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Emergence of an exotic strain of serotype O foot-and-mouth disease virus O/ME-SA/Ind-2001d in South-East Asia in 2015

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Summary

The O/Middle East-South Asia (ME-SA)/Ind-2001 lineage of foot-and-mouth disease virus (FMDV) is endemic in the Indian subcontinent and has been reported in the Middle East and North Africa, but it had not been detected in South-East Asia (SEA) before 2015. This study reports the recent incursions of this viral lineage into SEA, which caused outbreaks in Vientiane Capital of Lao People's Democratic Republic (PDR) in April 2015, in Dak Nong, Dak Lak and Ninh Thuan Provinces of Vietnam from May to October 2015, and in Rakhine State of Myanmar in October 2015. Disease investigations were conducted during the outbreaks and followed up after laboratory results confirmed the involvement of FMDV O/ME-SA/Ind-2001 sublineage d (O/ME-SA/Ind-2001d). Affected host species included cattle, buffalo and pig, and all the outbreaks resolved within 2 months. Animals with clinical signs were separated, and affected premises were disinfected. However, strict movement restrictions were not enforced, and emergency vaccinations were only implemented in Vientiane Capital of Lao PDR and Dak Nong and Ninh Thuan Provinces of Vietnam. Clinical samples were collected from each outbreak and examined by nucleotide sequencing of the FMDV viral protein 1 coding region. Sequence analysis revealed that the O/ME-SA/Ind-2001d isolates from Lao PDR and Vietnam were closely related to each other and similar to viruses previously circulating in India in 2013. Viruses collected from Myanmar were divergent from viruses of the same sublineage recovered from Lao PDR and Vietnam but were closely related to viruses present in Bangladesh in 2015. These findings imply that at least two independent introductions of O/ME-SA/Ind-2001d into SEA have occurred. Our study highlights the transboundary nature of foot-and-mouth disease (FMD) and reinforces the importance of improved FMD surveillance and promotion of safer cross-border trade in SEA to control the risk of introduction and spread of exotic FMDV strains.

KEYWORDS
disease investigation, exotic strain, foot-and-mouth disease, O/ME-SA/Ind-2001d, sequence analysis, South-East Asia

1 | INTRODUCTION

Foot-and-mouth disease (FMD) is a highly contagious vesicular disease that affects cloven-hoofed animals. The disease is endemic in large parts of Asia and Africa (OIE, 2017; Rweyemamu et al., 2008) and is caused by foot-and-mouth disease virus (FMDV), which is easily transmitted through movements of infected livestock or their products, contaminated fomites, persons and aerosols.

Foot-and-mouth disease viruses exist as seven immunologically distinct serotypes (O, A, C, Asia 1, Southern African Territories [SAT] 1, SAT 2 and SAT 3), and each serotype can be divided into a variety of genetically and antigenically distinct topotypes and within a topotype, into lineages (Knowles & Samuel, 2003; Samuel & Knowles, 2001). Globally, the circulation of FMDVs can be subdivided into seven regional pools, which contain distinct virus strains that evolve independently from viruses normally found in other pools (Paton, Sumption, & Charleston, 2009; Sumption, Domenech, & Ferrari, 2012). Trans-pool spread of FMDV into new regions may cause significant epidemics and challenge local and international control strategies.

In South-East Asia (SEA) that is within Pool 1, FMD is endemic in mainland countries (Cambodia, Lao People's Democratic Republic [PDR], Myanmar, Peninsula Malaysia, Thailand and Vietnam) (OIE, 2017; Rweyemamu et al., 2008). Serotypes O and A are prevalent every year, while serotype Asia 1 is detected only sporadically (de Carvalho Ferreira et al., 2017; OIE, 2017; Tum, Robertson, Edwards, Abila, & Morzaria, 2015). Foot-and-mouth disease poses substantial economic burdens on small- and large-scale livestock producers (Nampanya et al., 2015, 2016; Shankar, Morzaria, Fiorucci, & Hak, 2012; Young et al., 2016). Regional control of FMD is exacerbated by the frequent movements of susceptible livestock across international boundaries, the majority of which are poorly regulated and represent substantial risks of transboundary spread of the disease (Di Nardo, Knowles, & Paton, 2011; Nampanya et al., 2013; Poolkhet et al., 2016). Previous studies have shown close genetic relationships between FMDVs recovered from various countries in SEA (Abdul-Hamid et al., 2011; Khounsy et al., 2009; Knowles et al., 2012; Le, Vu, Duong, Than, & Song, 2016; Le, Nguyen, Lee et al., 2010), and good agreement between the proposed pathways of FMDV dissemination and livestock movements (Di Nardo et al., 2011; Qiu, Rodtian, Widders, & Abila, 2016). In mainland SEA, vaccination against FMD is usually limited to cattle and buffaloes in targeted districts that are considered as FMD hot-spot or high-risk areas except for Thailand, where routine vaccinations are implemented in large ruminants almost across the country and in some large intensive pig farms (Cleland, Chamnanpoodb, Baldock, & Gleeson, 1995; Gleeson, 2002; OIE-SEACFMD, 2016a).

