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ORIGINAL ARTICLE

Characterization of the O/ME-SA/Ind-2001d foot-and-mouth disease virus epidemic recorded in the Maghreb during 2014–2015

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Abstract

The O/ME-SA/Ind-2001d has been the main foot-and-mouth disease virus (FMDV) lineage responsible for FMD epidemics outside the Indian subcontinent from 2013 to 2017. In 2014, outbreaks caused by this FMDV lineage were reported in Maghreb, where it was initially detected in Algeria and Tunisia and later in Morocco. This was the first incursion of an FMDV type O of exotic origin in the Maghreb region after 14 years of absence. In this study, we report analyses of both VP1 and whole-genome sequences (WGSs) generated from 22 isolates collected in Algeria and Tunisia between 2014 and 2015. All the WGSs analysed showed a minimum pairwise identity of 98.9% at the nucleotide level and 99% at the amino acid level (FMDV coding region). All Tunisian sequences shared a single putative common ancestor closely related to FMDV strains circulating in Libya during 2013. Whereas sequences from Algeria suggest the country experienced two virus introductions. The first introduction is represented by strains circulating in 2014 which are closely related to those from Tunisia, the second one, of which the origin is more uncertain, includes strains collected in Algeria in 2015 that gave origin to the 2015 outbreak reported in Morocco. Overall, our results demonstrated that a unique introduction of O/Ind-2001d FMDV occurred in Maghreb through Tunisia presumably in 2014, and from then the virus spread into Algeria and later into Morocco.

KEYWORDS
foot-and-mouth disease, isolates antigenic profile, isolates phylogenesis, Maghreb, O Ind-2001 epidemic

1 | INTRODUCTION

Foot-and-mouth disease (FMD) is one of the most important infectious diseases of cloven-hoofed livestock; when an incursion occurs in FMD-free countries it causes enormous economic losses, while in endemically infected regions it hinders both agricultural and economic development (Knight-Jones & Rushton, 2013). The disease is caused by the FMD virus (FMDV), belonging to the *Picornaviridae* family, genus *Aphthovirus* (Grubman & Baxt, 2004). The viral genome consists of a positive-sensed, single-stranded RNA of ~8.4 kb in length, organized in

a single long open reading frame (ORF) flanked by 5′ and 3′ untranslated regions (5′UTR and 3′UTR, respectively). The 5′UTR plays an important role in cap-independent translation initiation of the viral polyprotein and viral genome replication (Gao et al., 2016; Lawrence & Rieder, 2009). It consists of an S (short) fragment of ~350–380 nucleotides (nt), a poly(C) tract of ~100–420 nt, and an L (long) fragment of ~700 nt, which includes a type- II internal ribosome entry site (IRES) (Carrillo et al., 2005; Mason et al., 2003). The ORF encodes for a polyprotein that is co- and post-translationally cleaved by viral proteinases into structural (VP4, VP2, VP3 and VP1) and non-structural proteins (L^{pro}, 2A, 2B, 2C, 3A, 3B, $3C^{pro}$ and $3D^{pol}$). The 3′UTR is about 90 nt long and is thought to contain cis-acting elements required for efficient genome replication (Gao et al., 2016; Lawrence & Rieder, 2009).

Due to its genetic and antigenic diversity (Domingo et al., 2003), FMDV is classified into seven distinct serotypes namely O, A, C, Asia 1, Southern African Territories (SAT) 1, SAT2 and SAT3. FMDV is circulating worldwide within seven geographical distinct reservoirs (FMDV pools), each including virus strains of at least three serotypes, which evolve generating variants that tend to remain confined within those specific regions (Paton et al., 2009). By looking at the sequence variability of the VP1/1D coding region, each serotype is classified into different geographically restricted topotypes, which can be further subdivided into genotypes, lineages and sub-lineages (Knowles & Samuel, 2003).

In endemic areas, FMDV lineages encounter a turnover by a continuous replacement of emergent or re-emergent strains that occur between epidemics waves (Di Nardo et al., 2021). However, some of these dominant lineages have been previously documented to have escaped from their geographical distribution of origin, causing exotic incursions in new areas and leading to significant epidemics. This was the case for the O/ME-SA/Ind-2001 FMDV lineage, which despite its official report dated 2001 from India (Hemadri et al., 2002), it was already circulating since 1997 in both India (Subramaniam et al., 2015) and the Middle East (Knowles et al., 2005). After sporadic episodes, it re-emerged in 2008, supplanting the O/ME-SA/PanAsia lineage predominant at the time within the Indian subcontinent, and evolving into five distinct sub-lineages designated as O/Ind-2001a, O/Ind-2001b, O/Ind-2001c, O/ Ind-2001d and O/Ind-2001e (Bachanek-Bankowska et al., 2018; Subramaniam et al., 2013). Starting from 2013, cases caused by the O/Ind-2001d sub-lineage were reported outside the Indian subcontinent from countries of the Middle East region (e.g. Saudi Arabia, UAE, Bahrain and Jordan), in North Africa from Libya and later from Tunisia (2014), Algeria (2014) and Morocco (2015) (Bachanek-Bankowska et al., 2016; Bachanek-Bankowska et al., 2018).

