

## Efficacy of Expired Foot-and-mouth Disease O Type Vaccines in Cattle and Buffalo in Lao People's Democratic Republic

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### Abstract

Lao People's Democratic Republic (Lao PDR) submitted a request to Japan for 200,000 doses of expired foot-and-mouth disease (FMD) O type vaccines that were in storage for emergency use. Approximately 100,000 animals, consisting of both cattle and Asian water buffalo (*Bubalus bubalis*), received the same vaccine twice within one month in Xieng Khouang province in the north-east area of Lao PDR. Concurrently, the efficacy of three-month expired FMD O type vaccine (6PD<sub>50</sub> O Manisa) was assessed in serum samples of 90 cattle and 31 buffalo from the field using a Liquid Phase Blocking-ELISA (LPBE) assay. Of these samples, 75 cattle (83.3%) and 24 buffalo (77.4%) were seropositive against the FMD virus (FMDV) O type before vaccination. Testing for non-structural protein (NSP) using the PrioCHECK FMD NS kit showed that many of the animals with high titers in the screening test before vaccination were FMDV-infected animals. Fifteen cattle and seven buffalo with titers 1:32 or under before vaccination exhibited high titers of antibody (1:45-1:1448) one month after the first vaccination and further increased titers (1:362-1:5792) one month after the second vaccination. Nearly all of the cattle (97.6%) had high titers to control FMD 14 months after the second vaccination. To date, no outbreak of FMD has been reported at the study site. Three-month expired FMD O type vaccines induced appropriate immune responses against FMD in both cattle and buffalo.

**Discipline:** Animal health

**Additional key words:** Foot-and-Mouth disease virus, Liquid-Phase Blocking ELISA, serology

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## Introduction

Foot-and-mouth disease (FMD) is a highly contagious and economically devastating transboundary viral disease that affects cloven-hoofed animals such as cattle, pigs, deer, goats, sheep and buffalo (World Organisation for Animal Health 2012). FMD is caused by the foot-and-mouth disease virus (FMDV) of the genus *Aphthovirus*, family *Picornaviridae*, and has seven distinct serotypes (World Organisation for Animal Health 2012). The FMD serotypes O, A, and Asia 1 are currently prevalent in Southeast Asia (Knowles et al. 2012), although no Asia 1 outbreak has been reported since 2006 (FAO 2014). Sequence data of viral protein 1 (VP1) nucleotide have been used for phylogenetic analysis, characterizing different FMDV lineages in Southeast Asia and tracking transboundary movements of the virus (Knowles 2012). These data showed that recent FMD outbreaks in East Asia, including Japan in 2010, originated from mainland Southeast Asia (Knowles et al. 2012). These data also emphasize that FMD control in mainland Southeast Asia, where regular outbreaks of FMD are reported, is necessary to reduce the risk of FMD excursion into other parts of the world like East Asia. In mainland Southeast Asia, including the Lao People's Democratic Republic (Lao PDR), FMD is endemic and causes severe economic losses in smallholder farms and village households. Although vaccination is a key control strategy in managing FMD outbreaks (Nampanya et al. 2013), it is not widely employed in mainland Southeast Asia due to the cost involved (Gleeson 2002). To address this financial problem, projects focusing on vaccine donation have been implemented in an effort to control the spread of FMD in mainland Southeast Asia, by international organizations such as the World Organisation for Animal Health (OIE) (Khounsy et al. 2008). In 2012, Lao PDR made a request under this system to the Government of Japan for 200,000 doses of expired FMD O type vaccines, which were being stored by Japan for emergency use. Following the presentation of a feasibility proposal to the OIE/Japan Trust Fund (OIE/JTF) as part of the project for FMD vaccination in Lao PDR, the donation of vaccines to Lao PDR was approved by Japan's Ministry of Agriculture, Forestry and Fisheries (MAFF). Approximately 100,000 animals, consisting of cattle and buffalo (Asian water buffalo: *Bubalus bubalis*), were vaccinated twice within a one-month period in Xieng Khouang (XK) province in the northeast area of Lao PDR between October and November 2012. Although a serological study on FMD in Lao PDR was previously reported (Nampanya et al. 2013), few reports have been published on pre- and post-vaccination serology for FMD vaccination in Southeast Asia. Here, we describe the efficacy of expired FMD O type vaccines for cattle and buffalo in Lao PDR using data from pre- and post-vaccination serology.

## Materials and methods

### 1. Selection of study sites

Two villages (Khungvieng and Naguen) located within the Kham district of XK province in Lao PDR were selected, as both had no history of FMD outbreaks, no vaccination as per official reports in Lao PDR, a large ruminant population exceeding 100 animals to allow for possible losses caused by trade or deaths during the study period, households and village officials willing to participate, and convenient road access. In addition, the staff in this province has experience with FMD vaccination due to major FMD outbreaks in the Pek district (Rast et al. 2010).

