



Risk factors for emergence of exotic foot-and-mouth disease O/ME-SA/Ind-2001d on smallholder farms in the Greater Mekong Subregion



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ABSTRACT

Foot-and-mouth disease (FMD) is a significant endemic transboundary animal disease in Lao People's Democratic Republic (Lao PDR) and throughout the Greater Mekong Subregion (GMS). The disease has been shown to perpetuate the cycle of smallholder poverty through reduced animal production, plus limitations on market access for trading in livestock and their products. Despite significant national and multilateral efforts to control FMD over the past two decades, endemic FMD viruses (FMDVs) continue to circulate in Lao PDR. Further, the threat from new and emerging FMDVs is increasing as transboundary movements in the region intensify in response to increasing regional demand for meat. Although the economic impacts of FMD on smallholder farmers in Lao PDR are significant, studies investigating household-level risk factors for FMD are lacking. Following an outbreak of a novel FMDV (O/ME-SA/Ind2001d) in Lao PDR in 2015, a questionnaire and serological study were conducted in Naxaythong District to identify household-level risk factors associated with this outbreak, as well as endemic circulating viruses in the outbreak area. Data were analysed using a multi-variable generalised estimating equation (GEE) model with a logit link function and associations were calculated as odds ratios (OR) with 95% confidence intervals (CI_{95%}). After adjusting for other variables, the practice of quarantining new livestock for a minimum of two weeks prior to introduction to a herd was found to be a significant protective factor during the 2015 outbreak (OR 0.225, CI_{95%} [0.06, 0.88], *p*-value 0.003). In addition, households owning one or more animals with titres to the non-structural proteins of FMDV, indicating prior infection, had 5.5 times the odds (CI_{95%} [6.16, 49.11], *p*-value < 0.001) of sharing communal grazing land with neighbouring villages. These findings indicate that implementing basic on-farm biosecurity and improved husbandry measures to minimise FMDV circulation at the household level are important and reinforce the need to enhance the education of smallholder farmers in infectious disease control.

1. Introduction

Foot-and-mouth disease (FMD) is the most significant economic transboundary animal disease globally. The disease impacts negatively on smallholder farmers in the Greater Mekong Subregion (GMS), particularly the developing agrarian economies including the Lao People's Democratic Republic (Lao PDR) (Young et al., 2014a; Nampanya et al., 2014b). Dependency on livestock for subsistence is a common feature of the lower income sectors in such countries and controlling FMD is considered a key focus for poverty reduction initiatives (Knight-Jones

and Rushton, 2013). Although FMD-related mortality is generally low, the economic impact of FMD on smallholders can be significant due to production losses and indirect costs involving treatment, loss of draught power and trade limitations (James and Rushton, 2002; Nampanya et al., 2014a, b).

In Lao PDR, smallholders produce more than 94% of all livestock products (Khounsy et al., 2008; Nampanya et al., 2014a). FMD presents a significant barrier for efficient livestock production in the country, with losses from FMD in 2011 estimated at almost 12% of the predicted farm gate value of the national large ruminant herd (Nampanya et al.,

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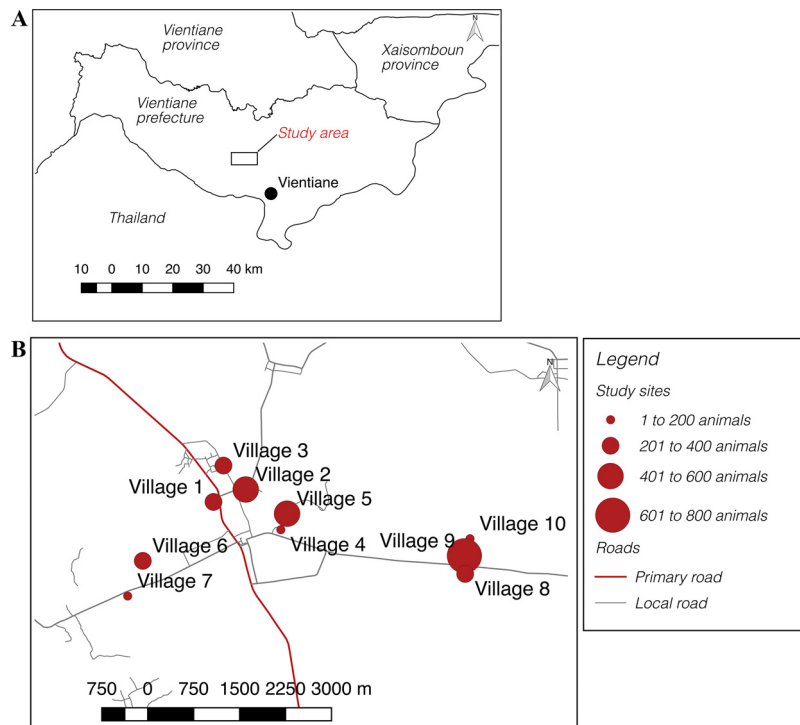


Fig. 1. a & b: Maps illustrating the (a) study area and (b) location of villages and size of large ruminant population.