Serotype O is the most frequently detected FMDV serotype in SEA and has three distinct cocirculating topotypes: the indigenous SEA topotype (O/SEA/Mya-98 lineage), the CATHAY (pig-adapted) topotype and the Middle East-South Asia (ME-SA) topotype (Abdul-Hamid et al., 2011; Brito et al., 2017; de Carvalho Ferreira et al., 2017). The ME-SA topotype originated from the Indian subcontinent (Pool 2) and is composed of several lineages, such as PanAsia, PanAsia-2, Iran-2001 and Ind-2001 (a, b, c, and d sublineages) (Knowles & Samuel, 2003; Subramaniam et al., 2015). Of these, only the O/ME-SA/PanAsia and O/ME-SA/PanAsia-2 lineages have previously been detected in SEA (Abdul-Hamid et al., 2011; Knowles, Samuel, Davies, Midgley, & Valarcher, 2005). However, the O/ME-SA/Ind-2001 lineage has recently caused increasing concerns due to its multiple transregional movements. It has spread to some of the Gulf States of the Middle East including Saudi Arabia and the United Arab Emirates in 2013 (Knowles et al., 2016), and Bahrain in 2015 (WRLFMD, 2015). This viral lineage has also been reported from North African countries including Libya in 2013 (Valdazo-Gonzalez, Knowles, & King, 2014), Tunisia and Algeria in 2014 (Knowles et al., 2016), and Morocco in 2015 (Bachanek-Bankowska et al., 2016).

The OIE South-East Asia and China FMD (SEACFMD) Campaign, which was initiated in 1997, seeks to coordinate FMD surveillance, control and prevention activities in SEA, China and Mongolia (OIE-SEACFMD, 2016a). The programme encourages and supports Member Countries to investigate and report FMD outbreaks, and promotes FMD risk control activities. Under the SEACFMD surveillance framework, the d sublineage of O/ME-SA/Ind-2001 (O/ME-SA/Ind-2001d) was detected from field outbreaks which occurred in 2015 in Vientiane Capital of Lao PDR (April), in three southern provinces (Dak Nong, Dak Lak and Ninh Thuan) of Vietnam (May–October), and in Rakhine State of Myanmar (October), representing the first detections of this strain in SEA. The objectives of this study were to describe the findings from outbreak investigations conducted in the three affected countries, and to determine the genetic relationships between the O/ME-SA/Ind-2001d isolates recovered from the outbreaks as well as compare them with sequences of viruses from other regions, with a goal to better understand the origins and risks of the O/ME-SA/Ind-2001d outbreaks in SEA.

2 | MATERIALS AND METHODS

2.1 | Disease investigation and sample collection

In this study, an outbreak was defined as a village or a farm from which one or more cases of FMD were reported, and in this article, only investigations of the laboratory-confirmed outbreaks caused by O/ME-SA/Ind-2001d in 2015 were described. These outbreaks were detected under passive and clinical surveillance programmes in Lao PDR, Vietnam and Myanmar. In brief, clinical diseases resembling FMD were reported by farmers or village animal health workers, and outbreak investigations were conducted by local veterinary services as soon as possible after notification. Disease data were collected through interviewing village chiefs, village animal health workers and farmers. The recorded data included animal age and species, livestock management, presence or absence of clinical signs, previous FMD infection and vaccination history, numbers of susceptible animals, cases and deaths, and the likely sources of the diseases. Disease status was followed up until the outbreak resolved. However, not all records had complete information. After the laboratory confirmation of the presence of O/ME-SA/Ind-2001d, joint retrospective investigations were carried out by the OIE and country veterinary authorities through field visits or telephone interviews, with an objective of collecting as much missing information as possible. A map was created using Quantum GIS version 2.16.0 to show the 9 villages/farms in which investigations were conducted.

