

Response to Foot and Mouth Disease (FMD) Vaccination among Local Malaysian Cattle of Various Vaccination Backgrounds from Endemic and Non-endemic FMD Areas

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ABSTRACT

A longitudinal study assessed the response to foot and mouth disease virus (FMDV) vaccination on sequential sera of local Malaysian Kedah-Kelantan cattle in two states of Peninsular Malaysia; Perlis, a foot and mouth (FMD) disease non-endemic state, and Kelantan, an endemic state for FMD. These cattle were from various vaccination backgrounds and some with unknown vaccination status. For the cattle in both states, the antibody against FMDV type O effectively increased to a strong protective level in the first week following vaccination, regardless of the vaccination background of the animals. In the endemic state, where vaccination was performed more routinely than the non-endemic state, the response had better magnitude and duration. In the non-endemic state, the antibody

response level was good but appeared to last for a shorter period of time before it significantly declined. For naïve cattle with no evidence of infection or vaccination, the response was rapid and reached a strong level immediately by the first week. However, the level was not sustained and significantly declined thereafter. All the cattle stayed healthy and clinically FMD-

ARTICLE INFO

Article history:

Received: 6 January 2014

Accepted: 23 August 2014

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free throughout the study, even when there was a transient evidence of natural field infection detected among the cattle. A marked difference was observed in the patterns of antibody response between cattle in the FMD endemic and non-endemic areas. However, the level of antibodies generally rose to a strong protective level within the stipulated 7-14 days post-vaccination. The vaccine used was effective in eliciting immune response when naturally challenged by the local field FMD virus.

Keywords: FMD, cattle, vaccination, antibody response, structural-proteins, non-structural proteins

INTRODUCTION

Foot and mouth disease (FMD) is an important trans-boundary and re-emerging infection of the ungulates that can result in devastating economic and trade losses (Forman *et al.*, 2009). FMDV serotypes O, A, and Asia 1 were reported to be endemic to seven countries in mainland Southeast Asia (Cambodia, Laos, Malaysia, Myanmar, the Philippines, Thailand and Vietnam) (Gleeson, 2002). Three countries in this region (namely, Brunei, Indonesia and Singapore) are recognized by the OIE as free of the disease without vaccination. Part of the Philippines (Mindanao, Visayas, Palawan and Masbate) and part of Malaysia (Sabah and Sarawak) are also recognized internationally as being free of FMD without vaccination (Rweyemamu *et al.*, 2008b). FMD viruses, for example, Cathay 'O' and the Pan-Asia 'O' topotypes which have been described to originate in South

China, could have possibly joined by Asia 1 topotype and evolved in 2005 (Valarcher *et al.*, 2005), crossed into South-East Asia across the border into Vietnam, and then spread westwards into Cambodia, Laos and eventually Thailand (Rweyemamu *et al.*, 2008b). According to a recent report by the World Organization for Animal Health, only 36 of the 178 OIE member countries are FMD-free without vaccination (OIE, 2011). Several countries that had been free from FMD for decades have reported FMD outbreaks in the recent years (Bouma *et al.*, 2003; Ellis-Iversen *et al.*, 2011; Park *et al.*, 2003). However, many have regained disease freedom status, with or without the use of vaccines. The control and prevention of the disease may be achieved following the outbreak via stamping out or culling of all affected animals and herds (with or without emergency vaccinations), accompanied with various animal movement controls. However, in countries where the disease is endemic and widespread, vaccination accompanied by movement controls and zoo-sanitary measures are recommended as a more economically feasible mode of preventing and controlling the disease (Geering & Lubroth, 2002; Paton *et al.*, 2009). Many FMD-endemic countries have implemented strategic vaccination in an effort to control clinical FMD and as a step in the progressive phase of achieving disease-freedom (Gleeson, 2002; Rweyemamu *et al.*, 2008a; Windsor *et al.*, 2011).