2014b). At the smallholder level, direct and indirect losses may amount to as much as 60% of the annual household income (Nampanya et al., 2013a). However, as underreporting of outbreaks in Lao PDR and other GMS countries is likely, the incidence and impact of FMD on smallholder farmers is also likely to have been underestimated (Nampanya et al., 2013b; Young et al., 2016).

Knowledge of FMD epidemiology in the GMS has expanded over the past two decades through national and multilateral donor project collaborations, and coordination activities led by the World Organisation for Animal Health (OIE) through the South-East Asia and China Foot and Mouth Disease (SEACFMD) campaign (OIE, 2016b) and the framework of the Progressive Control Pathway (PCP) (OIE and FAO, 2012). The SEACFMD campaign, initially established in 1997 by the OIE and its collaborative partners, is now in its fifth phase, with a regularly updated roadmap aimed at long-term sustainable FMD prevention and control (OIE, 2016b). However, despite such control efforts, endemic FMD viruses (FMDVs) continue to circulate in Lao PDR and threats from emerging viruses are increasing as transboundary movements in the region intensify (Madin, 2011; Smith et al., 2015). Successful FMD control will require considerable and sustained financial and political investment in GMS countries, and recent research has highlighted the importance of engaging with smallholder farmers to increase farmer husbandry and on-farm biosecurity knowledge and improve understanding of household-level risk factors (Nampanya et al., 2014a, b; Young et al., 2015, 2017). Several recent studies in the GMS have identified the need for village biosecurity training and implementation of change management practices to reduce the risk of infectious diseases occurring at the household level (Nampanya et al., 2010; Young et al., 2015, 2017).

In Lao PDR, transboundary trade in livestock occurs across national borders shared with multiple countries with endemic FMD infection (OIE SRR-SEA, 2016a), with the distribution and movement of FMD viruses reflecting this trade (Gleeson, 2002; Cocks et al., 2009; Smith et al., 2015). FMD control presents a particular challenge as the country is situated on a thoroughfare for animal movements between Myanmar and Thailand to China and Vietnam (Khounsy et al., 2008; Windsor, 2011; Smith et al., 2015). In recent years, rising market demand for

animal products in advancing regional economies has resulted in an increasing volume of unofficial livestock movements (Madin, 2011; Smith et al., 2015), resulting in trade routes from India and Bangladesh transiting through Lao PDR (Smith et al., 2015). Managing the risks of emerging FMDVs associated with increasing livestock and animal product movements is a critical issue if national and regional FMD control initiatives are to be sustainable.

In April 2015, the Lao Department of Livestock and Fisheries (DLF) detected the first outbreak of the South Asian strain of FMD type O/ME-SA/Ind2001d (the Ind2001d sub-lineage within the Middle East-South Asia topotype of serotype O, or O/Ind2001d) in the GMS (FAO, 2015; Qiu et al., 2017). The outbreak occurred in Naxaythong District in Vientiane Capital and was the first reported outbreak of FMD in this district since 2007 (OIE SRR-SEA, 2016a). The O/Ind2001d virus has since been detected regionally in Vietnam, Myanmar, Thailand, China and the Republic of Korea (FAO, 2017; Qiu et al., 2017). The continued spread of FMD O/Ind2001d reinforces the need for an understanding of the risk factors associated with disease transmission in order to develop the most appropriate evidence-based control strategies.

Although the economic impacts of FMD on smallholder farmers in Lao PDR are significant, studies investigating risk factors for FMD transmission at the smallholder level are limited (Cleland et al., 1996). This paper reports on a study that investigated the household-level risk factors associated with emerging and endemic FMD viruses in Naxaythong District, driven by the 2015 emergence of O/Ind2001d. The objective was to provide improvements in evidence-based management interventions for FMD control on smallholder farms in Lao PDR and the wider GMS.

