer of virus through cuts or abrasion on skin of infected animals and also via droplets or aerosol deposition into the respiratory tract of

susceptible animals. Physical contact with exceretions, secretions, or even epithelial cells with relatively large amount of FMD virus can facilitate the transmission via contact.

Different host species or strain of virus will result in different amount of virus shed by each route. For example, pigs produce large amounts of virus in aerosol, plus presence of large herds of infected pigs may cause increase in risk of airborne spread. The susceptibility against the virus is also different between species. For instance, much high doses of virus were required in pigs to be infected, but cattle are more susceptible to aerosolized virus (Aftosa, 2015).

2.3 Pathogenesis

Entry of the virus through droplets inhalation will allow it to multiply in the epithelium of the upper respiratory tract. This will result in viremia. Localization of the virus is usually at the epithelium of the mouth and feet. Since it has cryopathogenic nature, hydropic generation occur intracellularly and also necrosis of the infected cells. Vesicle will also be formed as clinical signs (Sarma, 2004).

2.4 Incubation Period

Incubation period is the time interval between exposure to an infective dose and the first appearance of clinical signs (Alexandersen *et al*., 2003). OIE

Terrestrial Animal Health Code 2015 has stated that the incubation period of FMD virus is 14 days. However, according to Aftosa (2015), there are several factors that may affect incubation period of FMD virus, which are the species of the animal, the dose of the virus, the viral strain as well as the route of infection.

2.5 Clinical Signs

The typical clinical manifestations observed in livestock with FMD are hyperthermia, hypersalivation, and vesicular formation on the oral mucosa, nose and the spaces between the digits as well as the coronary bands (Jamal and Belsham, 2013). Meanwhile, Aftosa (2015) added that vesicle can also be formed in and around the mouth and also the mammary gland while other locations such as prepuce, vulva or the pressure points on the legs can also be the sites of vesicular formation occasionally. The lesions can cause pain and discomfort and the animal will show inappetance, depression, anorexia lameness and hypersalivation. This would eventually reduce their performance. However, the clinical signs may vary between species. Mortality is rare in adults but common in calves due to multifocal myocarditis or starvation.

2.6 Serological Diagnosis

In order to certify animals for exports, to monitor immunity from vaccination, and to confirm suspected cases during outbreak, serological tests can be done. Different serological tests detect different types of protein of the virus. NSP ELISA can detect the non-structural protein (NSP) which is expressed during replication (Aftosa, 2015).

By using non-structural protein (NSP) Enzyme Linked Immunosorbant Assay (ELISA), the cattle infected with FMD virus can be differentiated from those vaccinated. Sørensen et al. (1998) suggested that the most reliable index of infection in vaccinated animals is by detecting antibodies against the NSP of FMD virus. According to annual report prepared by Institute of Animal Health, Pirbright Laboratory (2003), the tests for antibodies against 3ABC NSP are particularly reliable and are not specific to any serotype of the virus and able to detect antibodies from any strain. The antibodies against NSP in cattle can last for 560 days (more than 1 year) in an experiment conducted by Silberstein *et al.* in 1997 while Elnekave et al. (2015) found that antibodies can also be detected even until 1118 days or about 3 years after infected with FMD virus.

2.7 Livestock Importation

Malaysia has been importing live cattle from other countries such as Thailand and Myanmar. According to Arahan Prosedur Tetap Veterinar Malaysia (APTVM) 2011, produced and published by Department of Veterinary Services (DVS), quarantine is a process needed to avoid the entrance of animal disease and also zoonotic diseases from other countries by importation of live animals, and animal products.

In Malaysia, there are seven Government Quarantine Stations for Animals and imported animals can be quarantined in the three types of quarantine stations which are Government Quarantine Station, Temporary Quarantine Station, or Private Quarantine Facilities. Animals are allocated to either of the location after approval from the Director General of DVS which depends on the risk analysis to the animal species and the status of the country of origin (Arahan Tetap Veterinar Malaysia (APTVM), 2011).

The quarantine period depends on the species and health status of the animal referring to the country of origin. According to Regulations for the Importation of Cattle and Buffalo from Thailand into Malaysia, empowered under Section 8 of Animal Rules, 1962, upon arrival, the cattle and buffalo

shall be quarantined for 10 clear days at approved quarantine station and the first day is being counted after the last animal has been admitted.

Livestock will be observed for clinical signs and vaccination will be done. They will be tested serologically and antibodies against FMD are determined. Those with FMD lesions will be treated and released after quarantine period to the Malaysia-Thailand-Myanmar (MTM) Zone **(**Prosedur Operasi Piawai, 2009).

Results of serology test reflect the viral circulation and status of exporting country and does not explain the viral circulation in Malaysia (Smith, 2012). Usually every animal in the quarantine has a chance of 2.7% to have an inapparent form of FMD infection. Thus, it is likely that the animal will not be identified as infected. A study conducted by Wongsathapornchai, *et al.*, in 2008 revealed that, 2.9% animal accepted into Malaysia-Thailand-Myanmar Region for importation, have FMD.

Karuppanan and Naheed (2000) had conducted a study, revealed that, the seroprevalence of FMD in Padang Besar Animal Quarantine Station was higher compared to Rantau Panjang Animal Quarantine Stations from 1995 to 1998. However, this high number only reflected the viral circulation and status

of exporting country and not represented the viral circulation in Malaysia (Smith, 2012).