During the outbreak investigation, epithelial tissues were taken from fresh oral or foot lesions from animals showing suspected FMD symptoms. After collection, the samples were immediately placed in a transport medium composed of equal amounts of glycerol and 0.04 M phosphate buffer at pH 7.2–7.6, and shipped on ice to the National FMD Laboratory of Lao PDR or Myanmar or the Regional Animal Health Office No. 6 (RAHO6) of Vietnam. All the samples tested positive for serotype O by FMDV antigen ELISA at each respective laboratory.

2.2 | RNA extraction, reverse transcription-PCR and DNA sequencing

Samples with sufficient quantities and quality from Lao PDR ($n = 3$) and Myanmar ($n = 2$) were submitted for viral sequencing to the OIE FMD Reference Laboratory/Regional FMD Reference Laboratory for SEA (RRLFMD), at Pakchong, Thailand. At RAHO6 of Vietnam, viral sequencing was carried out on a half-yearly basis, and five samples collected from this study were sequenced. It should be noted that in all the outbreaks reported here, the samples were examined for molecular strain typing 6–10 months after they had been collected in the field. The viral protein 1 (VP1) coding region (639 nucleotides) was amplified and sequenced at RRLFMD or RAHO6 using the following approaches.

At RRLFMD, RNA was extracted from 10% epithelial tissue suspensions using Trizol® LS Reagent (Invitrogen, USA) as per the manufacturer's instructions. The RNA was first transcribed to cDNA using the NK61 primer (5′- GACATGTCCTCCTGCATCTG-3′) and M-MLV Reverse Transcriptase (Promega, USA). The reaction was performed at 42°C for 60 min, followed by denaturation at 95°C for 5 min. The cDNA was then amplified using the forward primer 1C-609 (5′-TAGTGCTGGTAAAGACTTTGAGCT-3′) and the reverse primer NK61, and the thermal conditions were set as follows: an initial denaturation at 94°C for 4 min; 30 cycles (denaturation at 94°C for 1 min; annealing at 55°C for 1 min; extension at 72°C for 1.5 min); a final extension at 72°C for 5 min. The PCR products were separated on a 1.5% agarose gel and purified using the QIAquick PCR Purification Kit (QIAGEN, Germany). PCR amplicons were sequenced using the BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, USA) as per the manufacturer's instructions and with the forward primer 1C-609 and the reverse primer NK72 (5′-GAAGGGCCCAGGGTTGGACTC-3′). The sequencing reactions were run on a 3500 Genetic Analyzer (Applied Biosystems, USA) as per the manufacturer's instructions.

At RAHO6, RNA was extracted from 10% epithelial tissue suspensions using InviMag® Virus RNA Kit/KF96/KFflex 96 (STRATEC Biomedical, Germany) according to the manufacturer's instructions. One-step reverse transcription-PCR (RT-PCR) was performed using the forward primer ARS4 (5′-ACCAACCTCCTTGATGTGGCT-3′) and the reverse primer NK61. The following thermal profile was used: reverse transcription at 50°C for 30 min; an initial denaturation at 94°C for 2 min; 40 cycles (denaturation at 94°C for 30 s; annealing at 55°C for 30 s; extension at 68°C for 2.5 min); a final extension at 68°C for 5 min. The amplification products were purified using the QIAquick Gel Extraction Kit (QIAGEN, Germany) and sequenced using the 454 GS Junior System (Roche, USA) as per the manufacturer's instructions.

2.3 | Genetic analysis of FMDV O/ME-SA/Ind-2001d isolates

The VP1 sequences obtained at RRLFMD and RAHO6 (GenBank accession numbers KY399460-61 and KY399463-70; Table 1) were shared with the OIE FMD Reference Laboratory/FAO World FMD Reference Laboratory (WRLFMD), at Pirbright, UK, for comparison with sequences of O/ME-SA/Ind-2001d isolates from other regions. At WRLFMD, alignment of the sequences was performed using BioEdit version 7.2.5 (Hall, 1999) and Clustal W (Thompson, Higgins, & Gibson, 1994). The most appropriate nucleotide substitution model (Tamura 3-parameter distance plus gamma distribution) was selected after testing 24 models using Molecular Evolutionary Genetics Analysis (MEGA) 6.06 (Tamura, 1992; Tamura, Stecher, Peterson, Filipski, & Kumar, 2013), and difference matrices were calculated. A maximum-likelihood phylogenetic tree was then generated using MEGA 6.06, and the robustness of tree topology was assessed using 1,000 bootstrap replicates.