The performance of most commercial FMD vaccines has been evaluated in countries where it is manufactured. Hence,

in situations and environments that differ, the vaccine may result in disparate levels of immunity and protection and should therefore be tested in the field situations where it is applied. Peninsular Malaysia, as one Southeast Asian country where the disease is generally endemic, has faced many outbreaks of FMD and used strategic vaccination and modified stamping out methods to control the disease, particularly in the northern part of the country. However, this policy was reviewed in the 1980's (Karuppanan & Naheed, 2000) due to the extensive nature of the FMD outbreaks and was discontinued in the early 1990s (Palanisamy *et al.*, 2000) when the disease became too widespread. Currently, FMD is endemic in many parts of the Peninsula. Only three serotypes are known to occur in Malaysia: serotype O, A and Asia 1. However, in the recent years, only the two former serotypes have been reported (Abbo *et al.*, 2010). As part of the national FMD prevention and control measures, vaccinations against the aforementioned serotypes using the FMD 'killed' (inactivated) vaccine (Merial Animal Health Limited) are performed regularly, although outbreaks have continued to occur (SEAFMD, 2007). It is important to note that even though the vaccine has been used for more than a decade, its performance based on local animal and field conditions has not been formally evaluated. Therefore, this study assessed the FMD antibody response levels following vaccination performed on the local Kedah-Kelantan cattle in the endemic and non-endemic FMD

areas of Peninsular Malaysia, with special emphasis on the challenges of performing vaccination in areas where recordings were sparse or poor.

MATERIALS AND METHODS

Study Design and Epidemiological Background

A longitudinal study was conducted where local cattle from Perlis and Kelantan, two states in the northern part of Peninsular Malaysia, were sampled between 17 November 2008 and 21st July 2009. The study was conducted in collaboration with, and under the approval of the Department of Veterinary Services Malaysia and was performed as part of the FMD vaccination and surveillance programme. The use of cattle in each farm was approved by the farm managers and owners. Although Perlis was not declared as FMD-free, based on the absence of FMD clinical outbreaks in the previous four years prior to the study (SEAFMD, 2007), it was considered as non-endemic for the purpose of this study. Meanwhile, Kelantan, which annually suffers outbreaks of the disease, was considered as FMD-endemic. The annual vaccination campaign for the states such as Perlis was aimed to vaccinate cattle at least once a year while for states such as Kelantan, twice a year. However, due to the various local cattle management styles and various degrees of knowledge of previous FMD vaccination, diverse vaccination backgrounds were anticipated in this study.

In order to determine the sample size, at least 70% of cattle were assumed to have

reached a strong protective antibody level if the vaccine was administered. At the desired precision of 10% and the confidence level of 95%, the number of animals required was 81 for each endemic and non-endemic state (Thrusfield, 2013). The study population consisted of local Kedah-Kelantan cattle (1.5-2.5 years) from: (1) eight villages in Perlis that were assembled in one large-scale Government beef farm (for the purpose of the study) and one small-scale beef farm, and (2) one large-scale Government cattle breeding farm in Kelantan. The cattle from the villages in Perlis were selected based on the permission by the animal owners and were temporarily placed in the government beef farm during the study period to facilitate animal management. Farms were selected based on the willingness of the farm managers to participate and the animals within the farms were initially selected using systematic sampling. All cattle were separated from the rest of the animals in the farm and remained as a closed unit until the study was completed.

Nonetheless, the researchers could not obtain any vaccination history from the animals that originated from the villages in Perlis as these animals were raised in an extensive management system. As for the other two farms, individual animal records were not accessible at the time of the study; however, it was reported that the last FMD vaccination was performed between 10-11 months prior to the study. Vaccination using inactivated highly purified trivalent vaccine containing serotype O₁ strain Manisa and O 3039 was performed as suggested by

the vaccine manufacturer (Merial Animal Health Limited). This vaccine was the only approved vaccine in use in Malaysia for more than a decade. Blood from selected cattle was collected before the vaccine was given, after which each animal was serially sampled four times within a period of 4 months. The samples were collected from the coccygeal vein using 10 ml plain vacuum tubes at pre-vaccination day 0 (round 0; R0) and post-vaccination day 7-14 (R1), day 15-27 (R2), day 28-100 (R3) and day >100 (R4). The serum was separated and placed in Eppendorf Safe-Lock tubes on the same day of collection and stored at -20 C° until further use.

Serological Analysis

All the serological analyses were performed at the National FMD Laboratory in Kota Bharu, Kelantan. Priocheck® FMDV type O Solid-Phase Blocking ELISA (SP-ELISA) was used to detect antibodies directed against the structural proteins (SP) of FMDV type O virus (Chénard *et al.*, 2003). This was followed by the Priocheck® FMDV Non-Structural Protein ELISA (NS-ELISA) to differentiate cattle infected with FMDV from those vaccinated via the detection of antibodies to one or more NS proteins (Sørensen *et al.*, 1998). These kits are presently manufactured by Prionics AG, Switzerland, and were formerly produced by Ceditest® Diagnostics. For both the tests, the optical density and the percentage inhibition (PI) of the reference and test sera at 450 nm were measured using an ELISA reader (Chénard *et al.*, 2003). In

this study, the levels of antibodies were categorised according to the SP, based on the work of Chénard *et al.* (2003), whereby PI > 90% (strong), 50-90% (weak) and < 50% (negative), and the levels of the antibodies against the NS virus proteins based on Brocchi *et al.* 2006), whereby PI \geq 50% (positive) and <50 (negative). In addition, referring to the work of Westbury *et al.* (1988), Smitsaart *et al.* (1998) and Palanisamy *et al.* (2000), SP PI>90% was used as a guide for the protective level against FMD infection.