In order to control and eradicate FMD progressively and to promote the collaborative working environment between countries in the area, the five years program involving Myanmar, Thailand and the Malaysia Peninsular, known as Myanmar-Thailand-Malaysia Peninsular (MTM) Campaign, was developed in 2001, establishing FMD-free zone (regionalization) on the MTM region (Wongsathapornchai, *et al.*, 2008). In Malaysia, the MTM zone involved are, Kedah, Kelantan, Perak, Perlis, Pulau Pinang, and Terengganu. According to Protokol Veterinar Malaysia, MTM region is included in FMD control zone.

As reported by Abila (2011), FMD outbreaks were reported to be high in 2010 in the South East Asia region. Outbreaks occurred in lower part of Peninsular Malaysia due to higher demands of live animals but low supply. Therefore, people opted to import from endemic countries, which are cheaper, but very risky.

Intensive measures taken by the government to control this disease by reducing the importations of live animals and increase beef importation in order to compensate the local demand including vaccination programs and improved surveillance strategy in the countries (Protokol Veterinar Malaysia, 2011).

3.0 MATERIALS AND METHODS

3.1 Seroprevalence Study

In this study, serology results from Non-structural Protein Enzyme-Linked Immunosorbent Assay (NSP-ELISA) test of all cattle and buffaloes in the northern border government quarantine stations; Rantau Panjang Animal Quarantine Station, Kelantan and Padang Besar Animal Quarantine Station, Perlis, from 1st January 2010 until 31st December 2015, were obtained from the Veterinary Diagnostic Laboratory, Kota Bharu, Kelantan.

3.1.1 Study Area

Map 1: Locations of Padang Besar (SKPB) and Rantau Panjang (SKRP) Animal Quarantine Stations in Peninsular Malaysia

3.1.2 Study Design and Data Collection

This study involved data review from January 2010 until December 2015. The data was the serology result from the database in Veterinary Diagnostic Laboratory, Kota Bharu, Kelantan from the software SIMMAK.exe. The data involved cattle and buffaloes in the northern quarantine stations; Rantau Panjang Animal Quarantine Station, Kelantan, and Padang Besar Animal Quarantine Station from January 2010 until December 2015.

Serology test results were obtained from Enzyme Linked Immunosorbent Assay (ELISA) done using the PRIONICS PrioCHECK® FMDV antibody test kit which were used to differentiate infected and vaccinated animals regardless of vaccination status.

3.1.3 Data Management and Analysis

The FMD serology results tested using NSP ELISA was managed using Microsoft Excel 2010 ® with the following information; submitter address, date of sample submission, herd age, herd sex, species, no. of serum sample sent, no. of serum sample tested, no. of serum sample tested positive. The data was imported to Statistical Package for Social Science (SPSS) version 22 for statistical analysis.

Descriptive statistical analysis was done after obtaining the seroprevalence of FMD by having the proportion of positive serum sample from the total serum sample tested from each quarantine station for each year from 2010 to 2015.

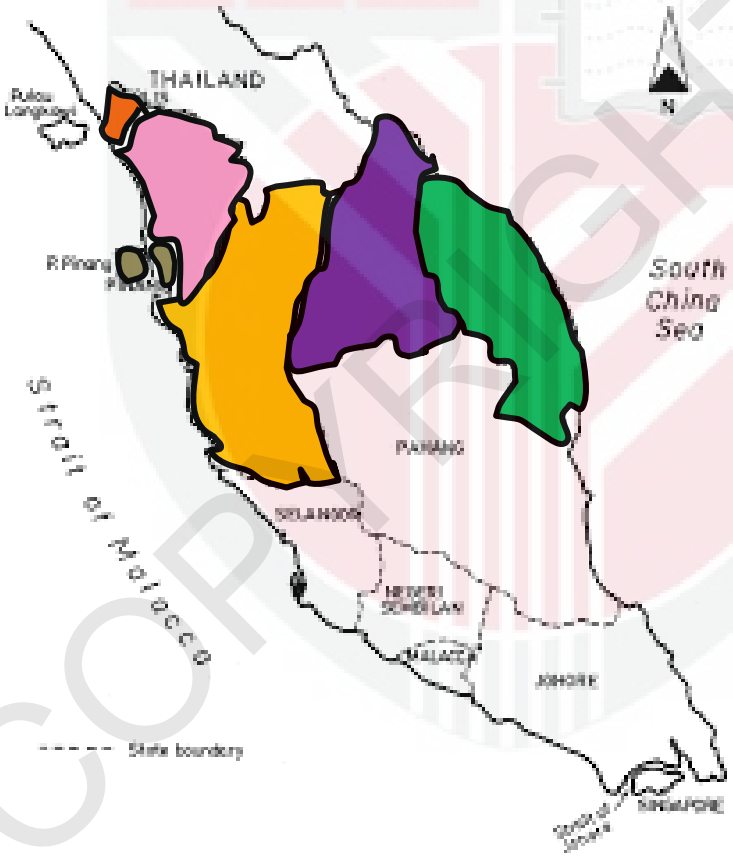
$$\text{Seroprevalence} = \frac{\text{Total number of positive sample}}{\text{Total number of cattle and buffaloes tested}}$$

Chi square and odd ratio was calculated to determine any association of risk factors with FMD (Nawaz *et. al*. 2014) and to know the strength of the association.

3.2 Outbreak Data Description

The outbreak data of six selected state which were Kedah, Kelantan, Perak, Perlis, Pulau Pinang and Terengganu, from 1st January 2010 until 31st December 2015 were obtained and tabulated.