2. Materials and methods

This research was conducted within the research project ‘Enhancing transboundary livestock disease risk management in Lao PDR’ administered by the University of Sydney and the DLF of Lao PDR, with approval of the University of Sydney Animal Ethics (2015/765) and Human Ethics (2014/783).

2.1. Sampling strategy

A cross-sectional study was designed to assess household-level risk factors in Naxaythong District, Lao PDR, associated with: (1) reported clinical FMD in smallholder herds during the outbreak of O/Ind2001d; and (2) sero-positivity for FMDV in smallholder herds as determined by the non-structural protein antibody (NSP-Ab) ELISA. The target population was smallholders with large ruminants (cattle and buffalo) in the outbreak area. The outbreak area was defined as the area encompassing the two villages, Nakhoun and Nasap, where laboratory-confirmed cases of FMD were recorded by the DLF during the 2015 outbreak, plus the eight surrounding villages within a 5 km radius that farmed large ruminants (Fig. 1a & b).

The outbreak duration reported to DLF was from April to June 2015. However, due to the widely suspected underreporting of disease outbreaks at the smallholder and village level, the study window of interest was expanded to ensure unreported cases were captured within four standard incubation periods (4×14 days) either side of the reported outbreak, as per the OIE Terrestrial Animal Health Code Chapter 8.8 (OIE, 2017). Thus, the study window of interest was February to August 2015.

Households were set as the epidemiological unit of interest, and the number of households required from each village was estimated using an approximated two-stage probability proportional to size sampling approach (Das and Mohanty, 1973; Skinner, 2016). Study households were then randomly selected using Naxaythong District Office records as a sampling frame, with eligibility of households confirmed at the time of visit. Eligible households were those that were physically accessible during the study period, had farmer consent for enrolment and owned large ruminants suitable for serological sampling on the day of the visit. Animals were considered suitable for serological sampling if they were owned prior to or during the outbreak window, and where farmer consent, operator safety and animal welfare standards were met as determined by the lead author. All large ruminants considered suitable for sampling on the day of the visit were included (Table 1).

2.2. Case definition and variables

Two outcome variables for household FMD infection status were investigated: (1) reported clinical FMD during the defined O/Ind2001d outbreak window; and (2) household FMD NSP-Ab ELISA sero-

Table 1

Summary of households surveyed and large ruminants sampled using two-stage probability proportional to size (PPS) sampling in Naxaythong District, Lao PDR.

Village ID	PPS ^a sampling (by estimated number of eligible households)			Household primary livestock keepers surveyed			Large ruminants sampled	
	N_i	$\pi_i = N_i/N$	$\pi_{j i} = c/N_i$	$\pi_{ij} = c/N$	Male	Female		Total
1	82	0.217	0.122	0.016	9	1	10	20
2	11	0.029	0.364	0.011	3	1	4	9
3	30	0.079	0.200	0.016	4	2	6	17
4	35	0.093	0.171	0.016	4	2	6	17
5	43	0.114	0.163	0.017	3	4	7	12
6	33	0.087	0.182	0.016	2	4	6	17
7	35	0.093	0.171	0.016	5	1	6	15
8	40	0.106	0.150	0.016	4	2	6	17
9	32	0.085	0.188	0.016	5	1	6	18
10	37	0.098	0.162	0.016	6	0	6	18
	378				45	18	63	160

^a PPS, or probability proportional to size sampling, is a multi-stage sampling method where the probability of selection for primary sampling units is proportional to their size (Das and Mohanty, 1973).

positivity.

The first outcome variable was dichotomised to households that had experienced clinical FMD during the study window and those that had not, where the case definition for clinical FMD was defined as the presence of vesicular lesions or associated ulcerations in the mouth, feet or udders of large ruminants, with or without accompanying lameness, inappetence, milk drop, decreased weight gain, and/or abortion. The case definition for clinical FMD was described to smallholder farmers, along with visual materials depicting these clinical signs, prior to questioning. Case households were defined as those that satisfied all of the following criteria: (1) the farmer described clinical lesions consistent with FMD in one or more large ruminants owned by the household; (2) these clinical lesions occurred during the study window of February to August 2015; and (3) one or more of these animals returned a percentage of inhibition (PI) $\geq 50\%$ indicating a positive titre for FMD NSP-Ab ELISA (Paton et al., 2006).