### 2. Vaccine

The donated FMD vaccine had been stored for emergency use in Japan and was an inactivated six 50% protection dose (6 PD<sub>50</sub>) O Manisa FMD vaccine purchased from foreign vaccine makers. The r<sub>1</sub> value between the vaccine strain and the O/JPN/2010 strain, SEA topotype (Mya-98 lineage) was greater than 0.3 in a preliminary serological examination by Japan's National Institute of Animal Health (NIAH). The vaccines were shipped to Lao PDR from Japan immediately after the storage periods ended. They were kept under suitable conditions and transferred to the vaccination sites with good cold chain until use. Because vaccines should generally be used before their expiration date, it is desirable that expired vaccines should also be used as soon as possible. About three months after their expiration date, vaccines were used to vaccinate cattle and buffalo at the study sites in XK province after being transported from Japan in the shortest period.

### 3. Blood sampling and vaccination

After cattle and buffalo were identified by ear tags, the first batch of blood samples was collected before the first vaccination, on the 1st and 2nd of October 2012. The second batch of blood samples was obtained from these identified animals about one month after the first vaccination, on the 6th and 7th of November 2012. Finally, the third batch of blood samples was collected from these cattle and buffalo about one month after the second vaccination, on the 5th and 6th of December 2012. A total of 189 pre-vaccination samples were collected from 139 cattle and 50 buffalo. One month after the first vaccination, 144 samples were collected from 106 cattle and 38 buffalo from the previously identified 139 cattle and 50 buffalo. In total, 121 samples from 90 cattle and 31 buffalo were successfully collected in all three sampling times, and were used in this study. Sera obtained from all blood samples were maintained in the National Animal Disease Diagnostic Laboratory (NADDL) in Vientiane, Lao PDR, until testing. All vaccinated animals were clinically observed throughout the study period for

potential side effects or outbreaks.

#### 4. Antibody detection assay

The antibody assay against FMD O type was conducted using Liquid-Phase Blocking ELISA (LPBE) (Biological Diagnostic Supplies Limited, Ayrshire, Scotland, UK) (Fukai et al. 2013). Serum samples of 90 cattle and 31 buffalo were examined to screen for antibodies against FMD O type by LPBE as per the manufacturer's instructions and the OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals 2012 (World Organisation for Animal Health 2012). The FMDV O Manisa strain was used as the antigen. For the titration test, serum samples from animals with titers 1:32 or under on screening by the first of three serum samples were selected from among the 90 cattle and 31 buffalo and tested using LPBE. Serum samples from animals with titers exceeding 1:32 on screening with the LPBE kit were examined using a PrioCHECK FMD NS kit (Prionics AG, Schlieren-Zurich, Switzerland) (Sørensen et al. 2005) for conducting the foot-and-mouth disease differentiation of infected and vaccinated animals (FMD DIVA) test.

#### 5. Residual FMD vaccine antibodies in cattle

To determine the presence of residual antibodies in cattle 14 months after the second vaccination, blood samples were collected from 41 identified cattle in Naguen village on the 22nd and 23rd of January 2014, and obtained sera were tested using LPBE.

### Results

Based on the screening of serum samples of 90 cattle and 31 buffalo, 75 cattle (83.3%) and 24 buffalo (77.4%) were sero-positive (antibody titers >1:32) against FMDV O type before vaccination. Fifteen cattle and seven buffalo with negative titers before the vaccination exhibited high titers of antibody (1:45-1:1448) one month after the first vaccination, and significantly increased titers (1:362-1:5792) one month after the second vaccination (Table 1). Based on screening tests conducted using samples from the third blood sampling one month after the second vaccination, all wells were clear compared to the results at before vaccination and one month after the first vaccination (Fig 1). After two vaccinations, the immunity of the 90 cattle and 31 buffalo was considered to be significantly greater than that before vaccination based on the visualized results of screening tests.

Seventy-five cattle and 24 buffalo with titers no less than 1:45 before vaccination by the screening test of LPBE were examined using the PrioCHECK FMD NS kit for the FMD DIVA test. Positive results were obtained for 54 cattle (72.0%) and 22 buffalo (91.7%), which were subsequently classified as FMD-infected animals.

For the samples of 41 cattle collected 14 months after the second vaccination, 39 exhibited high titers (>1:256) on LPBE. Of the remaining two cattle, one also exhibited a positive titer (1:128) while the other had a low titer (1:32). Nearly all cattle (97.6%) had sufficiently high titers to control FMD 14 months after the second vaccination.

No side effects, including abortion, were reported in response to this vaccination. Most importantly, no FMD outbreak was reported at either study site after vaccination.