3.2.1 Study Area



Map 2: Locations of the selected states; Kedah, Kelantan, Perak, Perlis, Pulau Pinang, Terengganu

Legend:

- Kedah
- Kelantan
- Perak
- Perlis
- Pulau Pinang
- Terengganu

3.2.2 Study Design and Data Collection

The outbreak data of six selected state which were Kedah, Kelantan, Perak, Perlis, Pulau Pinang and Terengganu, from 1st January 2010 until 31st December 2015 were obtained from the website (http://www.seafmd-rcu.oie.int/fmd_se_asia.php, assessed on 11 February 2016 and www.oie.int/wahis_2/public/wahid.php/Diseaseinformation/statusdetail assessed on 19 February 2016) based on the reports submitted by the Department of Veterinary Service (DVS), Putrajaya, Malaysia. The data comprised of the year and month of outbreak occurrence, number of outbreak, and FMD virus serotype involved according to states.

3.2.3 Data Management and Analysis

The data were tabulated in Microsoft Excel 2010 ® and described.

4.0 RESULTS

4.1 Seroprevalence Study

Table 1 Seroprevalence of FMD in two different quarantine stations

Quarantine Stations	No. of animals tested	No. of animals tested positive	Seroprevalence	p-value
Padang Besar	11685	4081	34.9%	0.000
Rantau Panjang	3900	1588	40.7%	
Total	**15585**	**5671**	**36.4%**	
χ^2= 43.5, df= 1, Odd Ratio= 1.3				

Out of 15,585 cattle and buffaloes tested in Padang Besar and Rantau Panjang Quarantine Stations, 5,671 were found to be positive against NSP antibodies and this represented 36.4% seroprevalence for those two quarantine stations. The number of animal tested for FMD from Padang Besar (n=11,715) was higher than Rantau Panjang (n=3,900). However, the number of animal tested positive from Rantau Panjang was more than Padang Besar. Therefore, the seroprevalence of FMD in Rantau Panjang Quarantine Station (40.7%) was identified as higher than Padang Besar Quarantine Station (34.9%).

Statistically, there was significant difference between seroprevalence and the quarantine stations as the p-value was less than 0.05. Padang Besar Animal Quarantine Station has 1.3 times more likely to be seropositive than Rantau Panjang Animal Quarantine Station (CI = 1.19-1.38).

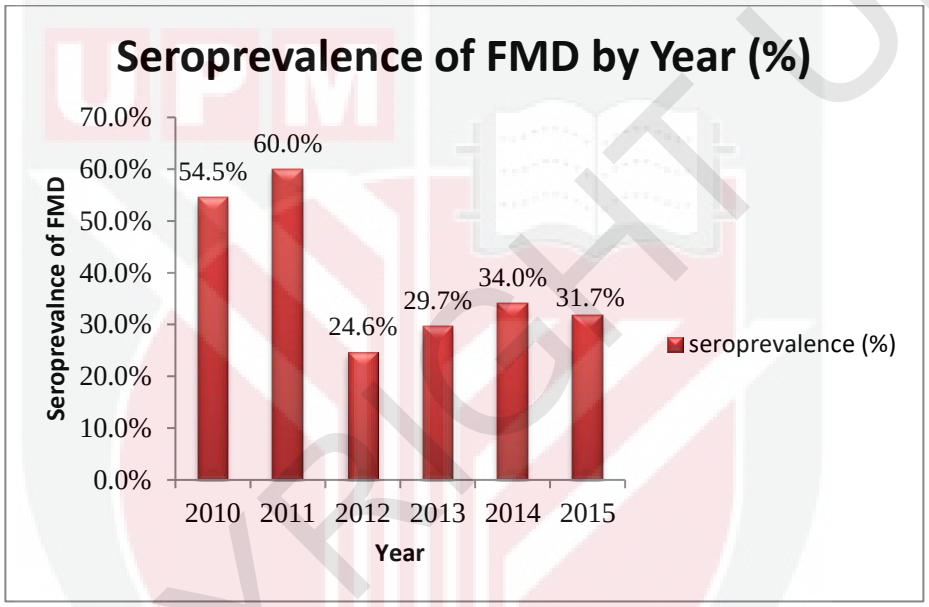


Chart 1 Seroprevalence of FMD in Padang Besar and Rantau Panjang Quarantine Stations from 2010 to 2015

From the year 2010 to 2015, the highest seroprevalence of FMD in both quarantine stations was on 2011 (60.0%) while the lowest seroprevalence was on 2012 (24.6%).