3 | RESULTS

3.1 | Outbreak investigation findings

Figure 1 shows the locations of the villages (in Lao PDR and Myanmar) or farms (in Vietnam) where an outbreak due to O/ME-SA/Ind-2001d was investigated and confirmed by this study. As far as we were aware, none of these sites had any previous FMD outbreak more recently than 2012. No FMD vaccination or other control measures had been implemented at any affected village or farm prior to the O/ME-SA/Ind-2001d outbreak. Information (location, starting and resolution dates, and numbers of susceptible animals, cases and deaths) of each outbreak is shown in Table 1. Typical clinical signs characteristic of FMD, including pyrexia, salivation, lameness and foot and mouth vesicles, were observed in animals of all age groups.

The first confirmed outbreak due to O/ME-SA/Ind-2001d was detected on 20 April 2015 in a village located approximately 16 km from Vientiane City, Lao PDR, and 2 weeks later the disease spread to a neighbouring village sharing common water and grazing resources. Backyard cattle and buffaloes were affected, but clinical signs were not observed in pigs or goats. The index village reported no introduction of new livestock or comingling livestock with herds showing FMD-like symptoms prior to the outbreak. Both affected villages had frequent human movements and trade of untreated products of animal origin from Vientiane City.

On 26 May 2015, an outbreak due to O/ME-SA/Ind-2001d was detected from a cattle farm in Dak Nong Province, Vietnam. This farm purchased 14 cattle from a livestock market located near the border between Nghe An Province and Lao PDR 2 days before

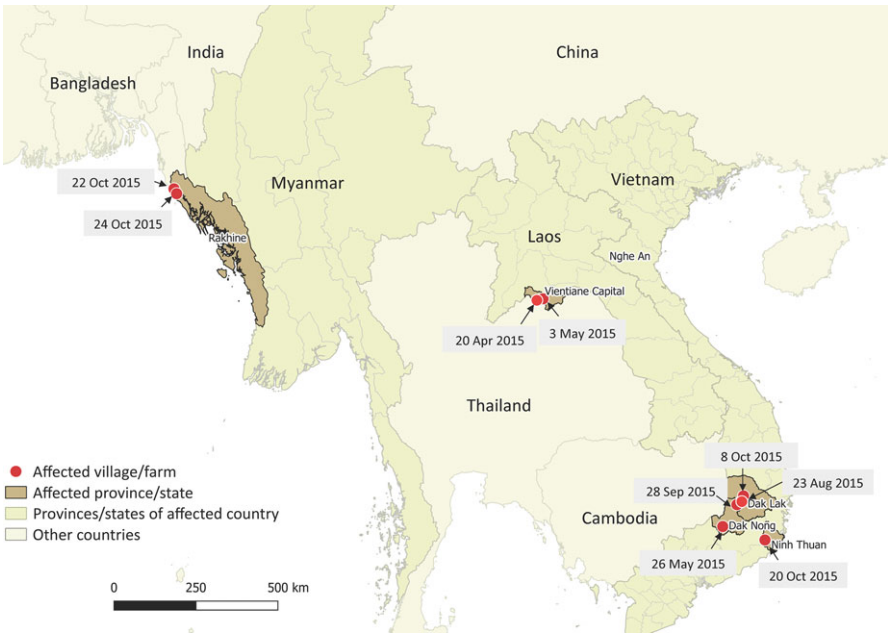

FIGURE 1 Locations and starting dates of the foot-and-mouth disease outbreaks due to O/ME-SA/Ind-2001d described in this study.

the outbreak. During August and September, additional outbreaks due to O/ME-SA/Ind-2001d were detected in two neighbouring pig farms near the border between Dak Nong and Dak Lak Provinces. Both pig farms reported regularly feeding pigs with untreated swill containing meat scraps. In October, O/ME-SA/Ind-2001d further caused outbreaks in two cattle farms in Dak Lak and Ninh Thuan Provinces, but the probable sources of the outbreaks were not determined. All the livestock in the five affected farms were kept for meat production, while cattle were additionally used for agriculture activities.