In this study, we investigated retrospectively the FMDV O/Ind-2001d epidemics that affected countries of the Maghreb region between 2013 and 2015 by analysing 22 FMDV isolates collected from Tunisia and Algeria, providing new insights into the network of viral circulation and correlation between outbreaks reported during the epidemic wave.

2 | MATERIAL AND METHODS

2.1 | Samples collection

A total of 22 samples from confirmed FMDV positive cases collected during O/Ind-2001d outbreaks that occurred in Tunisia and Algeria between 2014 and 2015 were used (Table 1).

Three samples consisting of epithelial tissue homogenates from cattle displaying clinical signs were sent in virus transport medium (50% glycerol/PBS, pH7.2) to the Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna (IZSLER), Brescia, Italy, by the Tunisian National Laboratory, Tunisia. Further 11 samples from Tunisia, including 10 epithelial tissue homogenates and one vesicular epithelium, were sent to the French Agency for Food, Environmental and Occupational Health & Safety (ANSES), Paris, France: the sample with the identification number 1030A was received both at IZSLER and ANSES.

Nine samples were collected in Algeria, of which four consisted of epithelial tissue homogenates from cattle sent in virus transport medium (50% glycerol/PBS, pH7.2) to IZSLER by the Algerian National Laboratory, Algeria; five samples consisted of vesicular epithelium/tissue debris from cattle and sheep, resuspended in a lysis buffer, containing guanidine isothiocyanate.

2.2 | Virus isolation and identification

Virus isolation was carried out at IZSLER using BHK-21 and IBRS-2 cell lines (de Castro, 1964; Stoker & Macpherson, 1964) as previously described (Alexandersen et al., 2003). Virus type was confirmed by using the antigen detection and serotyping ELISA kit (Grazioli et al., 2020). Virus isolation was carried out at ANSES using ZZ-R 127 cell line as previously described (Gorna et al., 2014).

2.3 | Whole-genome sequencing

The whole-genome amplification of 11 FMDV isolates from Tunisia was obtained by RT-PCR using a panel of five primer pairs encompassing the entire FMDV genome. Total RNA from 140 μl of infected cell culture suspension was extracted automatically by the QiaCUBE automated DNA/RNA purification system (Qiagen, Hilden, Germany) using the QIAamp Viral RNA mini kit (Qiagen, Hilden, Germany), according to the manufacturer's instructions. RNA was eluted in 50 μl of elution buffer provided in the extraction kit.

The RNA was reverse transcribed into cDNA by using the Transcriptor High Fidelity cDNA Synthesis Kit (Roche, Basel, Swiss) following the manufacturer's instructions. The cDNA was used for the amplification of a total of five overlapping amplicons covering the whole FMDV genome by using a set of forward and reverse primers (Table S3), the Q5® High-Fidelity DNA Polymerase (New England Biolabs Ipswich,

TABLE 1 List and details of the analysed viruses

Accession number	Name	Identification number	Province	Date	Species	Samples	Samples submission	Full genome amplification method	isolate	WGS length
OM160626[†]	O/TUN/2/2014	1114	Sidi Bouzid	7/5/14	Cattle	Vesicular epithelium	IZSLER	\	BHK21 2° passage	\
OM160610	O/TUN/3/2014	1030A	Nabeul	26/4/14	Cattle	Vesicular epithelium	IZSLER/ANSES	AMPLICONS	ZZ-R 127	8043
OM160627[†]	O/TUN/4/2014	1054	Sidi Bouzid	29/4/14	Cattle	Vesicular epithelium	IZSLER	\	\	\
OM160611	O/TUN/5/2014	1101	Al Munastir	5/5/14	Cattle	Vesicular epithelium	ANSES	AMPLICONS	ZZ-R 127	8046
OM160612	O/TUN/6/2014	1223	Sidi Bouzid	14/5/14	Cattle	Vesicular epithelium	ANSES	AMPLICONS	ZZ-R 127	8043
OM160613	O/TUN/7/2014	1412	Qafsah	28/5/14	Cattle	Vesicular epithelium	ANSES	AMPLICONS	ZZ-R 127	8043
OM160614	O/TUN/8/2014	1771	Manubah	1/6/14	Cattle	Vesicular epithelium	ANSES	AMPLICONS	ZZ-R 127	8043
OM160615	O/TUN/9/2014	1566	Bajah	9/6/14	Cattle	Vesicular epithelium	ANSES	AMPLICONS	ZZ-R 127	8043
OM160616	O/TUN/10/2014	1797	Safaqis	7/7/14	Cattle	Vesicular epithelium	ANSES	AMPLICONS	ZZ-R 127	8042
OM160617	O/TUN/11/2014	1846	Madaniyin	15/7/14	Cattle	Vesicular epithelium	ANSES	AMPLICONS	ZZ-R 127	8043
OM160618	O/TUN/12/2014	2152A	Nabeul	5/9/14	Cattle	Vesicular epithelium	ANSES	AMPLICONS	ZZ-R 127	8049
OM160619	O/TUN/13/2014	2152B	Nabeul	5/9/14	Cattle	Vesicular epithelium	ANSES	AMPLICONS	ZZ-R 127	8043
OM160620	O/TUN/14/2014	2157	L'Ariana	15/9/14	Cattle	Vesicular epithelium	ANSES	AMPLICONS	ZZ-R 127	7762
MG983683[§]	O/ALG/1/2014	1628	Constantine	29/7/14	Cattle	Vesicular epithelium	IZSLER	SISPA	BHK21[‡]/IBRS2 2° passage	7759
OM160622	O/ALG/2/2014	1631_001	Medea	29/7/14	Cattle	Vesicular epithelium	IZSLER	SISPA	BHK21[‡]/IBRS2 2° passage	7751
OM160623	O/ALG/3/2014	1631_002	Medea	29/7/14	Cattle	Vesicular epithelium	IZSLER	SISPA	BHK21[‡]/IBRS2 2° passage	7753
OM160628[†]	O/ALG/4/2014	1636	Alger	30/7/14	Cattle	Vesicular epithelium	IZSLER	\	\	\
OM160624	O/ALG/5/2014	1920/1459	M'Sila	20/9/14	Cattle	Tissue debris in lysis buffer	IZSLER	SISPA	\	7765
OM160629[†]	O/ALG/6/2014	1920/3642	M'Sila	21/9/14	Cattle	Tissue debris in lysis buffer	IZSLER	\	\	\
OM160630[†]	O/ALG/7/2014	1961/3422	Aïn-Defla	25/9/14	Cattle	Vesicular epithelium in lysis buffer	IZSLER	\	\	\
OM160625	O/ALG/1/2015	398/32/07/SB/02	El Bayadh	21/2/15	Ovine	Vesicular epithelium in lysis buffer	IZSLER	SISPA	\	7723
OM160631[†]	O/ALG/2/2015	398/32/07/SB/03	El Bayadh	22/2/15	Ovine	Tissue debris in lysis buffer	IZSLER	\	\	\