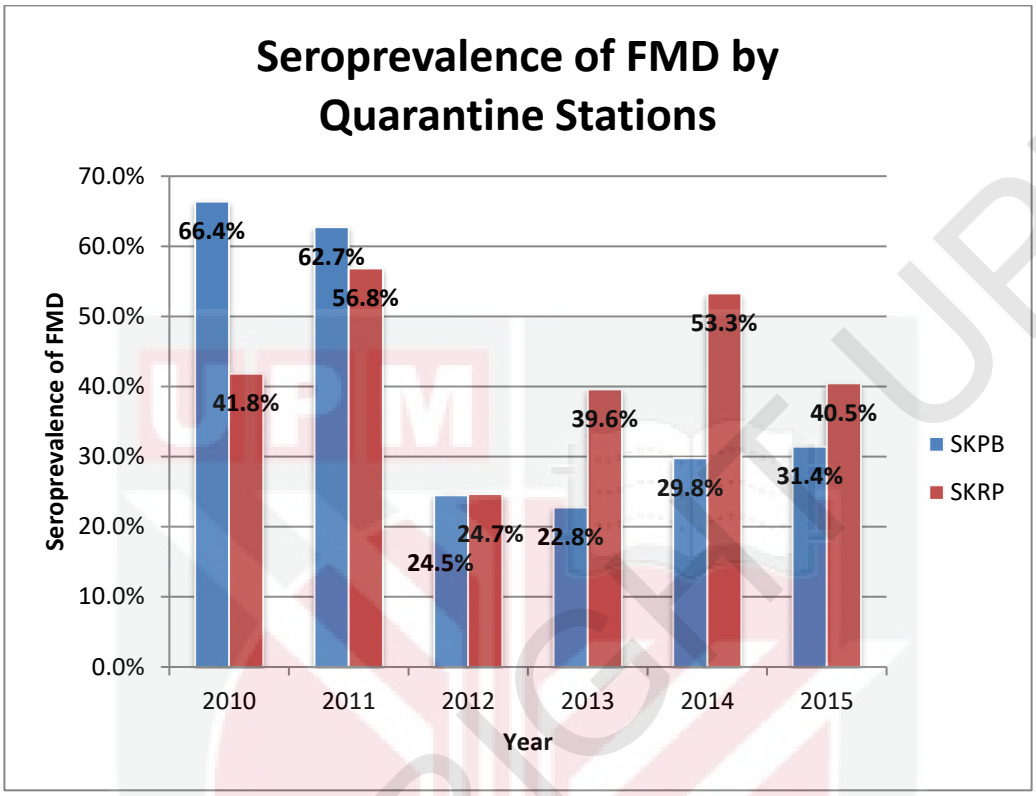


Chart 2 Seroprevalence of FMD according to Padang Besar and Rantau Panjang Animal Quarantine Stations from 2010 to 2015.

The highest seroprevalence in Padang Besar Animal Quarantine Station was on 2010 (66.37%) followed by the year 2011 (62.71%) while the lowest was on 2013 (22.76%). In Rantau Panjang Animal Quarantine Station, the highest seropositivity detected was on 2011 (56.83%) and the lowest was on the year after which was on 2012 (24.66%).

Table 2 Seroprevalence of FMD species-wise

Species	No. of animals tested	No. of animals tested positive	Seroprevalence	p-value
Buffaloes	508	156	30.7%	0.007
Cattle	15077	5513	36.6%	
Total	**15585**	**5669**	**36.4%**	
	χ^2= 7.28, df= 1, Odd Ratio= 1.3			

Of 15,077 cattle tested, 5,513 were found to be serologically positive against NSP FMD antibodies and this represented 36.6% for seroprevalence of FMD in both quarantine stations. Meanwhile, buffaloes had 30.7% seroprevalence for FMD. The seroprevalence of FMD was significantly higher ($p<0.05$) in cattle than in buffaloes. Cattle were 1.3 times more likely to be seropositive compared to buffaloes.

Table 3 Seroprevalence of FMD sex-wise

Sex	No. of animals tested	No. of animals tested positive	Seroprevalence	p-value
Male	10676	4178	39.1%	0.007
Female	4349	1320	30.4%	
Total	15025	5498	36.6%	
	χ^2=102.7, df= 1, Odd Ratio= 1.5			

Of 15,585 animals involved throughout 2010 to 2015, only 15,025 were of stated sex in the data sheet. The remaining 503 sex were not recorded. There were 10,676 males and 4,349 females. 39.1% males with seropositive towards antibodies against NSP FMD compared to females which was lower (30.4%). Statistically, it was significantly different. From this study, males wee 1.5 times more likely to be seropositive towards FMD.

Table 4 Seroprevalence of FMD according to species and sex

Species	Sex	No. of animals tested	No. of animals tested positive	Seroprevalence	p-value
Buffaloes	Male	502	150	29.9%	-
	Female	1	1	100%	
Cattle	Male	10174	4028	39.6%	0.000
	Female	4348	1319	30.3%	
	Total	**15025**	**5498**	**36.6%**	

Male cattle were found to have significantly higher ($p<0.05$) seroprevalence (39.6%) compared to female cattle (30.3%). Meanwhile, in buffaloes, the seroprevalence of males were incomparable to female buffaloes as the number of female buffalo was only 1.

