


Foot-and-mouth disease outbreaks due to an exotic serotype Asia 1 virus in Myanmar in 2017

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Abstract

In January 2017, two villages located in Rakhine State of Myanmar reported clinical signs in cattle suggestive of foot-and-mouth disease virus (FMDV) infection. Laboratory analysis identified the outbreak virus as FMDV serotype Asia 1, which represented the first detection of this serotype in Myanmar since 2005 and in the region of South-East Asia (SEA) since 2007. Genetic analysis revealed that the outbreak virus was different from historical viruses from Myanmar and was more closely related to viruses circulating in Bangladesh and India during 2012–2013, indicating that a novel viral introduction had occurred. The precise origin of the outbreaks was not clear, but frequent informal livestock trade with South Asia was reported. Responses to the outbreaks involved disinfection, quarantine and animal movement restrictions; no further outbreaks were detected under the present passive surveillance system. Detection of serotype Asia 1 highlights the complex and dynamic nature of FMDV in SEA. Active surveillance is needed to assess the extent and distribution of this exotic Asia 1 strain and continued vigilance to timely detect the occurrence of emerging and re-emerging FMDV strains is essential.

KEYWORDS

foot-and-mouth disease virus, genetic analysis, Myanmar, serotype Asia 1, South-East Asia

1 | INTRODUCTION

Foot-and-mouth disease (FMD) is a highly contagious viral disease affecting cloven-hoofed domestic and wild animals. It is endemic in mainland South-East Asia (SEA) comprising Cambodia, Lao People's Democratic Republic, Peninsular Malaysia, Myanmar, Thailand and Vietnam, and poses a significant burden to the agriculture sector owing to loss of animal production and access to international markets (Nampanya et al., 2016). Control of FMD in the region is constrained by gaps in disease surveillance and reporting, substantial unregulated livestock movements, and insufficient vaccination coverage (OIE-SEACFMD, 2016). FMD is caused by FMD virus (FMDV) that exists in seven immunologically distinct serotypes, namely O, A,

C, Asia 1, Southern African territories (SAT)1, SAT2 and SAT3. Serotype Asia 1 FMDV has been traced back to 1940s in Rawalpindi (now part of Pakistan) (Jamal, Ahmed, Hussain, & Ali, 2010) and was detected in India in 1951 (Dhanda, Gopalakrishnan, & Dhillion, 1957). In the following years, it was detected in countries both within and outside of South Asia, including those in SEA, East Asia, Central Asia, the Middle East and eventually Europe (Ansell, Samuel, Carpenter, & Knowles, 1994; Islam, Rahman, Adam, & Marquardt, 2001; Kitching, 2005; Schumann et al., 2008; Valarcher et al., 2009). This serotype has previously been reported in all countries in mainland SEA, where it co-circulated with serotypes O and A (OIE, 2018). Unlike the yearly occurrence of outbreaks caused by serotypes O and A, only sporadic outbreaks due to serotype Asia 1 were detected in SEA during 2000–2007, including in Myanmar in 2005 and in Vietnam during 2005–2007 (Abila, Gordoncillo, & Kukreja, 2012). While

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FIGURE 1 Map illustrating the locations of the foot-and-mouth disease outbreaks caused by serotype Asia 1 in Myanmar in 2017 [Colour figure can be viewed at wileyonlinelibrary.com]

serotype Asia 1 remains persistent in South Asia (Jamal, Ferrari, Ahmed, Normann, & Belsham, 2011; Mahapatra et al., 2015; Ullah et al., 2017), the apparent lack of field outbreaks due to this serotype in SEA since 2007 has been unexpected, albeit it cannot be excluded that this serotype has been circulating undetected below the level of the disease surveillance that is undertaken in the region (OIE-SEACFMD, 2016). During this period, antibodies against serotype Asia 1 were found in unvaccinated sheep and goats in Central Myanmar as determined by the liquid-phase blocking ELISA (Phyoe et al., 2017), but the majority of sera examined had antibodies reacting to more than one FMDV serotype, making it difficult to confirm the presence of Asia 1 due to serological cross-reactivity of the test used. This paper describes the detection of serotype Asia 1 from the field outbreaks in Myanmar in 2017, after 10 years epidemiological silence in SEA, and the corresponding field and laboratory investigations that were undertaken to help understand the origin of these outbreaks and onward risks of disease spread.