Data Analysis

A descriptive analysis was also performed in this work to examine the data in terms of normality, means and standard deviations. Then, Generalized Linear Model (GLM) two-way *repeated* ANOVA (Field, 2009) was used to evaluate the changes of the antibody response at different sampling times between the groups of cattle from Perlis and Kelantan. Mauchly's Test was used to test the assumptions of sphericity; if the assumption was not met, the Greenhouse-Geisser correction was then used for the analysis (Field, 2009). Meanwhile, pairwise comparison between the sampling times was analyzed for each subgroup using Bonferroni test. All the analyses were performed at a significance level of $\alpha = 0.05$ using SPSS ver. 19 (SPSS Inc, Chicago IL). Graphs were plotted using Microsoft Excel 2010 (Microsoft Office® 2010).

RESULTS

A total of 176 serum samples (88 each from Perlis and Kelantan) for each sampling time were analysed. Overall, at the pre-vaccination stage (R0), 92.6% (163) of the cattle from Perlis and Kelantan had some evidence of previous FMD vaccinations or virus exposure (PI > 50%) with 84.6% (149) cattle exhibiting a strong level of antibodies to SP (PI > 90%). Details on the antibody levels against SP for each sampling within groups of cattle are tabulated in Table 1. Cattle in Perlis responded rapidly to the vaccination, whereby an immediate increase in the proportion of cattle was detected with strong level of antibodies to SP (PI > 90%) was observed in the first week (R1), following vaccination (from 69% to 91%). However, the proportion waned in the subsequent weeks and by R4 only about 59.5% of animals had PI of >90%. The mean PI values increased from 85% to 96% by the 2nd week (R2) post-vaccination but then reduced to <90% by R3 and R4. All the cattle in Kelantan came into the study with the presence of a strong baseline level of antibodies to the SP. Vaccination increased the level of antibodies by R2, which then slightly declined by day 100 (R4). However, the mean PI remained very high (>95%) throughout the study period (Table 1).

The repeated measures ANOVA using the Greenhouse-Geisser correction determined that the PI values differed significantly between the sampling times ($F_{(3,1, 534.0)} = 12.3, P=0.001$) and between the endemic and non-endemic states ($F_{(1,$

$_{174}) = 122.7, P=0.001$). The Bonferroni pairwise comparisons revealed that vaccination response varied significantly between sampling times only for Perlis (non-endemic FMD state), whereby the antibody increased significantly from R0 (84.8 ± 1.7) to R1 ($95.8 \pm 0.8, P=0.001$) and R2 ($94.7 \pm 0.6; P=0.001$), followed by a significant decline from the peak response by R3 ($89.5 \pm 1.0; P=0.015$) and beyond ($87.5 \pm 1.1; P<0.001$). The cattle in Kelantan maintained higher antibody levels compared to cattle in Perlis and had a more gradual non-significant ($P>0.05$) incline from R0 (98.3 ± 1.67) to R1 (99.1 ± 0.78) and R2 (99.6 ± 0.61) and a slight non-significant ($P>0.05$) decline in R3 (98.5 ± 1.05) and R4 (97.8 ± 1.05) from the peak response (Fig.1).