4.2 Outbreak Description

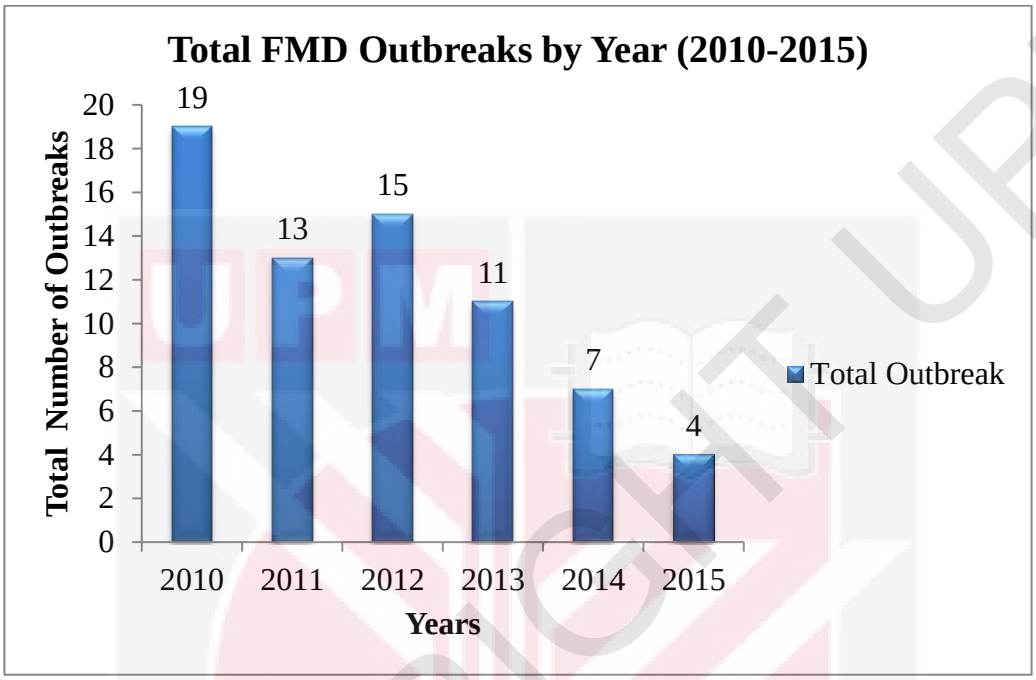


Chart 3 Total number of FMD outbreaks from 2010 to 2015 in selected states (Kedah, Kelantan, Perak, Perlis, Pulau Pinang, Terengganu) in Peninsular Malaysia.

The total number of FMD outbreak throughout the six years in those selected states in Peninsular Malaysia was 69. From that number, the FMD outbreaks mostly occurred in 2010, with 19 occurrences followed by in 2012 with 15 outbreaks. However, the FMD outbreaks reduced in number with only 4 reported cases in 2015.

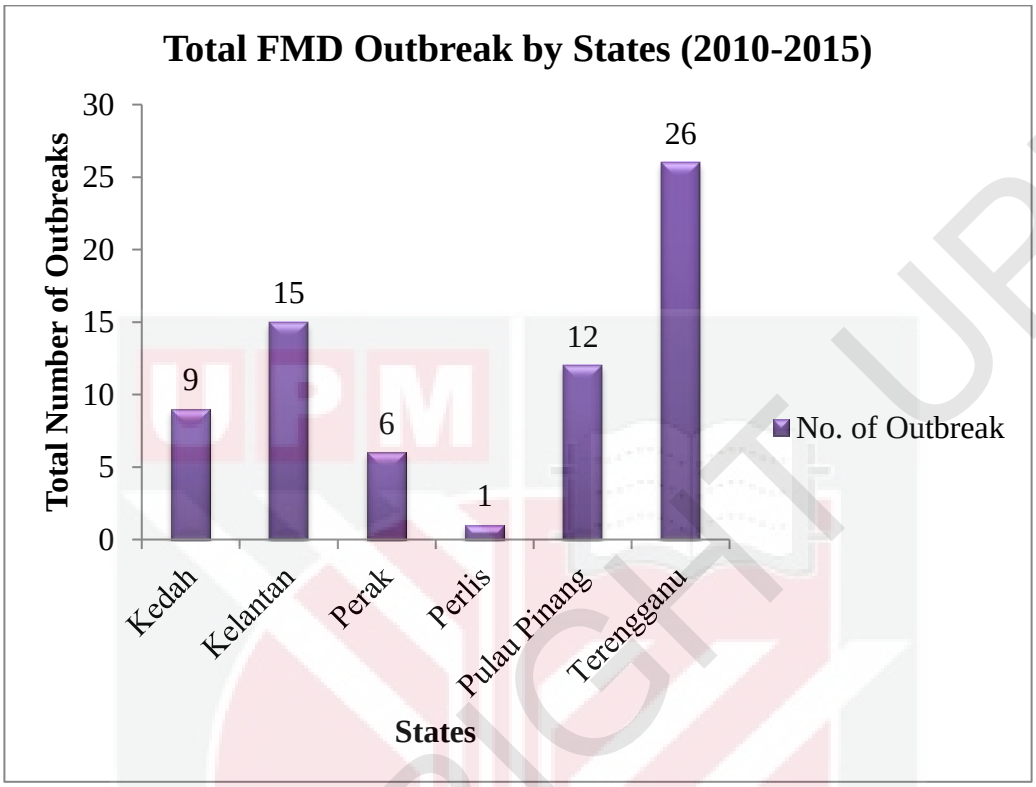


Chart 4 Total number of FMD outbreaks distribution by states from 2010 to 2015 in selected states in Peninsular Malaysia.

Out of six selected states in Peninsular Malaysia, Terengganu had the highest number of FMD outbreaks (26) followed by Kelantan (15) and the lowest was Perlis with only 1 recorded outbreak throughout 2010 to 2015.