2 | MATERIALS AND METHODS

2.1 | Field report

Surveillance for FMD in Myanmar is primarily through a passive approach, relying on notification of an outbreak through clinical presentation. On 15 January 2017, suspected clinical cases of FMD in cattle were reported in Sapar Htar village of Kyaukpyu Township in the northern part of Ramree Island within Rakhine State of Myanmar, as shown in Figure 1 (map created using Quantum GIS version 2.18.12). A day later the disease was detected in the neighbouring Kyun Gyaung village with shared grazing land. Free ranging and co-mingling of livestock during day time is a common practice in this area during the dry season (between October and May). The animals in the affected villages had never been vaccinated against FMD, and

no clinical cases of FMD had previously been recorded since 2001. Following the detection of serotype Asia 1, the Livestock Breeding and Veterinary Department of Myanmar implemented a programme to enhance awareness in the livestock sector across the country for the reporting and collection of samples from cases compatible with FMD.

2.2 | Molecular diagnosis

A bovine tongue epithelial specimen was collected from each affected village (total $n = 2$). The tissue suspensions (10% w/v in PBS) prepared from both samples tested positive for serotype Asia 1 by the FMDV antigen detection ELISA (IZSLER, Brescia, Italy; Grazioli, Brocchi, Dho, & Ferris, 2010) at the Myanmar National FMD Laboratory at Yangon. The samples were subsequently submitted to the SEA Regional Reference Laboratory for FMD (RRLFMD, Pak-chong, Thailand) and to the World Reference Laboratory for FMD (WRLFMD, Pirbright, United Kingdom). At both laboratories, the presence of FMDV in the two submitted samples was confirmed by the pan-serotypic real-time reverse transcription-PCR (RT-PCR) (Callahan et al., 2002; Shaw et al., 2007). At RRLFMD, the detection of FMDV serotype Asia 1 in both samples was further confirmed by the antigen ELISA as described elsewhere (Roeder & Le Blanc Smith, 1987). At WRLFMD, the viral protein 1 (VP1) coding region was amplified by RT-PCR and sequenced as previously described (Knowles, Wadsworth, Bachanek-Bankowska, & King, 2016).

2.3 | Sequence analysis

The VP1 coding sequence obtained was compared with sequences of Asia 1 viruses that are available on GenBank. Sequences were aligned using BioEdit v7.2.5 (Hall, 1999). A maximum-likelihood phylogenetic tree was generated by MEGA7 (Kumar, Stecher, & Tamura,

