

# ANTIGEN DETECTION OF FOOT-AND-MOUTH DISEASE AND SEROTYPES FROM SAMPLES SUBMITTED TO THE REGIONAL VETERINARY LABORATORY KOTA BHARU FROM 2012 TO 2016

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**ABSTRACT.** In Peninsular Malaysia, foot-and-mouth disease (FMD) has been reported since early 1860 which then became sporadic, causing outbreaks every year. Since then, Peninsular Malaysia has become endemic with FMD. The aim of this study is to provide findings of the current FMD occurrence and its serotyping in Peninsular Malaysia. An identification of Foot and Mouth Disease serotype was carried out in Peninsular Malaysia by the Regional Veterinary Laboratory Kota Bharu (RVLKB) only. Epithelial tissue samples were received from 10 states throughout Peninsular Malaysia from 2012 until 2016. Indirect sandwich ELISA was performed using ELISA kit for FMDV antigen detection supplied from the Institute for Animal Health, Pirbright Laboratory. All findings and results in this paper were based on samples received by RVLKB and does not reflect overall cases reported to State DVS or to DVS Malaysia. From the results, 2013 had the highest samples positive for FMDV (35% from 43 samples), followed by 2014 (31% from 80 samples), 2012 (24% from 122 samples), 2015 (21% from 39 samples) and the lowest is 2016 (17% from 194 samples). The FMDV serotypes detected throughout 2012 to 2016 from 110 positive samples were Serotype O (80%), followed by Serotype A (20%) and

none from Serotype Asia 1. Strict regulation, FMD vaccine evaluation by LPB ELISA and strict animal movement shall be considered to achieve FMD free for upcoming Year 2020.

*Keywords:* Foot and Mouth Disease, Serotype O, Serotype A, Serotype Asia 1, Peninsular Malaysia

## INTRODUCTION

Foot and mouth disease (FMD) is a viral disease that is caused by the *Aphthovirus* from the family of Picornaviridae. It primarily affects Artiodactyla or cloven-hoofed animals such as cattle, buffaloes, pigs, sheep, goats, deer, giraffes and antelopes (Grubman *et al.*, 2004). There are 7 serotypes of FMD which are A, O, Asia 1, Southern African Territories (SAT) 1, SAT 2 and SAT 3. The disease has been included in the OIE list of notifiable diseases. Animals infected with this virus will show signs of fever, loss of appetite, and vesicles in the mouth, on muzzles, teat as well as interdigital spaces. It is spread through direct contact and aerosols which can enter the body via inhalation, ingestion and open wound.

The initial measures of controlling FMD are early detection and a warning system as well as prevention and rapid response. For FMD-free countries, precise import and

cross border animal movement controls and surveillance are applied. In endemic areas, culling may be complemented by vaccination for susceptible livestock, so as to protect from the particular virus strain prevalent in the area. At the farm level, it is very important for the farm manager to maintain good biosecurity practices to prevent introduction or spread of the FMD virus into or out of the farm. These include controlling new animals introduced into the farm, controlling people and access of equipment, maintaining sanitation of the farms including appropriate disposal of carcasses and good drainage system, and the monitoring and reporting of the disease.

In Peninsular Malaysia, FMD has been reported since early 1860 which then became sporadic, causing outbreaks every year. Since then, Peninsular Malaysia has become endemic with FMD. A study on FMD outbreaks recorded that from 2001 to 2007, 270 outbreaks of FMD were documented. 89.95% was from serotype O and 7.7% was from serotype A (Ramanoon *et al.*, 2013). No further FMD occurrence and serotyping from 2007 afterwards have been documented. This study is to provide findings of FMD occurrences from 2012 to 2016, and its serotyping, in Peninsular Malaysia.