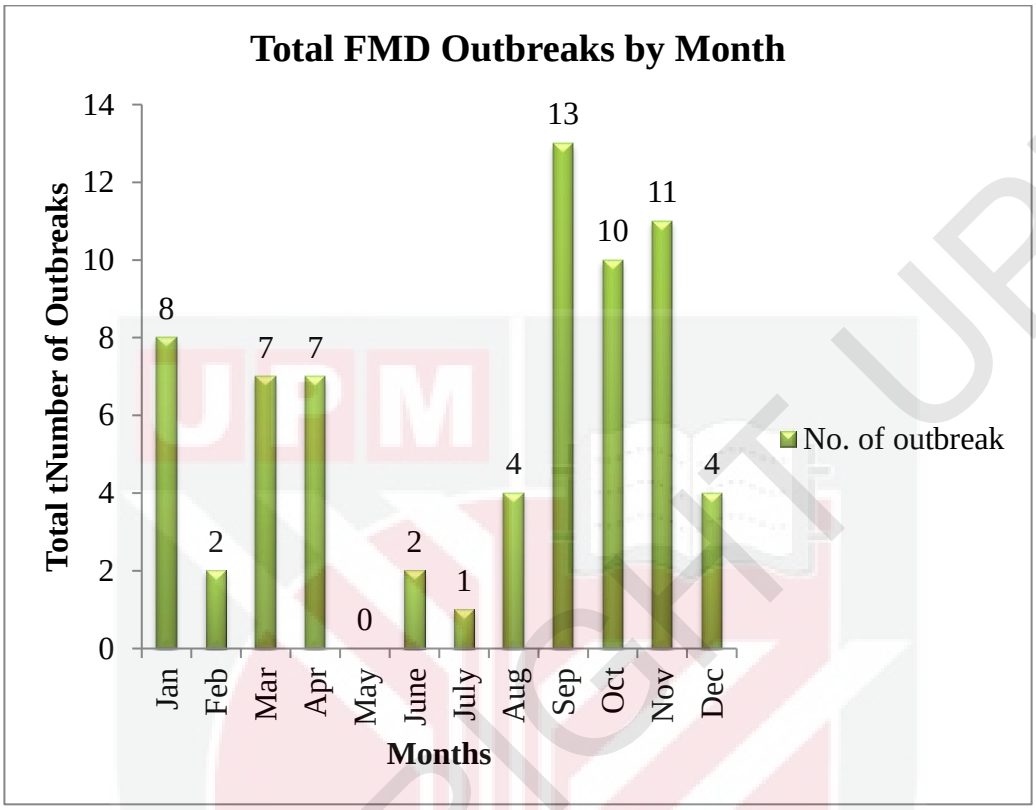


Chart 5 Monthly distributions of FMD outbreaks from 2010 to 2015 in selected states in Peninsular Malaysia.

The highest number of outbreak was in September (13) followed by November (11) and the lowest was in May with no outbreak throughout 2010 to 2015. Most of FMD outbreaks happened during the end and early of the calendar year.

Table 5 Serotype of FMD outbreaks in selected states between 2010 and 2015

Year	Kedah	Kelantan	Perak	Perlis	Pulau Pinang	Terengganu
2010	O	O	O	-	O,A	O
2011	O,A	-	-	NT*	O	O
2012	O	O	O	-	-	O
2013	-	NT*	NT*	-	-	O
2014	-	O	-	-	-	O
2015	-	-	-	-	-	O

NT*= Not Typed

The most common serotype of FMD virus causing FMD outbreak in six selected states in Peninsular Malaysia were serotype O and followed by serotype A. However, there were several cases of outbreak that were not typed. Terengganu was affected with serotype O throughout the year while serotype A affecting only Pulau Pinang and Kedah in 2010 and 2011 respectively.

5.0 DISCUSSION

The serum collected from cattle and buffaloes imported into Malaysia were tested for antibodies against the 3ABC non-structural protein (NSP) of FMD virus using Enzyme-Linked Immunosorbent Assay (ELISA) test. The test is used to detect the presence of antibodies against NSP of the FMD virus in infected susceptible animals, which is expressed during replication (Aftosa, 2015). Those animals infected with live FMD virus will produce antibodies against structural (SP) and non-structural protein (NSP) meanwhile those that are vaccinated will only produce antibodies against SP of the FMD virus (Jamal and Belsham, 2013). Therefore, in this study, the seroprevalence study was done by calculating those with positive antibodies for infections in susceptible animals in Padang Besar and Rantau Panjang Quarantine Stations from 2010 to 2015.

The overall seroprevalence for FMD in both quarantine stations in this study was 36.4%. This indicates the importance of FMD in the study area. The results from the serology test showed high FMD prevalence in cattle and buffaloes entering Malaysia which were quarantined in Rantau Panjang Animal Quarantine Station, Kelantan, compared to Padang Besar Animal Quarantine Station in Perlis.

This might be due to the proportion of animal coming through Rantau Panjang was smaller than in Padang Besar but had more positive cases coming in. This finding is contrary to a study conducted by Karuppanan and Naheed (2000), where the seroprevalence of FMD in Padang Besar Animal Quarantine Station was higher compared to Rantau Panjang Animal Quarantine Stations from 1995 to 1998.

Under current importation protocol, Malaysia does not reject those animals detected as seropositive for NSP antibodies test but relies on clinical observations and vaccination during quarantine period. Animals entering a quarantine station will be disinfected, vaccinated, and tagged. Daily examinations were done to check for any clinical signs of FMD. Cases of FMD signs will be isolated and sampled for the blood and epithelial tissues. They will be treated for the lesion and released after quarantine period to the Malaysia-Thailand-Myanmar (MTM) Zone **(**Prosedur Operasi Piawai, 2009).

The percentage of seroprevalence of FMD in both quarantine stations was the highest in the year 2011 with 60.0% and the lowest was in 2012 with 24.6%. From the report released by World Animal Health Organisation (OIE) on 2011, outbreaks were reported to be high in 2010 in the South East Asia region. Outbreaks in 2010 in South East Asia (SEA) countries can cause animals in exporting countries to have antibodies for NSP. Non-structural

protein (NSP) antibodies can be detected up to 560 days in animals after infected experimentally (Silberstein *et al*., 1997). Those antibodies can also be detected even until 1118 days or about 3 years after infected with FMD virus (Elnekave *et al.*, 2015). Therefore, this can cause higher seroprevalence detected in animal entering Malaysia on 2011.

The seroprevalence of FMD was detected to be higher in cattle compared to buffaloes. At the same time, cattle are 1.3 times more likely to be seropositive compared to buffaloes. Extensive exotic blood introduced and cross breeding among cattle can lead to high susceptibility of cattle towards FMD (Zulfiqar, 2003).