[†]Accession number referred to VP1 sequence.
[‡]Cell line from which the sequenced strains were isolated.
[§]The WGS was already present in GenBank; our sequence of 7759 nt showed 100% identity.

Massachusetts, USA) was used for the amplification according to the manufacturer's instructions.

For only five out of nine Algerian samples, the whole-genome amplification was carried out: two samples were provided in lysis buffer and from the other three, the FMD virus was isolated in cell culture. The protocol consisted of the Single-Primer-Amplification method (SISPA) (Table 1) (Djikeng et al., 2008).

The RNA from the two samples provided in the lysis buffer was extracted by QiaAmp RNA extraction kit (Qiagen, Hilden, Germany) following a DNAse (Roche, Basel, Swiss) treatment, according to the manufacturer's instruction. RNA virus extraction from the three BHK-21 cell culture isolates was performed after a prior immuno-capture of virus particles on a solid phase using an FMDV type O specific monoclonal antibody (MAb). Briefly, a 1/10 dilution of infected cell culture supernatant was distributed into three wells of a MAb-coated plate (200 μl/well, 600 μl of sample in total). After an incubation of 1 h at 37°C and a washing step with PBS, the immune-captured virus was lysed by adding a total of 350 μl of guanidine thiocyanate buffer in the respective wells and harvested. RNA was then ethanol precipitated and resuspended in 20 μl of RNAse-free water. For SISPA amplification was followed the protocol described by Djikeng et al. (2008).

The whole-genome sequencing was performed on an Illumina MiSeq platform (Illumina, San Diego, CA, USA). The Nextera XT DNA Sample Preparation Kit (Illumina, San Diego, CA, USA) was used to generate multiplexed paired-end sequencing libraries, according to the manufacturer's instructions.

The whole-genome sequence (WGS) de novo assembly was performed using SeqMan NGen and SeqMan Pro version 12 (Lasergene package; DNAStar, Inc., Madison, WI). The primers sequences were removed from the NGS reads that were obtained by amplicon-based protocol.

2.4 | VP1 sequencing

The VP1/1D coding region of the FMDV genome for all the samples from Algeria and for two samples from Tunisia of which the WGS was not available (Table 1) was sequenced by Sanger sequencing. RNA was extracted from the original samples using the QiaAmp RNA extraction kit (Qiagen, Hilden, Germany) and the complete VP1 region was amplified by conventional RT-PCR according to standard procedures (Knowles et al., 2016); amplicons were purified and sequenced on 3500xL Genetic Analyzer (Thermo Fisher Scientific, Waltham, Massachusetts, USA).

2.5 | Sequence analysis

The VP1 coding sequence, as well as the WGSs obtained, were identified and compared using BLAST against publicly available sequences deposited in GenBank. Alignment and analysis of the sequences were performed using the DNASTAR Lasergene package. Analysis of codons and synonymous-non-synonymous (syn/non-syn) substitution rates were calculated using SNAP (https://www.hiv.lanl.gov/content/sequence/SNAP/SNAP.html) (Rodrigo & Learn 2001).

Comparative analyses of the genome were conducted including the GenBank retrieved FMDV sequences TUN/1/2014 (MG983735) and MOR/1/2015 (KU291242), for a total of 18 almost complete WGSs, and six VP1 sequences, obtained by Sanger sequencing from strains for which the WGS was not available.

2.6 | Phylogenetic analysis

Two alignments were constructed using the FMDV sequences obtained from this study, in addition to contemporary O/Ind-2001 sequences from North Africa and the Middle East retrieved from GenBank: the first alignment included 16 completely obtained ORFs and 12 sequences from GenBank (n = 28 sequences of 6999 nt in length) (Table 1); the second included 22 sequences of the VP1/1D coding region and 17 sequences from GenBank (n = 38 sequences, 639 nt in length) (Table S4). Phylogenetic signal was evaluated for each alignment by likelihood mapping analysis, with 20,000 random quartets, as implemented in TREE-PUZZLE 5.2 (Strimmer & von Haeseler, 1997).