On 22 October 2015, an outbreak due to O/ME-SA/Ind-2001d was detected in a village in Rakhine State, Myanmar, and 2 days later in a neighbouring village. Backyard cattle were affected, but no cases were reported in pigs or goats. Both affected villages were located close to the border with Bangladesh and reported frequent unregulated cross-border movements of humans and animals.

3.2 | Disease control measures

In all these outbreaks, sick animals were separated and treated with antibiotics and herbal medicine, and affected premises were disinfected. Restriction of livestock movements during the outbreak was recommended by local veterinary authorities but not strictly enforced. In Lao PDR, ring vaccination was conducted in 15 villages within a 5 km radius from the affected villages 2 weeks after the first outbreak started. The vaccines used were inactivated FMD vaccines composed of O_1/Manisa, O/3039, A/Malaysia/1997 and A22/Iraq strains (≥six protective dose 50%) and were supplied by the OIE SEACFMD Vaccine Bank. Approximately 60% of the cattle and buffalo population in this area were vaccinated. In Vietnam, emergency vaccinations were conducted 2-3 weeks after the outbreak started in Tam Thang Commune of Dak Nong Province and in Ma Noi Commune of Ninh Thuan Province. Approximately 60%–70% of cattle and buffaloes of each commune were vaccinated with imported inactivated vaccines containing O_1/Manisa and O/3039 strains (≥three protective dose 50%). Animals of other species were not vaccinated. Emergency vaccination was not conducted in any other outbreaks described in this study.

3.3 | Genetic characterization of FMDV O/ME-SA/Ind-2001d isolates

Phylogenetic analysis of viral VP1 gene sequences showed that viruses recovered from the above outbreaks belonged to two different genetic clusters within the O/ME-SA/Ind-2001d sublineage (Figure 2). High pairwise nucleotide identities were observed among isolates within the same country (99.6%–100% identity within Lao PDR; 99.7%–99.8% identity within Vietnam; 99.8% identity within Myanmar). Notably, the isolates from Lao PDR and Vietnam were also closely related (99.1%–99.2% identity), and they were most similar (98.1%–98.4% identity) to a virus previously circulating in India in 2013 (KM264361). The O/ME-SA/Ind-2001d isolates from Myanmar were found most closely related (99.5% identity) to a virus present in Bangladesh in 2015 (KY077610), but divergent (92.0% identity) from the isolates collected from Lao PDR and Vietnam.

4 | DISCUSSION

Multiple lineages of serotype O FMDV, including O/SEA/Mya-98, O/ME-SA/PanAsia and O/CATHAY, are recognized to cocirculate in mainland SEA. In addition to these established lineages, this study reports the first detections of the O/ME-SA/Ind2001 viral lineage from field outbreaks in 2015 in three countries in SEA (Lao PDR, Vietnam and Myanmar). This viral lineage has also recently spread from the Indian subcontinent in a westerly direction to cause

TABLE 1 Overview of the foot-and-mouth disease outbreaks due to O/ME-SA/Ind-2001d described in this study

Country	Province/ State	Village/Farm	Date of outbreak (2015)		Species	Number of			Virus detected (Accession No.[a])
			Onset	Resolution		Susceptible animals	Cases	Deaths	
Lao PDR	Vientiane Capital	Nakhoun Village	20 Apr	20 Jun	Cattle	250	70	0	O/LAO/11/2015 (KY399460) O/LAO/12/2015 (KY399461)
					Buffalo	117	23	0	
					Pig	10	0	0	
					Goat	30	0	0	
		Naxup Village	3 May	20 Jun	Cattle	627	7	0	O/LAO/15/2015 (KY399463)
					Buffalo	756	7	0	
Vietnam	Dak Nong	A farm in Kien Thanh Commune	26 May	19 Jun	Cattle	15	12	0	O/VIT/9595/2015 (KY399464)
	Dak Lak	A farm in Thanh Nhat Commune	23 Aug	12 Oct	Pig	50	45	7	O/VIT/16451/2015 (KY399465)
	Dak Nong	A farm in Tam Thang Commune	28 Sep	9 Nov	Pig	328	306	78	O/VIT/17867/2015 (KY399466)
	Dak Lak	A farm in Cu Dlie M'nong Commune	8 Oct	30 Oct	Cattle	47	47	0	O/VIT/18280B/2015 (KY399467)
	Ninh Thuan	A farm in Ma Noi Commune	20 Oct	13 Nov	Cattle	5	5	0	O/VIT/19350/2015 (KY399468)
Myanmar	Rakhine	Kaingyi Village	22 Oct	15 Nov	Cattle	42	7	0	O/MYA/2493/2015 (KY399469)
					Pig	17	0	0	
					Goat	63	0	0	
		Chan Pyin Village	24 Oct	17 Nov	Cattle	38	5	0	O/MYA/2496/2015 (KY399470)
					Pig	27	0	0	
					Goat	58	0	0	