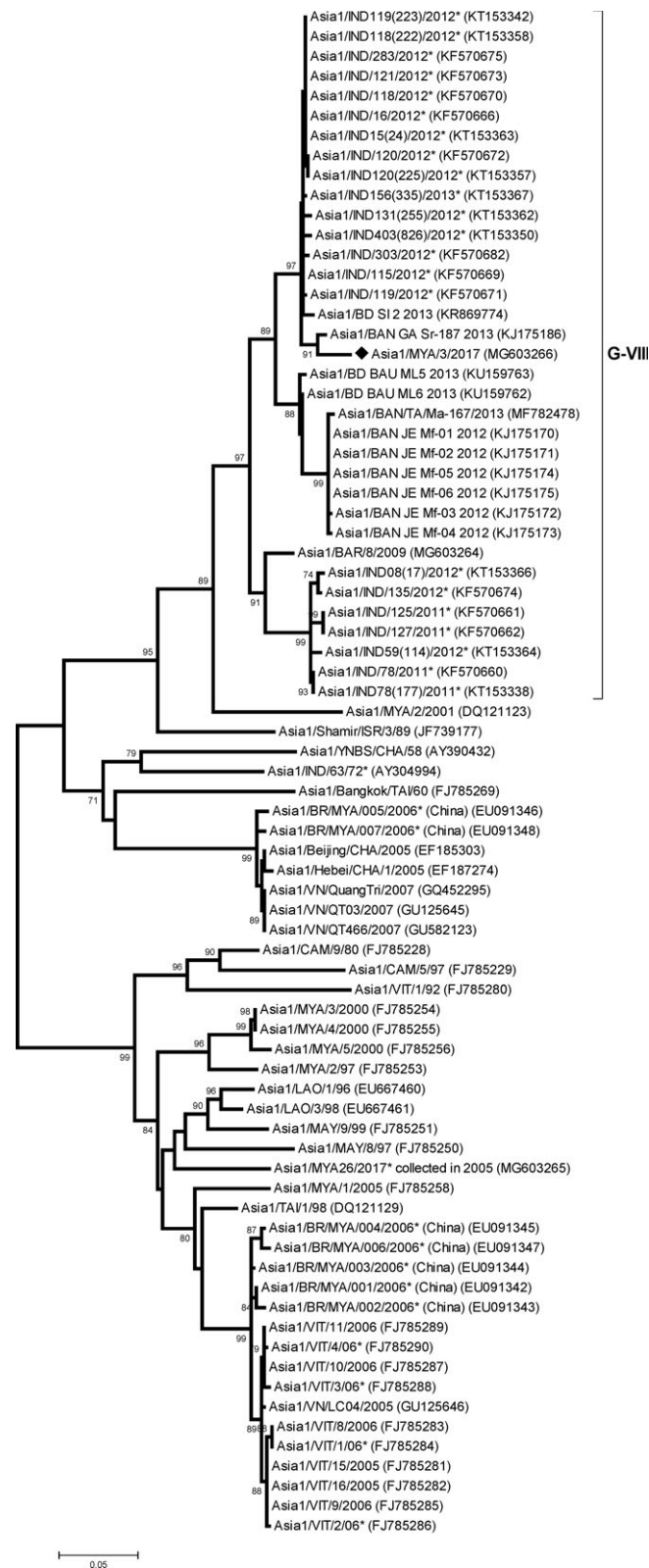


FIGURE 2 Maximum-likelihood phylogenetic tree based on viral VP1 coding sequences showing the relationship between the serotype Asia 1 foot-and-mouth disease virus circulating in Myanmar in 2017 (shown with a diamond symbol [◆]) and viruses previously circulating in South-East Asia, South Asia and China. Sequences with reference numbers that were not designated by the WRLFMD are marked with an asterisk (*)

2016) based on the Tamura-Nei nucleotide substitution model (Tamura & Nei, 1993). In order to establish the parameters for phylogenetic analyses, a discrete Gamma distribution was used to model evolutionary rate differences among sites [five categories (+G, parameter = 0.6101)] which allowed for some sites to be evolutionarily invariable [(+I), 27.1722% sites].

3 | RESULTS AND DISCUSSION

Clinical signs including excessive salivation, lameness, and blisters on tongue, mouth and hooves were observed in 20.3% (59/291) cattle at the two affected villages, but no clinical cases were exhibited in either buffaloes ($n = 22$) or goats ($n = 65$). Similar observations have been reported in outbreaks caused by the endemic FMDVs in SEA where clinical diseases have been reported mainly in cattle (OIE-SEACFMD, 2016). No deaths due to FMD were reported. Sick cattle were isolated and affected premises were disinfected immediately after the onset of the disease. Emergency vaccination was not implemented; however, quarantine and animal movement controls were applied at both affected villages from 20 January 2017 up until resolution of the last case on 6 February 2017. No further suspected FMD cases were reported from Kyaukpyu Township.