Time-stamped phylogenies were inferred using BEAST 2.5.0 software (Bouckaert et al., 2019). Substitution models with the lowest Bayesian information criterion (BIC), estimated from MEGA X (Kumar et al., 2018), were selected between those supported by BEAST 2: the TN model (Tamura & Nei, 1993) with gamma-distributed rate variation among sites (G) was selected for the ORFs alignment, while the HKY model (Hasegawa et al., 1985) was used for the VP1 sequences. A Markov Chain Monte Carlo (MCMC) of 20 million generations was set up for both alignments. The Effective Sampling Size (ESS) of posterior estimates were visualized in Tracer 1.6 (Rambaut et al., 2018) to ensure ESS values obtained for all parameters were at least equal to or greater than 200. Analyses with a relaxed lognormal clock were run for both alignments to estimate the coefficient of variation of the relaxed clock analysis (obtaining values of 0.24 and 0.14 for the VP1 and ORF alignments, respectively). Based on the obtained distributions the strict clock was rejected and the relaxed clock was used in both analyses, as suggested by Bouckaert and Drummond (2017).

To infer the timing of geographical transitions of O/Ind-2001d viruses moving between countries of the Maghreb, the VP1 data set was further analysed using the discrete phylogeography method as implemented in BEAST 2.5.0 (Lemey et al., 2009).

Maximum Clade Credibility (MCC) trees were obtained using Tree Annotator after removing 10% of the MCMC chain as burnin. Obtained trees were visualized and edited in FigTree 1.4.2 (http://tree.bio.ed.ac.uk/software/figtree/).

2.7 | Antigenic profiling by monoclonal antibodies (MAbs)

Forty-five specific MAbs, previously elicited against various FMDV serotype O strains, were used to characterize the antigenic profile

of 13 out of 22 FMD viruses analysed in the present study, in addition to three further isolates collected in Libya in 2013. Respectively, 24 MAbs were raised against O Manisa/Turkey/69, 5 against O1 Brent/Switzerland/65, 14 against O UK 31/01 and 2 against O Italy 93 (Nunez et al., 2006). Of the full set of MAbs, 22 were known to neutralize virus infectivity. The reactivity of field isolates with the 45 MAbs was tested using a trapping ELISA method as previously described (Samuel et al., 1991). Briefly, viruses grown in cell culture were incubated for 1 h onto microplates coated with rabbit immune serum anti-FMDV O type and, subsequently, each MAb was incubated, at a saturating concentration, with each virus for 1 h at room temperature. The binding between FMD viruses and MAbs was detected using anti-mouse immunoglobulins labelled with horseradish peroxidase. After incubation for 1 h, the reaction was revealed by using o-phenylenediamine as substrate and optical density was read at 492 nm wavelength. Cycles of three washing steps were performed between each incubation step.

The reactivity of field isolates with each Mab was expressed as a percentage of the corresponding reaction with MAbs homologous strain, which was assumed to be 100%. Three O/Ind-2001 FMDV isolates collected from Libya during 2013 were also included in the analysis.

3 | RESULTS

3.1 | Full genome sequencing

The FMDV genome of 16 (11 from Tunisia and five from Algeria) out of 22 samples was nearly completely sequenced, resulting in sequences between 7723 and 8049 nt in length. For 10 Tunisian samples, the amplicon protocol allowed to obtain 5′UTR sequences which include the S fragment, where deletion of 72 nt (already reported in other isolates of the same epidemic wave) was identified (Bachanek-Bankowska et al., 2016; Bachanek-Bankowska et al., 2018). For all 16 samples, the complete Internal Ribosome Entry Site (IRES) region was sequenced, while the PolyC tract within 5′UTR was not resolved. A single large ORF of 6999 nt, which was predicted to encode a polyprotein of 2333 amino acids, was obtained for all 16 strains. The 3′polyA region was not resolved, and partial sequences of 3′UTR ranging between 30 and 100 nt were obtained.

The 5′UTR (IRES portion) showed 95.6% of invariant nucleotides (Table S1); a total of 262 substitutions sites were distributed throughout the ORF (Figure 1), of which 59 were non-synonymous changes. All the substitutions sites presented two nucleotide variants except