## MATERIALS & METHOD

Epithelial tissue samples were received from animals showing vesicular lesions and suspected to be infected with FMD virus. Indirect sandwich ELISA was performed using ELISA kit for FMDV antigen detection supplied from the Institute for Animal Health, Pirbright Laboratory. Each epithelial

tissue sample was ground to make a 10% w/v suspension and centrifuged at  $1000 \times g$  for 10 minutes to get the supernatant. A microplate was then coated with trapping antibody stock and coating buffer (1:1000), incubated at  $\pm 4^\circ\text{C}$  overnight and washed. A control antigen and the test sample were then loaded into the microplate and incubated at  $\pm 37^\circ\text{C}$  for 1 hour before washing away. Detecting antibody stock and buffer B (1:100) were loaded respectively to serotypes O, A, and Asia 1 and incubated at  $\pm 37^\circ\text{C}$  for 1 hour and washed away. Conjugate and buffer B (1:200) were loaded to all wells and incubated at  $\pm 37^\circ\text{C}$  for 45 minutes. After the plate was washed away, substrate and chromogen (1:200) were loaded to all wells and left at room temperature for 15 minutes. Stopping solution was added to all wells and the plate was placed on a photometer carriage. Vales for each of the serotypes were read and recorded. A mean corrected optical density (OD) value of  $>0.1$  above background indicates a positive result. Data on the number of samples received, samples positive with FMDV together with their serotype (A, O or Asia 1) from 2012 until 2016, were extracted and percentages were calculated.

## RESULTS

In the results, 2013 has the most samples positive for FMDV (35% from 43 samples), followed by 2014 (31% from 80 samples), 2012 (24% from 122 samples), 2015 (21% from 39 samples) and the least is from 2016 (17% from 194 samples). The most common FMDV serotype detected throughout 2012 to 2016 from 110 positive samples was Serotype O

(80%), followed by Serotype A (20%) and none from Serotype Asia 1.

## DISCUSSION

The result of this study showing serotype O as the commonest FMD serotype occurring in Peninsular Malaysia from 2012 to 2016 is similar to the findings of Ramanoon *et al.* (2013). However, there is no data that can be used to determine whether the FMD virus was introduced from other neighbouring countries or it stayed circulating inside Peninsular Malaysia from year to year. Besides, the occurrence of FMD in the country cannot be concluded just by samples received by RVLKB as the samples do not represent all outbreak cases in Peninsular Malaysia, some of which may not have been reported to DVS Malaysia. None of the samples was positive for Serotype Asia 1. There were only two reported outbreaks in Malaysia caused by Serotype Asia 1 in 1997 and 1999 (Abdul Hamid *et al.*, 2011).

FMD is endemic in Peninsular Malaysia occurring throughout the year. As there has been no marked reduction in cases and samples received, strict regulations need to be enforced to ensure Peninsular Malaysia is free from the disease in upcoming Year 2020. In case the of animal movement, all animals must be confirmed to be negative for FMD lesions and NSP antibody before being moved to another state. This is because some animals may not develop fever and may not show obvious lesions. They may be infected and excrete the virus, thereby infect susceptible animals. Animals that have recovered also can remain persistently infected as the virus might stay inside the

oropharyngeal region for a certain period of time (Sutmoller *et al.*, 2002). The African buffalo may carry a virus for over five years, whilst cattle for over three years, sheep by up to nine months, goats and wild ruminants for shorter periods of time. Thus, FMD vaccine evaluation using Liquid Phase Blocking ELISA (LPBE) could be considered before moving the animals. LPBE can be used to evaluate antibody production and animal protection against contracting FMD. Animals that show protective values >90% for all the three FMD serotype can be considered for interstate movement. Protective value <50% (negative), 50-90% (weak) while >90% is strong towards FMDV (Chenard *et al.*, 2003). The present herd of animals should also be vaccinated and the antibody towards the vaccination should be evaluated so that the population has good herd-health immunity.

Besides, strict animal movement needs to be enforced to ensure no animals were moved without permit and health certificate from State DVS. This is because movement of the animals have been proves as the main source for outbreaks (Ramanoon *et al.*, 2013). The traders doing illegal animal transportation has to be charge for a high amount of fines. Furthermore, early detection and response of FMD cases, quarantine and control measure can to be done to ensure the disease are being contained effectively.

## CONCLUSION

Despite vaccination programmes, Peninsular Malaysia is still not free from FMD. Even though 2016 showed the lowest percentage of FMD positive samples, the are not

conclusive. FMD serotype O remains the common serotype detected in Peninsular Malaysia. Strict regulation, FMD vaccine evaluation by LPBE and strict animal movement should be considered to be FMD free for upcoming Year 2020.

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