The data were further stratified based on the background antibody levels to the SP detected at the start of the study and found 13 (7.4%) cattle (all from Perlis) were naïve for vaccination and natural infection, 14 (7.9%) had weak and 149 (84.6%) had strong baseline levels of antibodies. Fig.2 depicts the pattern of the responses based on the categories of cattle. The vaccination response significantly varied given sampling times and the initial background levels of antibodies against the SP ($F_{(5.6, 484.7)} = 59.6, P=0.001$). The 13 naïve cattle had a PI of 39% in R0 (39.0 ± 1.1), which then increased significantly and rapidly ($95.3 \pm 1.9; P=0.001$) by R1, then gradually waned by R2 ($94.4 \pm 1.7; P=0.1$) and significantly diminished from their peak by R3 ($84.9 \pm 2.9; P=0.04$) and R4 ($83.7 \pm 2.9; 0.03$). Among the cattle with weak baseline antibodies to the SP (71.2 ± 1.1),

the antibody level significantly increased in R1 ($88.9 \pm 1.9; P=0.01$) and R2 ($93.4 \pm 1.6; P=0.001$), before it insignificantly ($P>0.05$) declined in R3 (92.4 ± 2.8) and R4 (86.1 ± 2.8). Among the cattle with strong baseline antibodies to the SP (PI > 90%), vaccination insignificantly ($P>0.05$) increased the response from R0 (98.0 ± 0.3) to R1 (98.4 ± 0.5) and thereafter, insignificantly ($P>0.05$) declined in R2 (97.8 ± 0.5), R3 (94.9 ± 0.8) and R4 (94.1 ± 0.8).

Twenty-eight cattle came into the study with positive antibodies to NS proteins, indicating a prior natural FMDV field infection or exposure. However, none manifested clinical signs as they also possessed high levels of vaccination antibodies.

Sequential sampling at various points within the course of the study detected antibodies against the NS virus proteins in a proportion of cattle in both Perlis and Kelantan indicating exposure or infection to the field FMDV (Table 2). At every sampling, all the samples that were NS-positive were also SP-positive. The proportion of the infected cattle was consistently higher in Kelantan compared to Perlis. This coincides with the endemicity of FMD in Kelantan, where the animals are more likely to be exposed to field viruses. Nevertheless for most cases, antibodies against the NS proteins cleared up by the next sampling and none of the cattle manifested clinical FMD, which strongly indicated that the vaccine was efficacious in preventing the development of clinical disease following a natural field viral exposure.

TABLE 1
Percent inhibition (PI) of FMD Solid Phase Blocking ELISA (strong positive, weak positive, negative, mean PI and SD) on serum samples from cattle in Perlis and Kelantan

Sampling Rounds (days)	Perlis				Kelantan			
	Strong positive	Weak positive	Negative	Mean PI±SD	Strong positive	Weak positive	Negative	Mean PI±SD
R0(0)	61 (69.3)	14 (15.9)	13 (14.8)	84.76±22.01	88(100)	0	0	98.30±2.16
R1(7-14)	80 (90.9)	8(9.1)	0	95.87±10.26	88(100)	0	0	99.10±1.76
R2(15-27)	68 (78.2)	19 (21.8)	0	94.74±8.03	88(100)	0	0	99.66±1.37
R3(28-100)	64 (72.7)	24 (27.3)	0	89.48±13.87	88(100)	0	0	98.51±1.37
R4(>100)	50 (59.5)	34 (40.5)	0	87.50±13.75	88(100)	0	0	97.83±2.45

Strong +ve SPB-ELISA PI > 90%; Weak +ve SPB-ELISA PI 50-90; -ve SPB-ELISA PI 0-49

TABLE 2
Percent inhibition (PI) of FMD Non-Structural (NS) Proteins ELISA of serum samples from cattle in Perlis and Kelantan

Sampling Rounds (days)	Perlis			Kelantan		
	No Positive (%)	No Negative (%)	No Positive (%)	No Positive (%)	No Negative (%)	No Negative (%)
R0 (0)	6 (6.9)	81 (93.10)	22 (25.0)	66 (75.0)		
R1 (7-14)	9 (10.2)	79 (89.8)	39 (45.3)	47 (54.4)		
R2 (15-27)	6 (6.8)	82 (93.2)	37 (42.0)	51 (58.0)		
R3 (28-100)	18 (20.5)	70 (79.5)	13 (14.9)	74 (85.1)		
R4 (>100)	10 (11.4)	78 (89.6)	24 (27.6)	63 (72.4)		