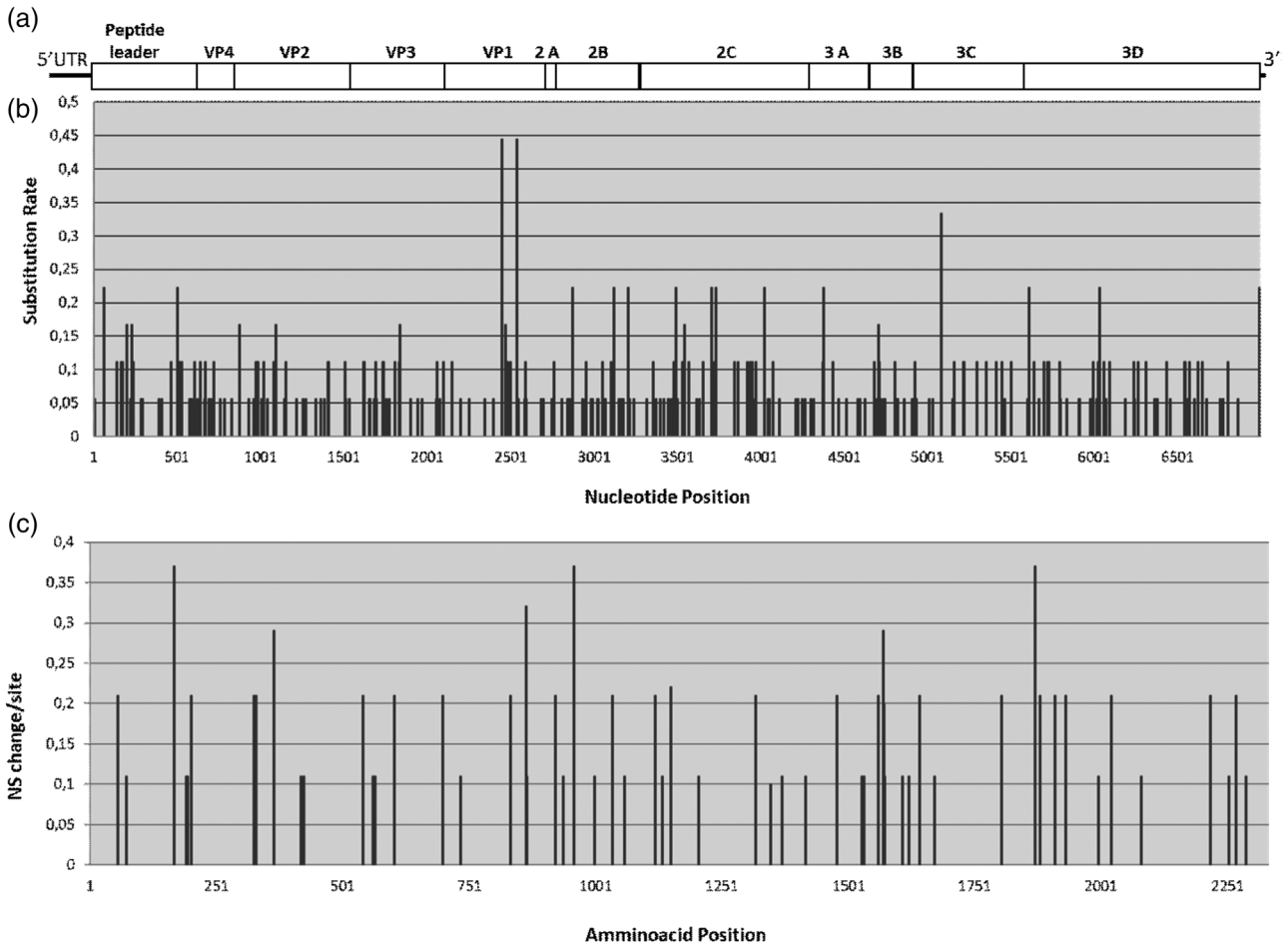

FIGURE 1 Coding region variability of eighteen FMDV WGS. (a) Schematic diagram of FMDV genome; showing, the protein-encoding regions within the polyprotein. (b) Graphic representation of rates of nucleotide substitution per site. (c) Graphic representation of non-synonymous substitutions per site as estimated using SNAP (https://www.hiv.lanl.gov/content/sequence/SNAP/SNAP)

the position 5082 within the 3C coding gene, where three different nucleotide modifications were present. The majority of nucleotide changes were strain-specific or in common between two strains, for 23 positions the nucleotide substitutions were in common to more than two strains. In particular, a nucleotide (nt) variation at positions 2448 and 2535 (VP1 coding region) was common in eight samples. The most conserved region, considering the nt variation in respect to the length of the gene, was the one encoding for the 3C protease (97.7%) while the region encoding for 2A resulted in the most variable (Table S1).

Non-synonymous substitutions were distributed across both structural and non-structural coding regions (Table S2 and Figure 1): 3D (twelve), 2C (eight), 3A (seven), VP1 and L (six each), VP2 and VP3 (five each), 2B (four), 3B (three), 3C (two) and 2A (one). The amino acid sequence encoding for the VP4 was found to be conserved. Thirty out of the 59 non-synonymous changes identified in the 18 WGS were unique to individual strains, while 26 were common to two samples, two to three strains and three to the four strains from Algeria 2014. For changes common to more samples, the aa substituted was identical except for one position (VP1 residue 140), where the aa variation was N to D for two samples and N to S for one sample. Five non-synonymous changes were non-conservative: three located respectively in the L^{pro}, VP2 and VP1 and two in the 3D proteins. The VP1 exhibited two out of six non-synonymous changes within the structural G-H loop (N140D, N140S and non-conservative E141G) where the linear neutralization site 1 has been mapped for the FMDV serotype O (Aktas & Samuel, 2000; Barnett et al., 1989; Kitson et al., 1990; Pfaff et al., 1988; Xie et al., 1987) as well as for other serotypes (Baxt et al., 1989; Bolwell et al., 1989; Grazioli et al., 2013; Mateu, 1995; Thomas et al., 1988); two out of five non-synonymous changes detected in VP2 (position C78Y non-conservative and I132V) involve residues belonging to the neutralization site 2; finally, out of five aa variations identified in VP3, the substitutions H56R and D60G map at the neutralization site 4 and also the more distant position 195 was described as part of this antigenic site in type A (Mateu, 1995). The only one aa change in 2A corresponds to the cleavage site at the junction with the VP1 (L1F), the 3A showed the lowest syn/non-syn substitution ratio and the highest number of non-syn substitutions with respect to the total aa number. Overall, the non-structural proteins 3A, 3B and 3D showed lower syn/non-syn substitution ratios than structural proteins (Table S2).