[a]Accession number of viral VP1 sequence; ME-SA, Middle East-South Asia; PDR, People's Democratic Republic.

outbreaks in the Middle East and North Africa (Knowles et al., 2016). The O/ME-SA/Ind2001d outbreaks in SEA affected cattle, buffaloes and pigs, where clinical manifestations of FMD were similar to those generated by the endemic FMDV lineages. However, clinical cases were not reported in goats, despite opportunities of direct and indirect contacts with infected large ruminants in a rural village setting. This is also a common finding for FMD outbreaks caused by the endemic FMDV lineages where subclinical infections in small ruminants are very common and difficult to diagnose (Rout, Subramaniam, Mohapatra, & Pattnaik, 2016; Sherman, 2011). Due to resource deficits, emergency vaccinations were not applied in the majority of outbreaks reported here. This, coupled with a lack of strict enforcement of movement control in the affected areas, may create opportunities for onward transmissions of this exotic strain in this region.

Genetic analysis of viral VP1 sequences showed that O/ME-SA/Ind-2001d isolates collected from Lao PDR and Vietnam were closely related, but divergent from the isolates from Myanmar, indicating that at least two independent introductions of this strain into the region have occurred. Due to the substantial under-reporting of FMD outbreaks, insufficient sampling and delay in molecular strain typing in this region (OIE-SEACFMD, 2016b), the primary outbreaks caused by O/ME-SA/Ind-2001d as well as the exact time and origins of the viral incursions remain unidentified. Yet, Bangladesh and India represent the most likely direct origins given the close genetic relationships between outbreak viruses, the geographic proximity and the close links via trade in animals and animal products with SEA (Di Nardo et al., 2011; Landes, Melton, & Edwards, 2016). The incursion of O/ME-SA/Ind-2001d into the western border of Myanmar is likely attributed to the frequent Bangladesh–Rakhine cross-border movements of livestock and humans, as supported by the close genetic relatedness between the isolates recovered from the two countries (Figure 2). A similar transboundary spread of FMDV of serotype A was detected in Rakhine State in 2010, the first recorded outbreak in Myanmar due to this serotype since 1978, and the causative virus was found to be closely related to viruses previously circulating in India in 2000 (Di Nardo et al., 2011). The O/ME-SA/Ind-2001 lineage has become dominant in India since 2008 and caused extensive FMD

