Transboundary and Emerging Diseases

SHORT COMMUNICATION

Outbreaks of Foot-and-Mouth Disease in Libya and Saudi Arabia During 2013 Due to an Exotic O/ME-SA/Ind-2001 Lineage Virus

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Summary

Foot-and-mouth disease viruses are often restricted to specific geographical regions and spread to new areas may lead to significant epidemics. Phylogenetic analysis of sequences of the VP1 genome region of recent outbreak viruses from Libya and Saudi Arabia has revealed a lineage, O-Ind-2001, normally found in the Indian subcontinent. This paper describes the characterization of field viruses collected from these cases and provides information about a new real-time RT-PCR assay that can be used to detect viruses from this lineage and discriminate them from other endemic FMD viruses that are co-circulating in North Africa and western Eurasia.

Foot-and-mouth disease (FMD) is a vesicular condition of cloven-hoofed livestock which may also occur in related wildlife species (Sutmoller et al., 2003). It is caused by FMD virus (family *Picornaviridae*, genus *Aphthovirus*) and occurs as seven immunologically distinct serotypes, namely O, A, C, Asia 1, SAT 1, SAT 2 and SAT 3 (Sutmoller et al., 2003). FMD is endemic in much of Africa and Asia and occurs in defined areas of South America (Rweyemamu et al., 2008). Each serotype has a different geographical range with type O being the most widespread (Knowles and Samuel, 2003). Additionally, within each serotype, genotypes may also be geographically restricted and are called topotypes (Knowles and Samuel, 2003). Eleven topotypes have been described for serotype O, but some may be extinct or occur rarely (Samuel and Knowles, 2001). The Middle East-South Asia (ME-SA) topotype mainly occurs in southern Asia and occasionally in Central and East Asia, North Africa and Europe (Samuel and Knowles, 2001). Genetic lineages within these topotypes may also be limited in their geographical distribution. Currently, the principal lineages within the ME-SA topotype are Ind-2001 (occurring in the Indian subcontinent, including Nepal and Bhutan, but excluding Pakistan), PanAsia (occurring in South-East Asian countries and causing recent exotic incursions into the People's Republic of China, the Russian Federation and Kazakhstan and PanAsia-2 (occurring in Western Asia, from Pakistan to Turkey). Although the Ind-2001 lineage (Subramaniam et al., 2013) is normally restricted to India,

Nepal, Bhutan and Bangladesh, it has occasionally spread westwards causing limited outbreaks, for example Jordan in 1995, Israel in 1996, Bahrain, Kuwait, Saudi Arabia and the United Arab Emirates (UAE) in 1997, Bahrain, Oman, Saudi Arabia and the UAE in 2001, Palestinian Autonomous Territories and Israel in 2002, the UAE in 2008–2009 and Iran in 2009. The Ind-2001 lineage has been divided into four sublineages, a, b, c and d. Although sublineages a and b differ by only 3.6% nt, the other sublineages differ by 7–10% nt. A recent isolate from Bangladesh (collected in 2013; GenBank accession KF985189) differs to representative members of the d sublineage by 4.9–7.8% nt and to lineage a, b and c by 7.2%, 7.4% and 9.3% nt, respectively.

During recent surveillance of FMD viruses in North Africa and the Middle East, the sequence of the genome region coding for one of the outer capsid polypeptides (VP1) of a number of type O virus isolates was determined using methods previously described (Knowles et al., 2005; Yuvaraj et al., 2013) (Table 1). Viruses from outbreaks in Libya (September to December 2013) and Saudi Arabia (August to November 2013) were found to belong to the ME-SA/Ind-2001 lineage. Phylogenetic analyses showed these viruses are most closely related to isolates from India and Bhutan collected in 2013 (Fig. 1) and provide evidence that the FMD outbreaks in Libya and Saudi Arabia have arisen through separate introductions of this virus strain into these two countries. The complete genome sequences of representative isolates from Bhutan, Saudi Arabia and Libya have also been determined and have been described elsewhere (Valdazo-González et al., 2014).