3.2 | Phylogenetic analysis

The likelihood mapping showed 15% and 25% of unresolved (uninformative) trees for the ORF and VP1 alignments, respectively (Figure S1). Both values were under 30% and were then considered reliable for phylogenetic analysis.

The topology of both trees (Figures 2 and 3) obtained by analysing, respectively, VP1 and complete ORFs sequences clustered the Tunisian, Algerian and Moroccan isolates in a single clade; in addition, results from the discrete phylogeography analysis supported the hypothesis of a single introduction in Tunisia (PP = 0.97), which, according to the time of most recent common ancestor (MRCA) estimated from the VP1 phylogeny, can be dated around April 2014 (3/4/2014, 95% HPD Interval 5/3/2014–26/4/2014). Algerian isolates were grouped in two distinct and well-supported clades in both VP1 and WGS phylogenies, one that includes viruses collected from Tunisia and the other including viruses descending from those present in Tunisia and including the first isolate from Morocco. This topological distinction between O/Ind-2001d viruses collected from Algeria is suggestive of two separate introductions from Tunisia (PP = 0.94 and 0.8, respectively), of which MRCA viruses were estimated to be circulating around March–April 2014, with estimated time of introductions in Algeria in June (first introduction) and December 2014 (second introduction) (Table 2). In addition, phylogenetic analysis based on the full ORF sequences grouped viruses circulated in Tunisia into three different clusters distributed in overlapping geographical areas. The main cluster included strains collected along the Mediterranean coast of Tunisia, with the two minor groups including strains from its central and the Northern regions. Using WGSs, the O/Ind-2001 FMDV lineages were estimated to have evolved within the timeframe of the epidemic at a mean rate of 2.36×10^{-4} nt substitution per site per year (95% HPD Interval 1.89×10^{-4}, 2.88×10^{-4}).

TABLE 2 Time of the most recent common ancestors for each of the reconstructed clades using the FMDV VP1 coding sequences

	Estimation	95% HPD	
North Africa	18/7/13	5/7/2013	23/8/2013
Tunisia/Algeria	3/4/14	5/3/2014	26/4/2014
Intr. in Algeria	23/6/2014	24/5/2014	18/7/2014
Algeria/Morocco	03/12/14	4/10/14	6/2/2015

3.3 | Antigenic profile

The reactivity profiles of the 16 field isolates with the panel of neutralizing MAbs were substantially homogeneous but, as expected, different from those exhibited by three reference viruses used to elicit the MAbs and belonging to lineages different from O/ME-SA/Ind-2001. In particular, the results highlighted that the neutralizing MAbs can be roughly assembled in three groups, irrespective of the MAb target site and the virus strain against which they were raised (Figure 4): one group is composed of neutralizing MAbs recognizing all 16 isolates, followed by a second group of MAbs which exhibited partial reactivity with several isolates indicating a modification, even if not substantial, of the target epitopes; finally, a third MAbs group includes mainly non-reactive MAb denoting a crucial change of the specific binding sites with respect to the homologous virus strains. Those MAbs showing diversified reactivity with the isolates, namely those of the second group, could suggest antigenic-based similarities or distinctions between isolates, which however do not seem related to the genomic variability nor with the aa substitutions found. For example, several MAbs raised against different viruses and identifying the same neu-