Based on serology results, a second outcome variable was examined to define the influence of explanatory variables on the likelihood of a household having serological evidence of FMD infection. Investigation of risk factors for NSP-Ab sero-positivity, regardless of clinical FMD status, enabled consideration of households with unreported or unrecognised FMD, and/or subclinical or carrier animals. Assessment of household NSP-Ab sero-positivity also facilitated investigation of infection due to historical outbreak events in the area, providing an insight into the functionality of reporting systems. The outcome was dichotomised to households that owned one or more NSP-Ab positive (PI $\geq 50\%$) animals at the time of sampling, and those that did not, regardless of reported clinical signs during the 2015 outbreak.

Analyses of household-level survey data were performed to determine the influence of putative risk factors on the likelihood of a household being infected, with respect to both outcome variables. Binary and continuous survey data were collected for explanatory variables that were considered potential risk factors or confounders of any observed statistical relationship (Table 2). These included information on: (1) herd size and composition (number and species of animals owned at the time of the outbreak); (2) restocking sources (stock purchased from within the village, neighbouring villages or livestock traders); (3) livestock husbandry practices (use of communal grazing land, water sources and feeding of animal products or products that have had contact with these, also known as swill feeding); (4) biosecurity practices (quarantine of new animals and isolation of sick animals); and (5) historical FMD infection and vaccination status.

2.3. Survey development and implementation

The household-level risk factor survey, consisting of 21 close-ended questions, was written in English, translated into Lao and Naxaythong District extension staff received training prior to implementing the survey. A total of 63 farmers were interviewed by a single interviewer. The interviewer received training prior to delivery to ensure a clear translation and understanding of survey questions and objectives. Surveys were conducted in study villages between October 19–27, 2016. Farmers were interviewed individually face-to-face to avoid group conformity, with each survey requiring approximately 20 min to complete.

2.4. Animal sampling and serological testing

Large ruminants were restrained using a portable bleeding pole (Fig. 2) and were examined by a veterinarian for clinical signs of active FMD prior to sampling. Blood was collected in 8.5 ml serum clot tubes (BD Vacutainer[®]) from 160 large ruminants and processed at the Lao National Animal Health Laboratory (NAHL) in Vientiane.

Presence of antibodies specific to NSP were detected using PrioCHECK[®] FMD virus NSP blocking ELISA (Prionics). Tests were performed according to the manufacturer guidelines. Sero-positive

Table 2
Definition of household-level outcome variables and risk factors for FMD infection in Naxaythong District, Lao PDR.

Outcome variables	
Clinical FMD	Households were dichotomised as cases or non-cases for clinical FMD reported during the outbreak window
NSP-Ab sero-positivity	Households were dichotomised as cases or non-cases for NSP sero-positivity based on laboratory-confirmed serological evidence of FMD infection
Continuous risk factor variables	
Herd composition	The number of cattle, buffalo, pigs and goats owned during the outbreak window
Binary risk factor variables	
Vaccinated	Cattle and buffalo in the herd were vaccinated within 6 months prior to the outbreak window
Purchased cattle	Purchased cattle one month prior to or during outbreak window
Purchased buffalo	Purchased buffalo one month prior to or during outbreak window
Purchased pigs	Purchased pigs one month prior to or during outbreak window
Purchased goats	Purchased goats one month prior to or during outbreak window
Purchased from village	Purchased livestock ^a from within the village one month prior to or during the outbreak window
Purchased from neighbour	Purchased livestock from a neighbouring village one month prior to or during the outbreak window
Purchased from trader	Purchased livestock from livestock traders one month prior to or during the outbreak window
Quarantine	Always quarantine new stock for at least two weeks prior to introduction to the herd
Isolate	Always isolate clinically unwell stock until recovered
Communal grazing	Livestock have direct contact with livestock from neighbouring villages while using communal grazing land
Communal water	Livestock have direct contact with livestock from neighbouring villages while using communal water sources
Purchase meat	Purchase meat from neighbouring markets at least once per month
Feed swill	Feed swill to pigs

^a Livestock are defined for the purpose of this research as cattle, buffalo, pigs and goats.



Fig. 2. Large ruminants are restrained using a bleeding pole in absence of fixed cattle handling yards.

samples (i.e. $PI \geq 50\%$) were re-tested and only samples found positive in two repeated tests were considered as positive. A sensitivity of 81.8% and a specificity of 99.2% were previously reported for tests using the described ELISA kit (Paton et al., 2006).