The viral VP1 coding sequence was retrieved from only one clinical sample (GenBank accession number MG603266), and was categorised as belonging to the G-VIII lineage of serotype Asia 1 (Figure 2). Genetic analysis of the VP1 coding sequence showed that this virus shared the closest sequence identity (97.1%–97.6%) to viruses present in India and Bangladesh during 2012–2013, and only 82.9%–83.7% identity with historical viruses of this serotype detected in Myanmar in 2005. These results indicate that the recent Myanmar outbreaks are due to a novel introduction of Asia 1 virus that likely originated from a South Asian country, rather than a continual viral circulation within Myanmar, albeit the limited information about the more recent Asia 1 viruses from the neighbouring countries in South Asia makes it difficult to trace the precise origin of this incursion. The affected Kyaukpyu Township reported frequent trade of livestock with mainland Myanmar and Bangladesh. However, these movements were informal, and quarantine and biosecurity measures were rarely applied. Due to the gaps in surveillance of FMD and infrequent monitoring of animal movements in this area, the exact time and precise route of the incursion of Asia 1 cannot be conclusively established, and the index case may have occurred somewhere else in Myanmar and earlier than the detected outbreaks.

Since serotype Asia 1 had not been detected in the past 10 years anywhere in SEA, many countries including Myanmar have progressively withdrawn the Asia 1 strain from the vaccine composition, leaving a substantial livestock population susceptible to infection with serotype Asia 1. Currently, there are no reports under the passive surveillance system to indicate that serotype Asia 1 is still circulating in Rakhine State, or has spread to other locations in Myanmar or other countries in SEA. However, continued outbreaks

may go unnoticed due to the gaps in disease surveillance and reporting in the region. In Central Myanmar, vaccines used for mass vaccination in large ruminants have been recently adjusted to incorporate a widely used vaccine strain - Asia 1/Shamir. The inability to isolate “live” FMDV for these cases from Myanmar excludes the possibility to test samples using serological matching methods; however, previously undertaken *in vitro* vaccine-matching analyses using the virus neutralisation assay indicate that there is a close antigenic relationship between the Asia 1/Shamir vaccine strain and two related Asia 1/G-VIII lineage FMDVs ($r-1$ values of 0.80 and 0.78 for BAR/8/2009 and BAR/9/2009, respectively) (OIE/FAO FMD Reference Laboratories Network, 2009). In the absence of specific data for the FMDV causing the outbreaks in Myanmar, these findings indicate that the Asia 1/Shamir vaccine should be expected to confer effective protection against the G-VIII viral lineage.

Rakhine State of Myanmar borders Bangladesh and extensive uncontrolled cross-border animal movements occur, making it a “gateway” for new disease incursions from South Asia into SEA. In addition to the recent Asia 1 outbreaks, multiple trans-regional movements of FMDVs from South Asia to Rakhine State have previously been reported, including serotype Asia 1 virus in 2001 (Valarcher et al., 2009), serotype A virus in 2010 (WRLFMD, 2010), and the O/ME-SA/Ind-2001 strain in 2015 (Qiu et al., 2017). FMDVs from the O/ME-SA/Ind-2001 lineage with close genetic relationships to the Myanmar viruses have been recently detected in some other countries in SEA including Thailand, Vietnam and Malaysia (WRLFMD, 2016, 2017b, 2018) as well as more widely in China and South Korea (WRLFMD, 2017a,c). A recent risk analysis study further demonstrates the significant risk of incursions of exotic FMDVs from the countries to the west of SEA (Bartels, Afonso, Sieng, & McLaws, 2017). Therefore, close monitoring of the FMD situation, especially in the high-risk areas for the entry and spread of exotic strains, is essential for the region to adjust control measures including vaccine compositions. Also, enhanced multinational collaboration is needed to support high-risk countries, in order to strengthen their capacity for surveillance and response and to minimise the regional impacts of FMD.

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epidemiological investigation. KSL processed clinical samples and performed FMDV antigen-detection ELISA. SU, NJK, JW and DPK contributed to the diagnosis confirmation, viral sequencing and genetic analysis. YQ coordinated this study and wrote the manuscript in consultation with RA, DPK and NJK.

CONFLICT OF INTEREST

None.

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