In this study, males were found to have higher seroprevalence compared to females and this was agreeable with studies conducted by Megersa *et al.*, 2009 and Awan *et al*., 2009. However, according to Nawaz *et al.* in 2014, in the study, females were found to have higher seroprevalence of FMD compared to males due to physiological stresses such as pregnancy, oestrus, and lactation.

There are several states in Peninsular Malaysia that are considered as endemic states and classified as an area in which eradication of FMD is possible (Wongsathapornchai, *et al.,* 2008).. These areas are called the MTM

Zone, which the participants from Peninsular Malaysia are the Northern States including, Kedah, Kelantan, Perak, Perlis, Pulau Pinang and Terengganu.

Throughout 2010 to 2015, there were 69 total number of FMD outbreaks in those selected states. The highest was on 2010 with 19 outbreaks and reduced gradually until 2015 with only 4 reported outbreaks. As reported by World Animal Health Organization (OIE), on 2011, outbreaks of FMD were reported to be high in 2010 in the South East Asia region, and this explained the high number of FMD outbreaks. The reduction by year was due to intensive measures taken by the government to control this disease by reducing the importations of live animals and increase beef importation in order to compensate the local demands. Other measures also include vaccination programs and improved surveillance strategy in the countries (Protokol Veterinar Malaysia, 2011).

Terengganu was found as the state with the highest number of outbreaks among 5 other states, between 2010 and 2015. Terengganu is located at the eastern part of Peninsular Malaysia and southern of Kelantan with part of its districts are closely connected to Kelantan. It is reported that outbreaks occurred in lower part of Peninsular Malaysia due to higher demands of live animals but low supply. During that time, Australia had increased the price of their imported cattle and so Malaysia had to import cattle and buffaloes from

cheaper source, such as Thailand and Myanmar. This imposed risk to import infectious livestock. Other than that, illegal movements of animal inter-state can also lead to spread of disease (Abila, 2011).

Monthly distribution of FMD outbreaks in those selected states revealed that most outbreaks occurred on September with a total of 13 reported outbreaks followed by November with 11 cases and October, 10 cases. Foot and Mouth Disease (FMD) outbreaks clustered in northern part of Malaysia during the end and early calendar year (Abila and Foreman, 2006). In Malaysia, cultural and religious festivals were celebrated between September and November of 2010 to 2015. Therefore, there would be the higher demand of live cattle and buffaloes to be imported for slaughter (Ramanoon *et al*., 2013).

The most common serotype of FMD virus causing outbreaks in those selected states were serotype O, followed by serotype A. This is similar to a study reported by Ramanoon *et al*., conducted on 2013, that FMD virus was predominantly found to be involved in FMD outbreaks in Peninsular Malaysia and the emerging one was serotype A. The high prevalence of outbreaks associated with serotype O described that it was the most aggressive serotype (Kitching, 2005). The sequence data for serotype O and A suggested that the source of FMD infection in Malaysia was via the entrance of infected animals or products (Abdul-Hamid, *et al.*, 2013).

Peninsular Malaysia is known as natural receiver of imported livestock especially from the mainland of South East Asia which is Central Myamar. Movement of animals was the main source of the 66% of the outbreaks data that occurred from 2001 to 2007 (Ramanoon et al., 2013),

6.0 CONCLUSION AND RECOMMENDATION

Seroprevalence of FMD in two quarantine stations; Padang Besar and Rantau Panjang, was high. However, serological survey against NSP did not represent the level of viral circulation within Malaysia but only reflected the level of disease in source country. Malaysia is still endemic with FMD and measures need to be taken by enhancing the control and surveillance strategy in order to eradicate this disease and obtain more benefits in livestock trade internationally.

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APPENDICES

Table 1 Seroprevalence of FMD from 2010 to 2015

Year	No. of animals tested	No. of animals tested positive	Seroprevalence
2010	2421	1319	54.5%
2011	874	524	60.0%
2012	2060	506	24.6%
2013	989	294	29.7%
2014	4204	1420	34.0%
2015	5067	1608	31.7%
Total	15615	5671	36.4%

Table 2 Seroprevalence of FMD according to quarantine station by year

Year	No. of animals tested	No. of animals tested positive	Seroprevalence
SKPB			
2010	1249	829	66.37%
2011	464	291	62.71%
2012	1042	255	24.47%
2013	580	132	22.76%
2014	3461	1038	29.99%
2015	4889	1536	31.42%
Total	**11685**	**4081**	**34.85%**
SKRP			
2010	1172	490	41.81%
2011	410	233	56.83%
2012	1018	251	24.66%
2013	409	162	39.61%
2014	713	380	53.30%
2015	178	72	40.45%
Total	**3900**	**1588**	**40.72%**

Example of the Data Management table in Microsoft Excel 2010®

Case No.	Import Date	Quarantine Station	Species	Sex	No. of Serum Sent	No. of Serum Tested	No. of Tested Positive	%

Figure 1: Quarantine Procedure (MAQIS, 2009)