2.5. Data management

Survey data were translated into English and entered into a Microsoft Excel spreadsheet with dichotomous (yes/no) answers converted to '0' and '1' for binary analysis. Once processed, laboratory results were matched to household survey data in Microsoft Excel using identification numbers assigned to individual animals during the study.

2.6. Statistical analysis

Statistical analyses were completed using IBM® SPSS® Statistics (version 24). Descriptive analysis was performed to calculate frequency distributions and measures of central tendency of continuous and binary risk factors. Explanatory variables with a large number of missing observations ($> 10\%$) or limited variability were excluded from further analysis. Unconditional associations between each risk factor and each outcome variable were assessed with univariable logistic regression analyses. Variables with p -values of < 0.3 were shortlisted for multivariable analysis. Biologically plausible

confounders were considered, and collinearities between pairs of variables to be included in the multivariable model were assessed using Spearman's rank correlation coefficient with a cut-off of > 0.9 .

Associations between household FMD infection status and the examined risk factors were calculated as an odds ratio with 95% confidence intervals ($CI_{95\%}$) using a generalised estimating equation (GEE) with a binomial distribution, logit link function and unstructured working correlation matrix to produce population-averaged models for both outcome variables (Liang and Zeger, 1986; Dohoo et al., 2009; Elnekave et al., 2015). To address clustering by villages, the subject variable of households clustered by villages was inserted. A multivariable model was fitted to the data for each outcome variable using a backward stepwise approach for risk factors shortlisted during the univariable analyses. All biologically plausible interactions were tested for the variables in the final models and retained if statistically significant (p -value < 0.1). The goodness-of-fit of the final models were evaluated using the quasi-likelihood under the independence model criterion (QIC) for representative model selection (Imori, 2013).

3. Results

3.1. FMD infection and vaccination status

A total of 83% ($n = 52$) of the total households surveyed ($n = 63$) owned one or more large ruminants serologically positive to the NSP of FMDV. Of the total households, 36.5% ($n = 23$) reported clinical FMD in one or more large ruminants during the study window of interest. All households reporting clinical FMD owned at least one sero-positive animal. However, only 44.2% ($n = 23$) of the NSP-Ab positive households reported experiencing clinical FMD, with the remaining 54.7% ($n = 29$) stating their animals were clinically unaffected during the outbreak window.

No households reported vaccinating against FMD prior to the outbreak window, although 23.8% ($n = 15$) confirmed participation in the government emergency vaccination response in May 2015. During this response, approximately 60% ($n = 3241$) of the large ruminant population within the study villages were vaccinated (OIE SRR-SEA, 2016a) using a purified, high potency, inactivated FMD vaccine composed of O1/Manisa, O/3039, A/Malaysia/1997 and A22/Iraq (Qiu et al., 2017). No evidence of active clinical FMD was found during clinical examination of the large ruminants included in this study at the time of sampling, and no further outbreaks following the outbreak window were reported by smallholder farmers, village chiefs or DLF staff.

Table 3

Descriptive analysis of household herd composition by species owned during the 2015 Ind2001d outbreak window in Naxaythong District, Lao PDR.

Species owned	Total households	Total animals	Mean (SE)	Median	Range
Cattle	33	579	9.00 (0.99)	6.00	0–33
Buffalo	22	120	1.90 (0.56)	0.00	0–22
Goats	15	44	0.70 (0.02)	0.00	0–15
Pigs	1	1	0.02 (0.30)	0.00	0–1

3.2. Herd composition

The numbers of FMD-susceptible livestock owned by households during the outbreak window were recorded by species. A total of 93.7% ($n = 59$) of households owned cattle and 23.8% ($n = 15$) owned buffalo. Descriptive analysis of herd composition revealed a right skewed distribution of the number of cattle (median 6.00, range 0–33) and buffalo (median 0.00, range 0–22) owned. Limited variability in ownership of other species was noted, with 11.1% ($n = 7$) of households owning goats (median 0.00, range 0–22) and only one household with pigs (Table 3).

3.3. Livestock sources

Of the 63 households surveyed, 23.8% ($n = 15$) purchased FMD-susceptible livestock in the month prior to or during the outbreak window. Of these, 17.5% ($n = 11$) of households purchased cattle, 4.8% ($n = 3$) purchased buffalo or pigs, and one household purchased goats. Only 7.9% ($n = 5$) of households reported purchasing livestock one month prior to or during the outbreak window from other households within their village, with 14.3% ($n = 9$) purchasing livestock from neighbouring villages. No visits by, or purchases from, livestock traders were reported (Table 4).