Positive PI ≥ 50, Negative PI < 50

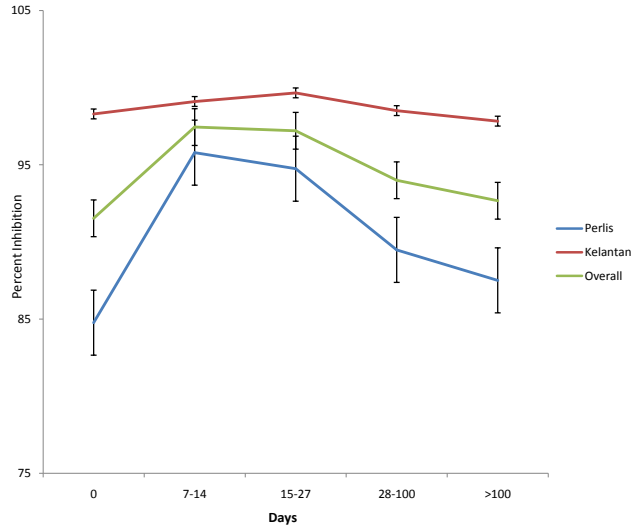


Fig.1: Percent inhibition (PI) of structural proteins SP-ELISA type O FMD throughout the study period for cattle in Kelantan (endemic), cattle in Perlis (non-endemic) and cattle from both states. Vertical lines indicate the 95% confidence interval

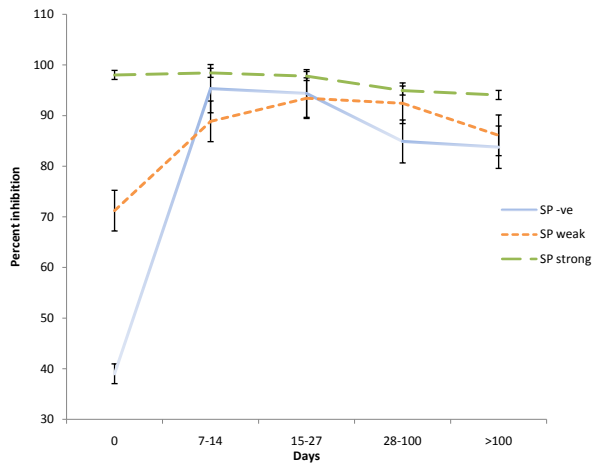


Fig.2: The antibody response patterns following FMD vaccination for groups of cattle with existing strong antibodies to structural proteins (SP), SP strong (PI >90%); weak antibodies, SP weak (PI=50-90%) and negative antibodies; SP -ve (PI<50) at the start of study. Vertical lines indicate the 95% confidence interval.

DISCUSSION

In this study, the ELISA kit that detected the SP of FMDV type O was used to assess the level of protection against the most common serotype affecting local animal populations. The baseline antibodies against these proteins indicated that 149 (84.7%) sera were strongly positive even before the vaccine was administered and only 13 (7.4%) were negative. The antibodies were generally due to previous vaccination as only 28 (15.9%) were also positive for the antibodies to NS virus proteins. We found this interesting because the last reported vaccination for each farm (excluding the cattle from villages in Perlis) was performed between 10 to 11 months before the study began. The finding suggests that the vaccine is efficient and effective in conferring the animals' immunity against FMD infection, which is consistent with Doel's review that suggests repeated vaccine will elicit prolonged protective immunity even without annual booster doses (Doel, 2003). However, since recording is rather poor for individual animals involved in this study, we refrained from drawing any conclusion about the duration of immunity conferred from previous vaccinations. Our study found that a single dose of FMD vaccine was generally adequate to protect the animals against FMDV type O ideally until the administration of the second annual vaccine dose in the FMD-endemic areas. This was apparent as the majority of vaccinated animals had antibodies against the vaccination proteins with PI>90%, even

4 months post-vaccination in Kelantan. Maintenance of strong antibody response is vital to ensure that animals remain protected from clinical disease especially in areas where FMD is highly endemic. At the end of the sampling period, the antibody levels among the cattle in Kelantan remained strong (>90% PI), while the level significantly dropped for the cattle in Perlis. The observation in Kelantan is consistent with the report of routine biannual FMD vaccinations, which may have contributed to the lasting antibody response (Doel, 1999, 2003).

When the data were stratified based on the pre-existing levels of vaccination antibodies detected at the start of the study (R0), a significant difference was found in the response pattern between the groups. The pattern of response from the 13 naïve cattle (see Fig.1) is similar to those suggested by Doel (1999, 2003), where the response reached a strong protective level within less than 7 days and maintained at that level for about 4-5 weeks before declining. This response is considered adequate for a primary dose in naïve populations after which a booster dose (at 4-5 weeks following the primary dose) was suggested to be administered to stimulate more sustainable antibody production (Doel, 1999) to protect susceptible animals in highly-endemic FMD areas. All the 13 cattle remained free from natural FMD infection (NS proteins -ve) until the end of the study. The group of cattle with the initial weak antibodies to SP responded to the vaccination similarly by a rapid antibody increase and then a gradual

decline towards the end of the study. These findings support that the vaccine confers immediate immunity against clinical FMD within less than 1 week post-vaccination (McCullough *et al.*, 1992). It also indicated that the application of the vaccine would effectively prevent disease in emergency situations, especially among herds that have not been vaccinated regularly or have never been vaccinated. In addition, it emphasized the need for a booster dose in naïve populations so as to ensure that the herd remained protected from clinical FMD because the level of immunity would decline rapidly rendering the animal susceptible to a re-infection (McCullough *et al.*, 1992).