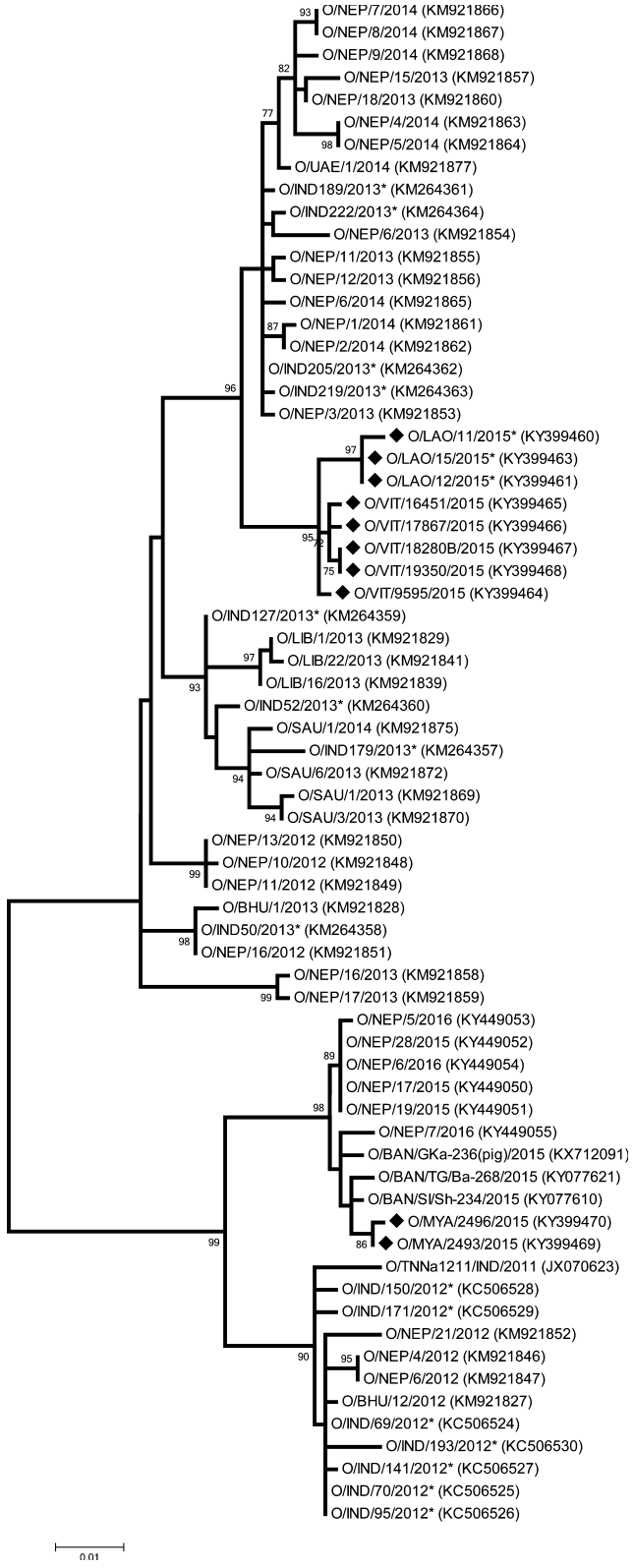

FIGURE 2 Maximum-likelihood tree based on viral VP1 sequences showing the relationships between foot-and-mouth disease virus O/ME-SA/Ind-2001d isolates collected from South-East Asia in 2015 and from South Asia, the Middle East and North Africa during 2011–2016. The sequence generated in this study is shown with a diamond symbol (♦). The sequence that does not have a WRLFMD reference number is marked with an asterisk (*)

outbreaks in this country during 2013–2014 (Subramaniam et al., 2015). The Indian isolates from this epidemic were closely related to the O/ME-SA/Ind-2001d isolates recovered from Lao PDR and Vietnam (Figure 2). However, it remains unclear whether the outbreaks in the two neighbouring SEA countries are epidemiologically linked or due to separate incursions from similar sources in India. A recent study on livestock movements in the Greater Mekong Sub-Region has shown that many live cattle and buffaloes from India and Bangladesh move through Myanmar, Thailand and Lao PDR towards the high-value markets in Vietnam and China annually (Smith et al., 2015). However, the management of animal movements is quite variable across SEA, and many of the cross-border movements are informal (Poolkhet et al., 2016; Smith et al., 2015), which directly impacts inter- and intraregional spread of various animal diseases, including FMD caused by O/ME-SA/Ind-2001d.

Apart from live animals, there is also well-developed trade of bovine products from India into SEA. Retrospective investigation in Vientiane Capital found that frozen buffalo tongue was officially imported for domestic consumption in December 2014 from Uttar Pradesh of India, where O/ME-SA/Ind-2001d is endemic (Subramaniam et al., 2015). Multiple importations of frozen buffalo deboned meat from India during 2014–2015 were also documented by the government of Lao PDR, but the risk of transmitting FMD via deboned meat is generally considered very low given that the acidification during rigour mortis is normally sufficient to inactive FMDV in muscle tissues (Paton, Sinclair, & Rodriguez, 2010). Vietnam also frequently imports buffalo products from India, both legally and illegally, with the majority of commodities being frozen deboned meat and a small amount of offal (e.g., tongue and heart) and bone-in meat. Foot-and-mouth disease viruses can survive for long periods in offal, lymph nodes and bone marrow (Cottral, 1969). Although the possible associations between the imported high-risk buffalo products and the field outbreaks have not yet been demonstrated in both Lao PDR and Vietnam, previous reports have shown that importation of such high-risk materials from FMD endemic countries can result in introductions of FMD into free countries (USDA, 1994; Valarcher et al., 2008).