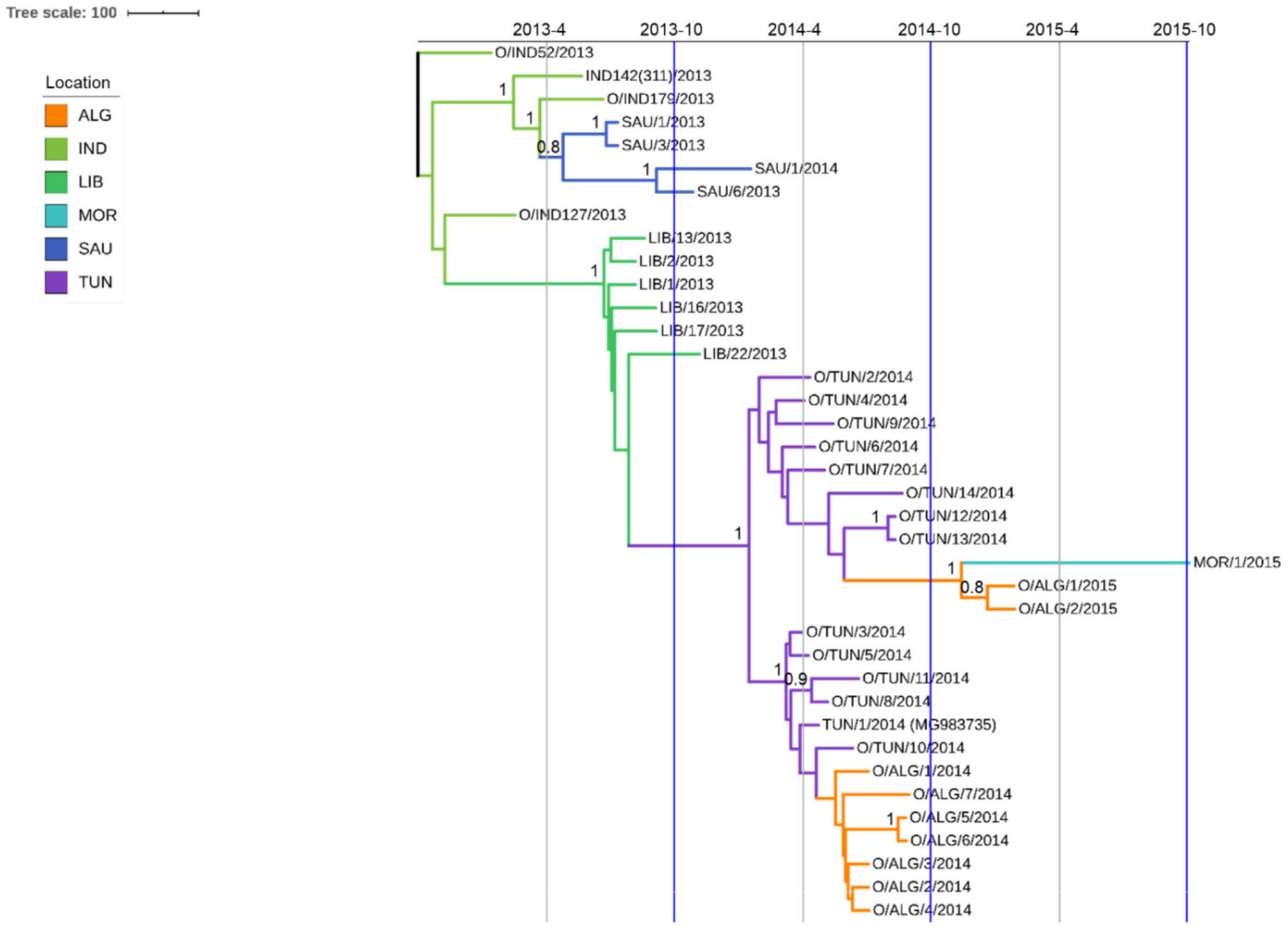

FIGURE 2 Time stamped tree reconstructed using the FMDV sequences encoding for the VP1/1D region. Horizontal axis represents time, with ticks (lines) set at intervals of 6 months. The colour of branches defines the geographic origin assigned by the discrete phylogeography analysis. Only posterior probabilities equal to or higher than 0.8 are showed to the corresponding node

tralizing site 2 (Figure 4) showed different reactivity profiles with the field isolates, confirming the complexity of this site which involves several critical VP2 residues (Aktas & Samuel, 2000; Grazioli et al., 2013; Kitson et al., 1990; Lea et al., 1994).

The target sites of non-neutralizing MAbs are expected to be less subject to immune pressure and therefore antigenically more stable. This seems to be confirmed by the homogeneous reactivity of the reference viruses that elicited the various non-neutralizing MAbs; interestingly, the used panel of MAbs identified both epitopes conserved among all the 16 field isolates (maintaining complete reactivity), and epitopes showing partial loss of reactivity, thus modified with respect to those displayed by strains homologous to MAbs.

4 | DISCUSSION

Sequencing and analysis of the VP1/1D coding region of FMDV are routinely used to characterize the global epidemiology of FMD and it is the target genome region used for FMDV taxonomy and classification (Knowles et al., 2016). More recently, the use of WGS to reconstruct the transmission pathway of FMD viruses during an outbreak has been shown to provide higher resolution to support known patterns of virus diffusion at the country level, as well as to disclose likely transmission links hidden to the classical epidemiological field investigation (Jamal & Belsham, 2018; Valdazo-Gonzalez et al., 2012).

The introduction of the O/ME-SA/Ind-2001 FMDV lineage in the Maghreb during 2014 was a significant epidemiological event, not only because the O/ME-SA is an exotic topotype to the region, but also because the Maghreb region has not reported any circulation of type O FMD viruses since 1999 (Bouguedour & Ripani, 2016; Samuel et al., 1999).

In this study, a total of 16 WGSs have been generated from samples collected during O/Ind-2001 outbreaks in countries of the Maghreb region, with the comparison of the polyprotein coding region revealing a minimum pairwise identity of 98.9% and 99% at the nucleotide and amino acid levels respectively. A total of 16 non-synonymous substitutions were found within the structural proteins, with some of them resulting in the proximity of known neutralizing sites. However, the reactivity of the field isolates with MAbs mapping to neutralizing epitopes did not show a specific correlation with the detected amino acid substitutions; the antigenic profiles of the epidemic isolates appeared substantially homogeneous, with only minor differences in the