At the time of sampling, 19.0% ($n = 12$) of households owned one or more large ruminants sourced from another village, while the majority (81.0%, $n = 51$) of households only owned large ruminants that had been born in the same village. Of the 52 households owning one or more large ruminants sero-positive to the NSP, 7.7% ($n = 4$) of households owned animals originating from outside Naxaythong District, all of which had been purchased from Vientiane Capital.

3.4. On-farm biosecurity and husbandry practices

In total, 30.2% ($n = 19$) of households stated they quarantine new livestock for a minimum of two weeks prior to introduction to the herd, with 31.7% ($n = 20$) isolating clinically unwell livestock from the herd

Table 4

Descriptive analysis of household-level binary risk factors in Naxaythong District, Lao PDR.

Risk factor	Household exposure frequency	
	No (%)	Yes (%)
Vaccinated	63 (100.0)	0 (0.0)
Purchased cattle	52 (82.5)	11 (17.5)
Purchased buffalo	60 (95.2)	3 (4.8)
Purchased pigs	62 (98.4)	1 (1.6)
Purchased goats	60 (95.2)	3 (4.8)
Purchased from village	58 (92.1)	5 (7.9)
Purchased from neighbour	54 (85.7)	9 (14.3)
Purchased from trader	63 (100.0)	0 (0.0)
Quarantine	44 (69.8)	19 (30.2)
Isolate	43 (68.3)	20 (31.7)
Communal grazing	18 (28.6)	45 (71.4)
Communal water	18 (28.6)	45 (71.4)
Purchase meat	12 (19.0)	51 (81.0)
Feed swill	62 (98.4)	1 (1.6)

Table 5

Univariable logistic regression analysis of short-listed household-level risk factors for the outcome variable of clinical FMD.

Risk factors	β	SE(β)	p -value	Exp(β)	95% CI
Number of cattle	0.053	0.034	0.119	1.055	0.99, 1.13
Number of buffalo	0.062	0.059	0.296	1.064	0.95, 1.20
Purchased buffalo	1.312	1.254	0.295	3.714	0.32, 43.40
Purchased from village	1.047	0.954	0.272	2.850	0.44, 18.48
Quarantine	−1.492	0.698	0.033	0.225	0.06, 0.88

until recovered. Sharing of communal grazing land and water points with neighbouring villages was reported by 71.4% ($n = 45$) of households and a majority of households (80.1%, $n = 51$) reported purchasing meat from markets in neighbouring villages at least once a month. Swill feeding was practiced by the one household that owned a pig (Table 4).

3.5. Univariable and multivariable regression analyses

Following descriptive analysis, four explanatory variables were found to have a large number of missing observations (> 10%) or limited variability and were excluded from further analysis. All biologically plausible confounders were considered during study design, but on descriptive analysis were found to be constants (i.e. there was no variation in identified variables between households). No biologically plausible interactions were found to be statistically significant (p -value < 0.1). Two pairs of variables with Spearman rank correlation coefficients above the cut-off > 0.9 and with p -values < 0.05 were considered to have significant collinearity and the redundant variables were removed from further analysis. Tables 5 and 7 detail the explanatory variables short-listed for inclusion in subsequent multivariable analyses.

For the outcome variable of clinical FMD during the study window, a total of five of the 18 explanatory variables met the < 0.3 p -value cut-off point and were included in the backward selection GEE model (Table 5). The final model for the outcome of clinical FMD included only one significant variable, identifying that quarantining of new livestock for a minimum of two weeks prior to introduction to a herd was a significant protective factor during the 2015 outbreak (OR 0.225, CI_{95%} [0.06, 0.88] and p -value 0.003), after adjusting for other variables (Table 6).

For the outcome variable of NSP-Ab sero-positivity, a total of three of the 18 explanatory variables met the < 0.3 p -value cut-off point and were included in the backward selection GEE model (Table 7). The final model for the outcome of NSP-Ab sero-positivity also included only one significant variable and found that households that share communal grazing land with neighbouring villages had 5.5 times the odds (CI_{95%} [6.16, 49.11] and p -value < 0.001) of owning one or more animals with a laboratory-confirmed positive titre (PI \geq 50%) for NSP-Ab, after adjusting for other variables (Table 8).

4. Discussion

This report details a study investigating risk factors for FMD

Table 6

Multivariable analysis of household-level risk factors for clinical FMD during the 2015 outbreak in Naxaythong District, Lao PDR. Multivariable analysis was performed using generalized estimating equation (GEE) models with a binomial distribution and logit link function and villages set as random variable.