The detections of O/ME-SA/Ind-2001d have motivated countries in SEA to facilitate molecular strain typing of field isolates collected since 2015. While preparing this article, viruses belonging to the O/ME-SA/Ind-2001d sublineage have been identified as causing FMD outbreaks in cattle, buffaloes and pigs from several northern provinces (Son La, Bac Kan, Lao Cai, Yen Bai, Lang Son and Nghe An) of Vietnam from June 2015 to September 2016 (WRLFMD, 2017a). This strain further spread to Thailand in September 2016 and has caused widespread outbreaks in cattle, buffaloes and pigs since then (OIE, 2017; WRLFMD, 2016). Additional outbreaks caused by O/ME-SA/Ind2001d were detected in cattle in Sagaing, Ayewaddy and Yangon States of Myanmar in January 2017 (WRLFMD, 2017b). Further investigations of these outbreaks and analysis of these newly generated viral sequences are needed to disclose their links with those described in this study. The spread of O/ME-SA/Ind-2001d over large distances in a short time period is similar to the earlier observations in the Middle East and North Africa (Bachanek-Bankowska

et al., 2016; Knowles et al., 2016; Valdazo-Gonzalez et al., 2014). Although so far, the O/SEA/Mya-98 viral lineage has continued to dominate the serotype O outbreaks in SEA (OIE, 2017; OIE-SEACFMD, 2016b), it is difficult to speculate whether O/ME-SA/Ind-2001d will only seed a limited number of onward outbreaks as occurred in the Middle East (Knowles et al., 2016; Valdazo-Gonzalez et al., 2014), or establish itself in the region and outcompete other endemic FMDV lineages as it has in India (Yuvaraj et al., 2013) and Bangladesh (Nandi, Rahman, Momtaz, Sultana, & Hossain, 2015). Constant monitoring and characterization of field isolates are required to better understand the epidemiology of FMD in this region and adjust control measures.

In SEA, Thailand and Myanmar produce FMD vaccines and any vaccines used in other countries are imported. Virus-neutralization assays and cross-protection challenge studies have shown that viruses incorporated in the imported FMD vaccines—O_1/Manisa and O/3039—can confer effective protection against viruses of the O/ME-SA/Ind-2001d sublineage (Fishbourne et al., 2017; Singanallur et al., 2016). Further research is needed to examine the antigenic match between O/ME-SA/Ind-2001d and vaccine strains used by Thailand and Myanmar. Also, close monitoring of the antigenic evolution of O/ME-SA/Ind-2001d and vaccine effectiveness in the field is needed to ensure a sustainable risk mitigation by vaccination.

Multiple trans-pool movements of FMDV from the Indian subcontinent to SEA have been previously recorded. The O/ME-SA/PanAsia lineage was introduced from India into SEA in the late 1990s and has been circulating since then (Knowles et al., 2005). In addition, the O/ME-SA/PanAsia-2 lineage originating from India caused outbreaks in Peninsular Malaysia during 2003-2009 (Abdul-Hamid et al., 2011). Moreover, the Asia 1 serotype extended from the Indian subcontinent to SEA before 1996 and had caused sporadic outbreaks until 2007, and it was reintroduced into Rakhine State of Myanmar in 2017 (Le, Nguyen, Park et al., 2010; Valarcher et al., 2009; WRLFMD, 2017c). The incursions of O/ME-SA/Ind-2001d once again highlight the porous borders between SEA and the Indian subcontinent as well as the continuous threat posed by FMD as a transboundary disease. The sharp rising demand for meat in China and Vietnam is expected to continue to drive substantial movements of livestock from the Indian subcontinent and different parts of SEA (Smith et al., 2015; Thornton, 2010), posing a significant challenge to controlling the risks of transboundary diseases including FMD. The OIE, through its SEACFMD Campaign, is coordinating multinational collaboration to monitor cross-border animal movements and developing approaches to promote safe and regulated international trade to mitigate such risks. In addition, governments in SEA are under increased pressure to allow importation of meat from India to meet domestic demands. It is highly recommended to base import decisions on a scientific risk assessment, in accordance with the OIE Terrestrial Animal Health Code, to minimize the risk of exotic animal disease incursions. Furthermore, improved surveillance of FMD and diagnostic capacity are essential for the rapid detection of emergence of any new strain and the timely institution of control and prevention measures, such as quarantine and movement control along risk pathways, shut down of livestock markets at risk, selection of appropriate vaccine strains for use and storage in emergency vaccine reserves. All of these can only be achieved by continued political commitment coupled with proper allocation of needed resources from countries and international donors.

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