### Discussion

In this study, the efficacy of expired FMD O type vaccines (6PD<sub>50</sub> O Manisa) was examined using the serum samples of 90 cattle and 31 buffalo obtained from the field in Lao PDR, and then tested using LPBE. The test results showed that, three months after expiration, FMD O type vaccines still effectively induced an antibody level sufficient to ward off FMD in both cattle and buffalo. Antibody titers then remained sufficiently high in these cattle for at least 14 months after vaccination. The duration of immunity endowed by the 6PD<sub>50</sub> O Manisa vaccine appears to be longer than that of ordinary FMD vaccines with lower potency.

Approximately 80% of animals were already sero-positive against FMDV O type before vaccination. We first considered these high LPBE titers to be due to unofficial vaccinations, as there were no official reports of any FMD outbreaks or vaccination regimens at the selected study sites. However, FMD DIVA tests using a PrioCHECK FMD NS kit showed that a high proportion of the examined animals with high titers before vaccination were FMD-infected animals that were merely asymptomatic. This study demonstrated that the FMD DIVA test differentiated well between FMD-infected and vaccinated animals using the serum samples from both cattle and buffalo in the field. Despite a lack of official reports of FMD outbreaks at the selected study sites, FMD is considered to be widely endemic in Lao PDR (Blacksell et al. 2008, Khounsy et al. 2008, Khounsy et al. 2009, Rast et al. 2010). Major outbreaks and vaccinations were reported in the Pek district of XK province in 2009 (Nampanya et al. 2013, Rast et al. 2010). Given that the selected study site is close to the Pek district and movements of cattle and buffalo are not controlled in this area (Nampanya et al. 2013, Rast et al. 2010), animals with high titers on LPBE titers might have been introduced from villages involved in the recent FMD O type outbreaks. It was reported that FMD outbreaks in Lao PDR have been introduced from the eastern border and had rapidly spread west, along the principal trading routes for pigs, cattle and buffalo (Perry et al. 2002). Given that our selected study sites were located on a trading route near the border with Vietnam to the west, the antibodies detected using LPBE in this study might be residual antibodies in animals involved

**Table 1. Titration test of antibody titers of cattle and buffalo with negative titers by LPBE**

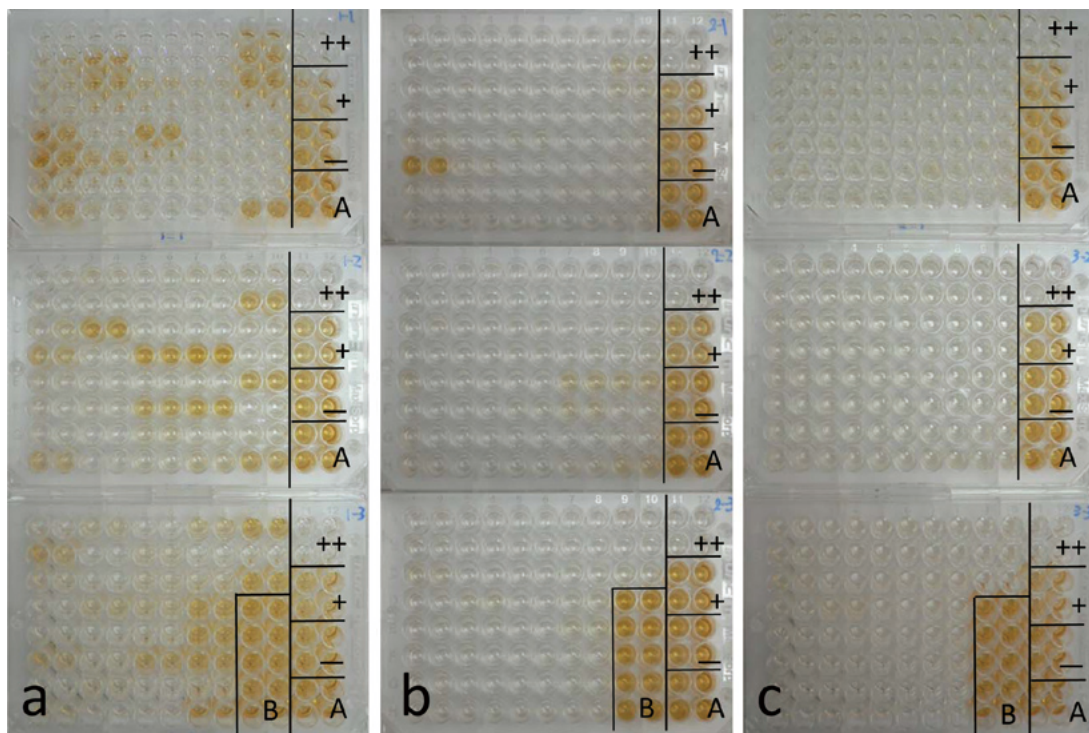
Number of animals	Animal	Sampling time <sup>1</sup>		
		<i>Pre-v.</i>	<i>1 month post-v.</i>	<i>2 months post-v.</i>
6	Cattle	<32	90	2896
12	Cattle	<32	362	724
16	Cattle	32	181	1448
33	Cattle	<32	181	724
52	Cattle	<32	45	724
53	Cattle	<32	90	1448
75	Cattle	<32	181	5792<
93	Cattle	<32	512	5792<
172	Cattle	<32	724	2896
178	Cattle	<32	724	2896
180	Cattle	<32	362	4096
182	Cattle	<32	724	2048
184	Cattle	<32	362	2896
186	Cattle	<32	181	1448
189	Cattle	<32	181	1448
116	Buffalo	<32	724	2048
118	Buffalo	<32	362	1448
124	Buffalo	<32	1448	2896
126	Buffalo	<32	64	362
131	Buffalo	<32	1448	2048
134	Buffalo	<32	128	724
137	Buffalo	32	90	362