Risk factors	β	SE(β)	p -value	Exp(β)	95% CI
Quarantine	−1.492	0.698	0.033	0.225	0.06, 0.88
Constant	−0.182	0.303	0.547	0.833	–

Table 7

Univariable logistic regression analysis of short-listed household-level risk factors for the outcome variable of NSP sero-positivity.

Risk factors	β	SE(β)	<i>p</i> -value	Exp(β)	95% CI
Number of cattle	0.075	0.056	0.185	1.078	0.97, 1.20
Purchase goats	−2.428	1.277	0.057	0.088	0.01, 1.08
Communal grazing	4.007	1.117	< 0.001	5.498	6.16, 49.10

Table 8

Multivariable analysis of household-level risk factors for NSP sero-positivity in Naxaythong District, Lao PDR. Multivariable analysis was performed using generalised estimating equation (GEE) models with a binomial distribution and logit link function and villages set as random variable.

Risk factors	β	SE(β)	<i>p</i> -value	Exp(β)	95% CI
Communal grazing	4.007	1.117	< 0.001	5.498	6.16, 49.10
Constant	−0.223	0.474	0.638	0.800	–

circulation and emergence at the household level in Lao PDR, and the reliability of animal disease reporting systems in the study area. Although several countries within the GMS have been members of the SEACFMD campaign for over twenty years, FMD remains endemic and a regional challenge, with unreported outbreaks known to occur frequently. While the benefits associated with preventive vaccination campaigns in the north and south of the country are recognised (OIE SRR-SEA, 2015), in much of Lao PDR, emergency vaccination and associated outbreak response measures are the primary FMD control activities. Such reactive approaches to animal disease control require strong and efficient surveillance systems to remain effective and are severely hampered by delayed or incomplete outbreak reporting. It is also recognised that preventive health measures are limited in the traditional husbandry practices of smallholders in Lao PDR (Nampanya et al., 2010). Encouragingly, recent in-country projects in the GMS have confirmed that an increase in farmer knowledge leads to enhanced large ruminant health and productivity, and applied change management principles have been advocated to improve biosecurity practices (Young et al., 2014a, b; Young et al., 2017). However, an improved understanding of household-level risk factors is necessary to ensure control initiatives are founded on evidence-based interventions and regularly reviewed to remain valid.

Results from this study suggest that considerable underreporting of clinical FMD may have occurred at both the village and household levels. Over a third of households (36.5%) were impacted by clinical FMD during the 2015 outbreak of O/Ind2001d in Naxaythong District, with multiple households from all 10 study villages affected. Prior to this study, only two villages were known by DLF to have been affected by the outbreak. Further, despite absence of formal reports of FMD outbreaks in Naxaythong District since 2007, our serological results confirm that FMD is endemic, with 52 (82.5%) of the households sampled owning one or more large ruminants with positive NSP-Ab titres. As only 4 of these households imported large ruminants from other districts, it is likely the majority (92.3%) of households with NSP-Ab positive animals were likely exposed to FMD within Naxaythong District. We conclude that endemic circulation of FMDVs in the study area appears considerably higher than formal outbreak reports to the DLF would indicate.

Interestingly, only 23 (44.2%) of the 52 NSP-Ab positive households reported experiencing clinical FMD during the 2015 outbreak of O/Ind2001d. It is unclear whether this disparity is the result of a lack of clinical recognition, the presence of subclinical infection, an aversion to reporting, or is, as considered more likely, a combination of these factors. Previous exposure to FMDV prior to the study window, or inoculation with non purified vaccines could also impact these results. Research in Lao PDR indicates that baseline smallholder knowledge of

animal health and husbandry is relatively low (Nampanya et al., 2010, 2012; Windsor et al., 2015). The failure of farmers to recognise and recall clinical signs of FMD may have contributed to low reporting sensitivity and the apparent underreporting observed in this study. A lack of awareness of available reporting pathways may also have contributed to underreporting, although investigation of smallholder knowledge of reporting pathways and processes was outside the scope of this study. However conversely, all households reporting clinical FMD were found to own at least one animal with a positive NSP-Ab titre. Despite the time lapse since the end of the outbreak window and anticipated recall bias, the specificity of farmer reporting of clinical FMD was high (~100%). Findings suggest a potential utility for smallholder reporting systems, particularly if education initiatives were to improve recognition of clinical signs and awareness of reporting pathways. Importantly, over half (55.8%) of the households with FMD seropositive animals and 8 of 10 villages failed to report clinical FMD during the 2015 outbreak of O/Ind2001d. This suggests that an effective emergency disease reporting and response system accompanied by rigorous investigation and surveillance of in-contact animals in surrounding villages for clinical and subclinical FMD, was required to more fully identify the extent of this outbreak.