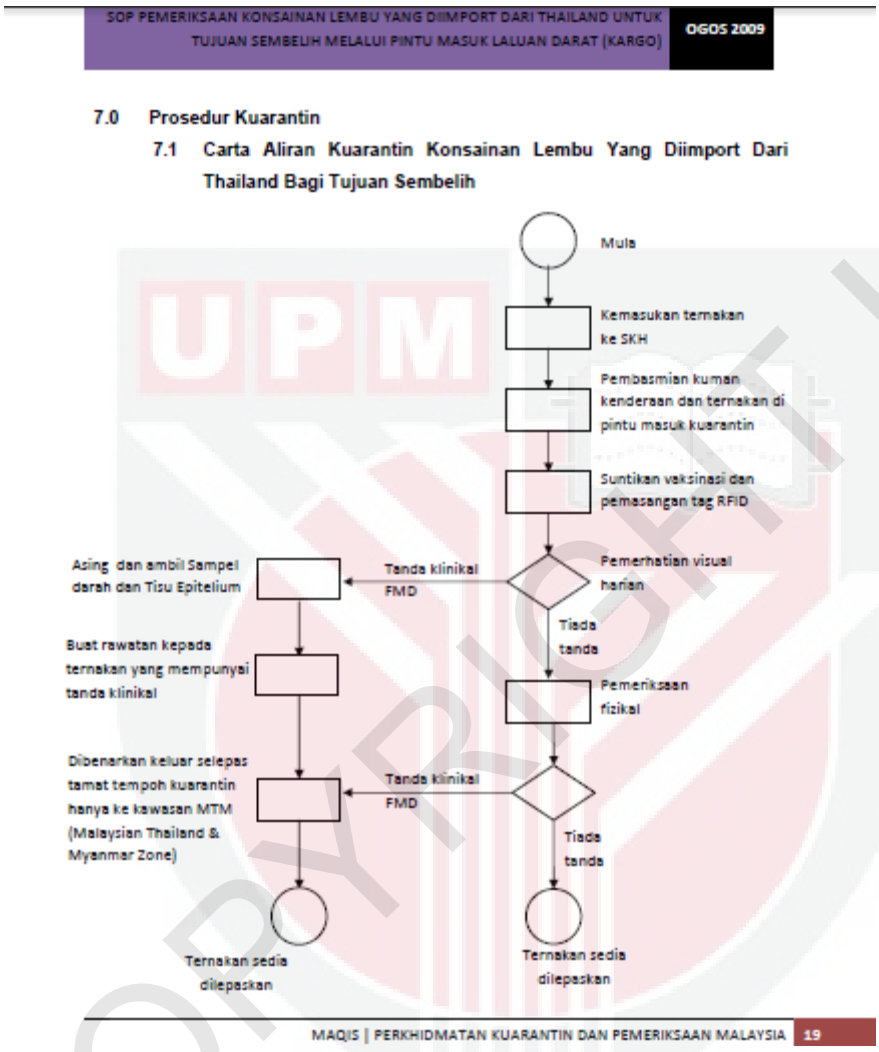

Figure 2: Sampling Procedure (MAQIS, 2009)

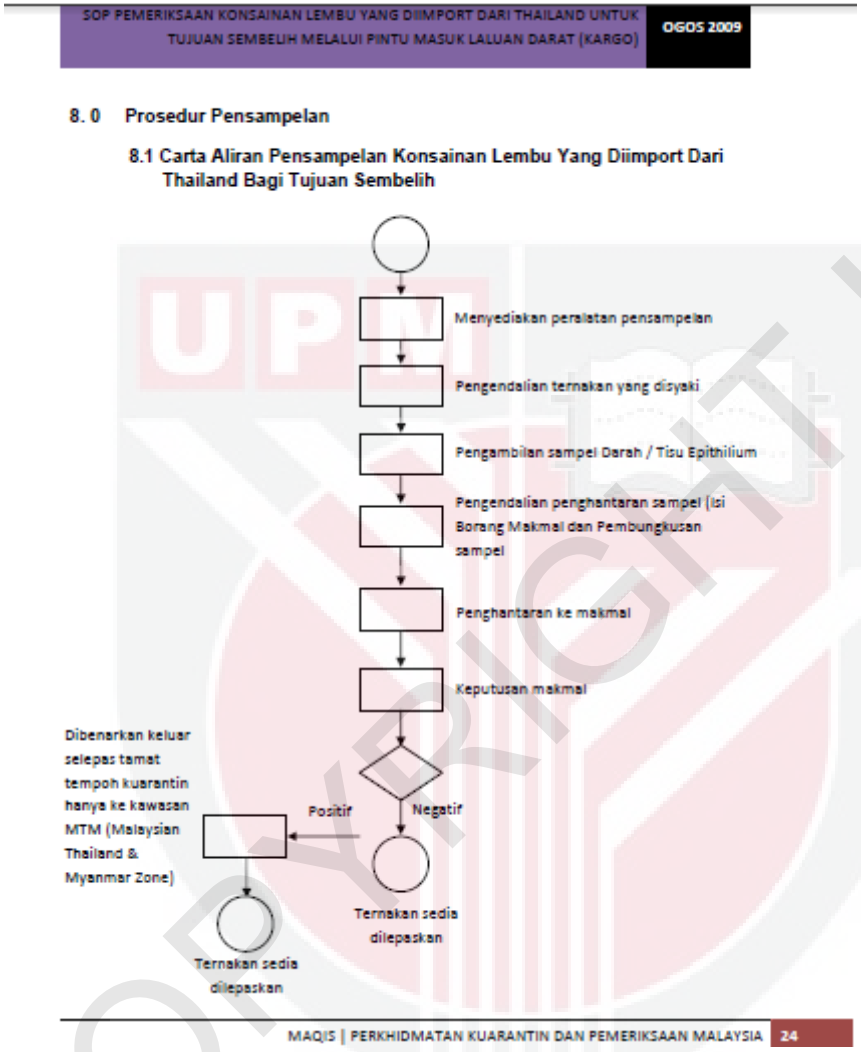

Figure 3: Quarantine Releasing Procedure (MAQIS, 2009)