Although a real-time RT-PCR (rRT-PCR) assay has recently been developed for the detection of FMD viruses which are endemic in the Middle East (Reid et al., 2014), it

Table 1. Origin of recent samples collected in Bhutan, India, Libya, Saudi Arabia and the United Arab Emirates[a]

WRLFMD Ref. no.	Geographic origin	Date of collection	Accession no.[b]
O/BHU/1/2013	Chukha, Bhutan	06/06/2013	KM921828
O/IND50/2013[c]	Karnataka, India	31/01/2013	KM264358
O/IND52/2013[c]	Karnataka, India	08/02/2013	KM264360
O/IND127/2013[c]	Karnataka, India	14/03/2013	KM264359
O/IND179/2013[c]	Karnataka, India	17/07/2013	KM264357
O/IND189/2013[c]	Karnataka, India	27/07/2013	KM264361
O/IND205/2013[c]	Karnataka, India	14/08/2013	KM264362
O/IND219/2013[c]	Uttar Pradesh, India	08/09/2013	KM264363
O/IND222/2013[c]	Odisha, India	25/07/2013	KM264364
O/LIB/1/2013	Zliten, Murqub District, Libya	01/09/2013	KM921829
O/LIB/2/2013	Zliten, Murqub District, Libya	01/09/2013	KM921830
O/LIB/3/2013	Zliten, Murqub District, Libya	01/09/2013	KM921831
O/LIB/4/2013	Zliten, Murqub District, Libya	01/09/2013	KM921832
O/LIB/5/2013	Zliten, Murqub District, Libya	01/09/2013	KM921833
O/LIB/6/2013	Zliten, Murqub District, Libya	01/09/2013	KM921834
O/LIB/7/2013	Zliten, Murqub District, Libya	08/09/2013	KM921835
O/LIB/11/2013	Tripoli District, Libya	12/09/2013	KM921836
O/LIB/12/2013	Tripoli District, Libya	12/09/2013	KM921837
O/LIB/13/2013	Tripoli District, Libya	14/09/2013	KM921838
O/LIB/16/2013	Tripoli District, Libya	30/09/2013	KM921839
O/LIB/17/2013	Misrata District, Libya	01/10/2013	KM921840
O/LIB/22/2013	Tripoli District, Libya	01/12/2013	KM921841
O/SAU/1/2013	Al-Kharj, Central region, Saudi Arabia	07/08/2013	KM921869
O/SAU/3/2013	Al-Kharj, Central region, Saudi Arabia	07/08/2013	KM921870
O/SAU/4/2013	Al-Kharj, Central region, Saudi Arabia	07/08/2013	KM921871
O/SAU/6/2013	Dhurma, Central region, Saudi Arabia	22/11/2013	KM921872
O/SAU/7/2013	Dhurma, Central region, Saudi Arabia	27/11/2013	KM921873
O/SAU/8/2013	Dhurma, Central region, Saudi Arabia	27/11/2013	KM921874
O/SAU/1/2014	Al-Kharj, Central region, Saudi Arabia	01/02/2014	KM921875
O/UAE/1/2014	Abu Dhabi, United Arab Emirates	08/01/2014	KM921877
O/UAE/2/2014	Abu Dhabi, United Arab Emirates	08/01/2014	KM921878

[a]All samples were collected from cattle, except the UAE samples which were from gazelles.
[b]Accession numbers of other VP1 sequences determined in this study are KM921813–KM921827, KM921842–KM921868 and KM921876.
[c]PD-FMD reference number.