Evidence of field exposure to the FMD virus was detected transiently throughout the study. According to Sørensen *et al.* (1998), the detection of antibodies to the FMD virus NS-proteins is the most reliable index of infection in vaccinated animals. In this study, a higher proportion of NS protein-positive was detected in the cattle in Kelantan and the proportion appeared to fluctuate over the study period, which is consistent with the endemic status of FMD in Kelantan. In Perlis, although the disease had not been reported in the previous 4 years prior to this study, the evidence of natural infection was detected, albeit at a lower proportion than that of Kelantan. This is consistent with the fact that Perlis borders Thailand, which is highly endemic for FMD. None of the animals succumbed to the infection and for the majority of animals, the antibodies were not sustained for more than one sampling interval, indicating that the vaccine was

effective and efficacious in protecting the animals against natural virus challenge even when the antibody response diminished to $PI < 90\%$. Most FMD vaccines available do not confer sterile immunity, which prevents infection and carrier status (Barnett *et al.*, 2004) and even with protective immunity some levels of viral replication occur in vaccinated cattle upon exposure to field viruses (Barnett & Carabin, 2002; Golde *et al.*, 2005). In addition, cattle protected by vaccination can become transient FMD virus carriers or even become persistently infected without ever showing any clinical symptoms (Alexandersen *et al.*, 2003; Barnett & Carabin, 2002; Doel *et al.*, 1994). In this study, if the subclinical cattle were to be moved to a naïve herd, they could potentially become the source of a new FMD outbreak.

CONCLUSION

In this study, the sample of the local cattle from two large cattle farms in Perlis and Kelantan and several villages in Perlis may not be representative of the cattle population in Malaysia. However, our study indicated that the FMD vaccine used was effective in conferring immunity towards the virus and efficacious in preventing clinical FMD even when naturally challenged by field FMDV. Nonetheless, the magnitude and sustainability of the immunity elicited were significantly affected by the background levels of vaccination. Therefore, record keeping is pertinent to determining the precise vaccination status of individual animals within a herd so that vaccination

can be tailored to suit the population where it is being administered. The success of any vaccination programmes not only depends on use of effective vaccines but also on the vaccination coverage rates whereby at least 80% (Barteling *et al.*, 2004) of the cattle within a population must be vaccinated to ensure herd-level immunity. The coverage rates of vaccination in Peninsular Malaysia over the past decade have not achieved the suggested target rate (Abbo, 2010). Furthermore, the potency of a vaccine also depends on other factors such as vaccine cold-chain management, storage conditions of the vaccine prior to use, vaccine administration route, the level of skill and training of the vaccinators in terms of dose, rate and technique, vaccine preparation prior to use, animal species to be vaccinated and their health status, usage of expired vaccines and animal vaccinating related-problems (Merial, 2008). Moreover, adhering to handling and storage recommendations can be challenging in many tropical countries; non-adherence typically results in the compromising of the vaccine's quality and potency (ASEAN, 2009).

The findings of the current study have provided more knowledge and better understanding of the response of cattle towards the FMD vaccine in a tropical field situation where animal vaccination and infection status or backgrounds may be uncertain. These findings may be used to improve the way vaccines are administered and in making decisions about vaccination strategies.

ACKNOWLEDGEMENTS

The authors wish to thank the Department of Veterinary Services officers, Dr. Mohammed Naheed from DVS Headquarters in Putrajaya, all staff from DVS Perlis, Pusat Ternakan Haiwan Pantai Timur and National Foot and Mouth Disease Laboratory in Kelantan, as well as farm managers and their staff of the livestock centres for their contributions to this project. The authors also thank Dr. Steven E. Krauss of Universiti Putra Malaysia for reading, and Dr. M. A. Sadiq for formatting the manuscript. This project was supported by the Ministry of Agriculture Malaysia via Sciencefund Grant No 05-01-04-SF1013 and was published in collaboration with DVS, Malaysia.

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