Multivariable regression analysis indicated households sharing communal grazing land with neighbouring villages have 5.5 times the odds (CI_{95%} [6.16–49.11], *p*-value < 0.001) of owning one or more animals with a laboratory-confirmed positive titre for NSP-Ab, after adjusting for other variables. An absolute collinearity (CC 1.000, CI_{95%} [1.00–1.00], *p*-value < 0.001) was identified between use of communal grazing land and communal water sources, likely influenced by a combination of village location, resource availability and improved husbandry practices being adopted concurrently. These results are consistent with previous findings that practices increasing direct contact between livestock, particularly the use of communal grazing and water sources, increase the likelihood of FMD transmission (Cleland et al., 1996; Young et al., 2014b). These findings confirm the importance of implementing basic on-farm biosecurity and improved husbandry measures to minimise FMD circulation at the household level. A widespread public awareness campaign is recommended to enhance smallholder education, thereby empowering producers to implement improved on-farm biosecurity and infectious disease control.

Furthermore, the multivariable regression analysis confirmed that implementation of basic biosecurity measures reduced the odds of clinical FMD at the household level during the O/Ind2001d outbreak in Naxaythong District. The quarantine of new livestock for at least two weeks prior to introduction to the herd was found to be a significant protective factor during the outbreak (OR 0.225, CI_{95%} [0.06–0.88], *p*-value 0.003), after adjusting for other variables in the model. There was a strong collinearity (CC 0.741, CI_{95%} [0.54–0.90], *p*-value < 0.001) between practicing quarantine of new livestock and isolation of sick livestock. It is likely that farmers implementing these measures had a higher general knowledge of FMD transmission and biosecurity practices.

Movement of livestock is widely recognised as the most important means of transmission of FMD in endemic regions (Ferris et al., 1992; Perry et al., 2002; Windsor, 2011). Strategies that reduce the likelihood of introduction through livestock purchases and decrease the opportunity for disease transmission have the greatest impact on reducing FMD spread between and within villages (Cleland et al., 1996). Confirmation of the source of the O/Ind2001d outbreak was not possible during this study due to the limitations in sample size and the time that had elapsed since the outbreak. Nevertheless, results confirm 23.8% of households purchased FMD-susceptible livestock in the month prior to the study window. It is difficult to comment on the practice of swill feeding to pigs in this study, given only one pig was owned by the surveyed farmers. This survey suggests that livestock movements and subsequent direct contact at communal grazing and water points was the main risk pathway for entry and transmission of O/Ind2001d virus in this FMD

outbreak. While the association between purchase of FMD-susceptible livestock and FMD infection was not statistically significant in the multivariable regression analysis, strategies to reduce the likelihood of FMD introductions through livestock purchases are strongly recommended (Cleland et al., 1996). Further research is recommended targeting smallholder pig farmers to understand the risk of FMD transmission including swill feeding. It is acknowledged that although a sampling frame was used to randomly select smallholder households, inclusion was based on households and farmers being both accessible and available on the day of investigation. Therefore, selection and participant recall bias should be considered as possible limitations to this study. Future studies could increase the sample size to help improve the study design and minimise potential sources of bias.

In conclusion, the findings from this study emphasise the need to engage smallholder farmers in programmes that increase their husbandry and animal health knowledge and practices, thereby improving uptake of on-farm biosecurity measures and animal disease surveillance. These findings are consistent with the expanding literature recommending further support for education-based extension programmes and applied change management interventions at the smallholder level. Such interventions have been found to be most effective in the GMS when delivered simultaneously with efforts that improve large ruminant productivity (Nampanya et al., 2012; Windsor et al., 2015). Further research is recommended to investigate farmer knowledge of reporting pathways, quantify the sensitivity and specificity of smallholder reporting capacity, and evaluate the impacts of education-based intervention strategies. In addition, studies to identify motivations and aversions to reporting at the household and village level are recommended to support effective and targeted delivery of evidence-based interventions, in-turn leading to improved rural smallholder livelihoods.

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