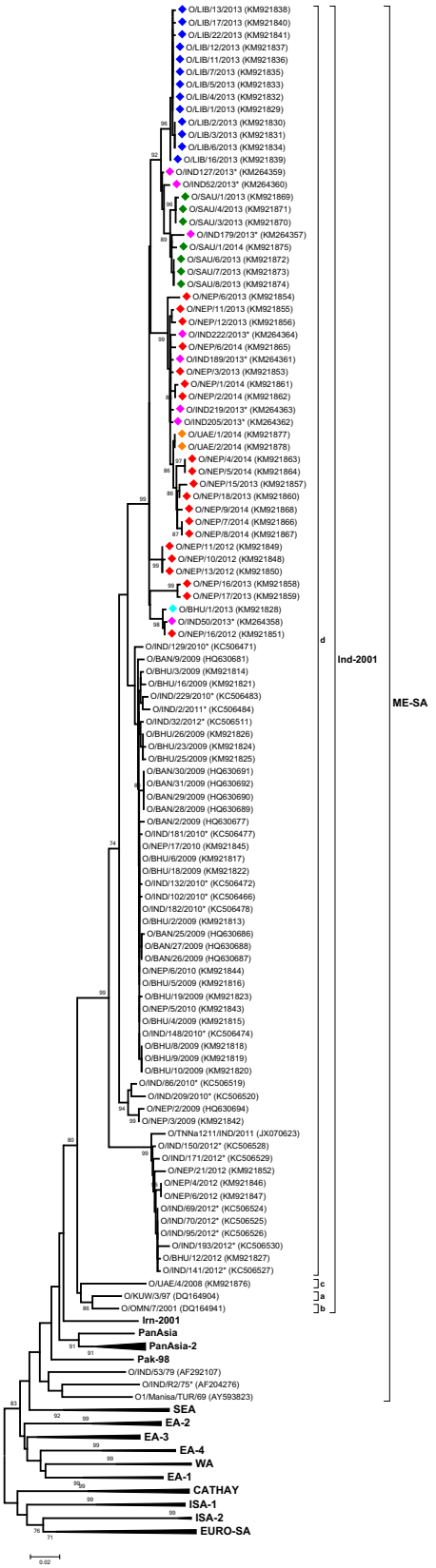

Fig. 1. Midpoint-rooted Neighbour-joining tree showing the relationships between the FMDV VP1 sequences studied. The tree was produced in MEGA 6.06 (Tamura et al., 2013) using the Kimura 2 parameter nucleotide substitution model. Sequences most closely related to the North African and Saudi Arabian viruses are colour-coded by country: Bhutan (cyan), India (pink), Libya (blue), Nepal (red), Saudi Arabia (green) and the United Arab Emirates (orange).

has not been extensively tested using O-Ind-2001 viruses. Therefore, to facilitate rapid identification of this lineage in FMD laboratories in North Africa and the Middle East, we have developed an Ind-2001-specific rRT-PCR assay. rRT-PCR primers and probes to detect FMDV type O Ind-2001 lineage were designed to target conserved regions within the VP1 coding sequence. The probe (5′-CTG CTC GCC ATT CAC CCG-3′) labelled with FAM and BHQ-1 at 5′ and 3′ ends, respectively, was designed to anneal specifically to sequences of the Ind-2001 lineage, while the primers (FMDV/O484-502F 5′-CCT CCT TCA AYT ACG GTG-3′ and FMDV/O/620-601R 5′- GCC ACA ATC TTY TGT TTG TG-3′) can anneal to a wider range of FMDV type O virus sequences. cDNA was synthesized with 5 μl RNA in 25 μl total volume reaction mix containing 0.8 μM total concentration of each primer and 0.3 μM total concentration of the probe, 12.5 μl 2× reaction buffer and 1 μl Superscript III/Platinum Taq enzyme mix (Life Technologies, Paisley, UK). Amplification conditions (MX3005p; Agilent Technologies, Stockport, UK) were identical to those used for pan-serotypic detection of FMDV (Reid et al., 2009): 60°C for 30 min, 95°C for 10 min followed by 50 cycles of 95°C for 15 s and 60°C for 1 min. Fluorescence was collected at the 60°C annealing/extension step.