<sup>1</sup>Pre-v., pre-vaccination; Post-v., post vaccination

in transboundary movement. Alternatively, unrecognized or unreported outbreaks of FMD O type may have left residual antibodies in animals that survived the outbreak. In any case, the FMD DIVA test results in this study emphasize the fact that the difficulty in controlling animal movement is a major problem in preventing the spread of FMD in Southeast Asia.

The most appropriate approach to FMD control in Southeast Asia is considered the prevention of infected animals from entering the principal trading routes for livestock, along with the protection of livestock systems adjacent to those trading routes by means of vaccination (Perry et al. 2002). XK province is located in the northeast area of Lao PDR and is an important location, as livestock move along the trade route into neighboring Vietnam (Nampanya et al. 2013). However, the movement of traders and livestock

in XK province is uncontrolled (Rast et al. 2010). This difficulty in controlling the movement of animals across international borders into Lao PDR makes vaccination the preferred FMD control option in this country (Rast et al. 2010). However, vaccination is not routinely used in Southeast Asia due to the high cost and additional related expenses (Gleeson 2002). In addition, many farmers fear that vaccinations cause abortion and complain that assembling animals for a vaccination is difficult (Cleland et al. 1995). The results of this study show that the vaccine effectively controls FMD in both cattle and buffalos with no side effects, including abortion. To date, no FMD outbreak has been reported at the study sites. The donation of good vaccines and the dissemination of information on the efficacy of vaccination and its economic impact might improve the cooperation of farmers, local veterinarians, and



**Fig. 1.** Screening of serum samples of 90 cattle and 31 buffalo by LPBE. Samples were diluted at 1:32 on screening. The clear wells showed sero-positive ( $32 <$ ). The colored wells showed negative ( $32$  or under). a) Samples from first blood sampling before vaccination. Many wells were sero-positive against FMDV O type before vaccination. b) Samples from the second blood sampling one month after the first vaccination. One month after the first vaccination, majority of the wells were clear. c) Samples from the third blood sampling one month after the second vaccination. All wells were clear as compared to one month after the first vaccination. After two vaccinations, the immunity of all tested animals increased significantly as compared to that before vaccination. ++: positive; +: weakly positive; -: negative; A: antigen; B: wells without sample (blank)

village officials. Vaccination coverage might also need to be significantly increased to effectively control FMD (Cleland et al. 1995). Therefore, continuous programs involving the donation of effective vaccines are required for the effective control of FMD in Southeast Asia.

Of note, the FMD outbreaks in Southeast Asia should not be regarded as something that could not happen elsewhere. Phylogenetically, the virus O/JPN/2010, isolated in Miyazaki, Japan, in 2010, was clustered with SEA topotypes that are usually restricted to mainland Southeast Asia (Knowles et al. 2012). Moreover, recent phylogenetic studies show the territorial expansion and continuous threat of this topotype in East Asian countries (Knowles et al. 2012). This evidence supports the need for regional cooperation, the sharing of information, and transparent reporting on disease. FMD control in mainland Southeast Asia is crucial to reducing the risk of FMD invasion into East Asian countries. Emphasis should be placed on the continuous provision of effective FMD vaccines to control FMD in mainland Southeast Asia, so as to possibly prevent FMD incursion into East Asian countries, including

Japan. Projects involving vaccine donations by international organizations have been established to control the spread of FMD in mainland Southeast Asia (Khounsy et al. 2008). After the present efficacy test for expired FMD vaccines, 100,000 doses of expired FMD O type vaccines were donated to Myanmar in 2013, and another 100,000 doses of expired FMD O type vaccines and 100,000 doses of expired FMD A type vaccines were also donated separately to Lao PDR in 2014 under the OIE/JTF project. The next donation of expired FMD vaccines is planned for 2015. We consider the donation of expired FMD vaccines in storage for emergency use in Japan as an effective tool for the continuous provision of effective FMD vaccines with little issue of cost.

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