Representative RNA from 55 virus isolates, including FMDV type O lineages previously detected in Libya (O/ME-SA/PanAsia-2 and EA-3), and viruses recently circulating in the region were tested by this 'Ind-2001' lineage-specific rRT-PCR, and the pan-specific assay detecting the 3D region of the genome (Callahan et al., 2002). All samples were identified as FMDV positive with the '3D' assay, while only those 35 samples from the O/ME-SA/Ind-2001 lineage (Fig. 2) were also amplified by the 'Ind-2001' specific assay. Although further validation of this assay may be warranted, these preliminary results provide evidence that this newly developed assay can accurately identify FMDV type O viruses of the emerging O/ME-SA/Ind-2001 lineage.

It is now important to define the precise viral sources of these outbreaks to identify transmission routes that may pose potential threats for future trans-regional movements of this viral strain. Based on the previous occurrences of O/ME-SA/Ind-2001 that have occurred outside of the Indian subcontinent, it is possible to speculate that these outbreaks in Libya and Saudi Arabia will only seed a limited number

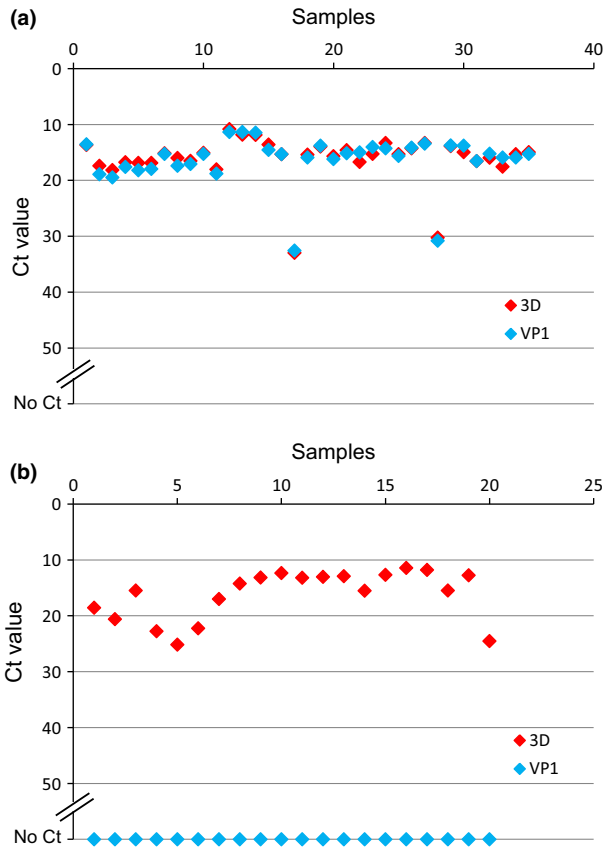

Fig. 2. Comparison of the Ct values from the VP1 and 3D real-time RT-PCR assays; (a) O/ME-SA/Ind-2001 lineage viruses; (b) O/ME-SA/PanAsia-2 (samples 1–19) and O/EA-3 (sample 20) lineage viruses.

of onward outbreaks. However, if this new sublineage was able to establish itself in the region, as it has in India (Yuvaraj et al., 2013) and Bangladesh (Nandi et al., 2013), it could potentially out-compete indigenous FMDV O/ME-SA lineages such as O/ME-SA/PanAsia-2. It is encouraging that preliminary vaccine-matching experiments (by virus neutralization) indicate that some vaccine strains normally used in the Middle East would probably offer effective protection against the O-Ind-2001d lineage viruses (R. Statham and A. Ludi, personal communication). To monitor the situation in more detail, increased and specific surveillance targeted at O/ME-SA/Ind-2001d should be instigated. In 2014, while preparing this paper, viruses belonging to the O/ME-SA/Ind-2001d have been found in gazelles in Abu Dhabi (United Arab Emirates). These viruses were most closely related to isolates from India and Nepal, suggesting a possible independent introduction (Fig. 1). Also in 2014, viruses belonging to the Ind-2001b lineage have spread to cattle, sheep and goats in Tunisia and Algeria. Viruses isolated from samples recently received by the WRLFMD from Sri Lanka (collected since December 2013) also belong to the Ind-2001d lineage, the first time that this virus lineage has been found in this country.

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