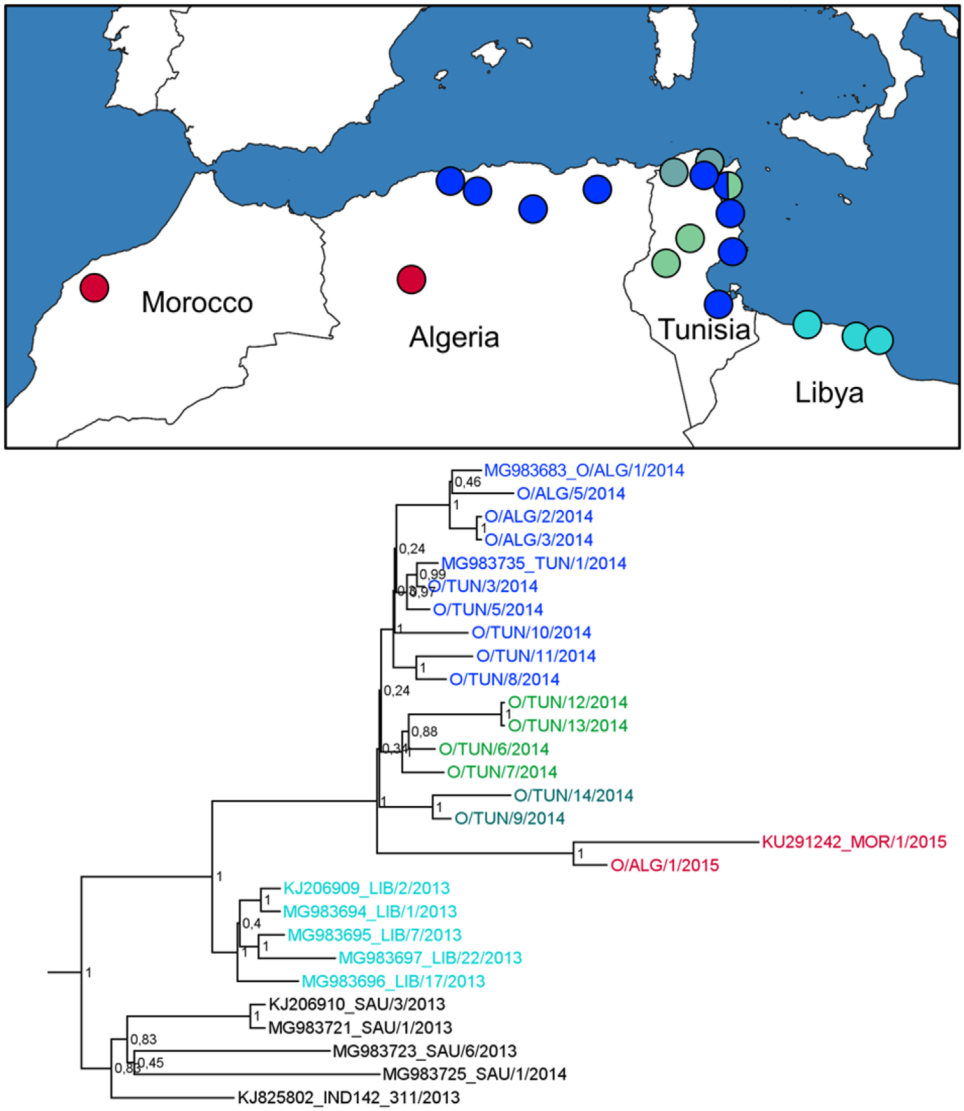

FIGURE 3 Map showing the location of strains sequenced in this work, corresponding to the phylogenetic clades reconstructed using FMDV complete ORFs sequences

reactivity of some of those isolates with a few neutralizing MAbs, which supports for randomly occurring mutations that did not fix in the field. Differently, both 3A and 3B non-structural proteins exhibited higher variability than structural proteins, suggesting that these proteins are subjected to a selective pressure distinct from that characterizing the non-structural region, as previously described (Carrillo et al., 2005).

FMD spread throughout Tunisia from the 25 April 2014 (the date of the first outbreak declared in Nabeul Governorate), until the 4 November 2014, when the Tunisian Veterinary Services declared the epidemic over. During this period a total of 150 outbreaks were reported with clinical signs observed in 646 cattle (20% of cattle population in the affected herds), 64 outbreaks in goats, 641 in sheep and 5 in a not specified small ruminant population (10% of sheep and goat population in the affected herds) (OIE-WAHIS, Tunisia 1st semester report, 2014; OIE-WAHIS, Tunisia 2nd semester report, 2014).

Phylogenetic analysis based on VP1 sequences indicated a single introduction of O/Ind-2001d in Tunisia (estimated around April 2014), probably from Libya where outbreaks caused by this FMDV lineage were already reported during 2013 (Valdazo-González et al., 2014; Bachanek-Bankowska et al., 2018). The analyses of the polyprotein sequences, here reported, further substantiated this hypothesis, with its phylogeny revealing a unique common ancestor of all O/Ind-2001d viruses collected from Maghreb. In addition, the viruses from Tunisia were all isolated from samples collected from cattle and the viral spread in the country was strongly consistent with the animal movements within Tunisian territories, where flows are concentrated mainly in the centre and north of the country (Zrelli et al., 2018) connected by livestock trade. It is noteworthy that some Tunisian phylogenetic clusters included isolates collected in governorates where important livestock markets are present (i.e. Sidi Bouzid, Qafsah and Bajah), which could have played a key role as hubs of FMD spread.

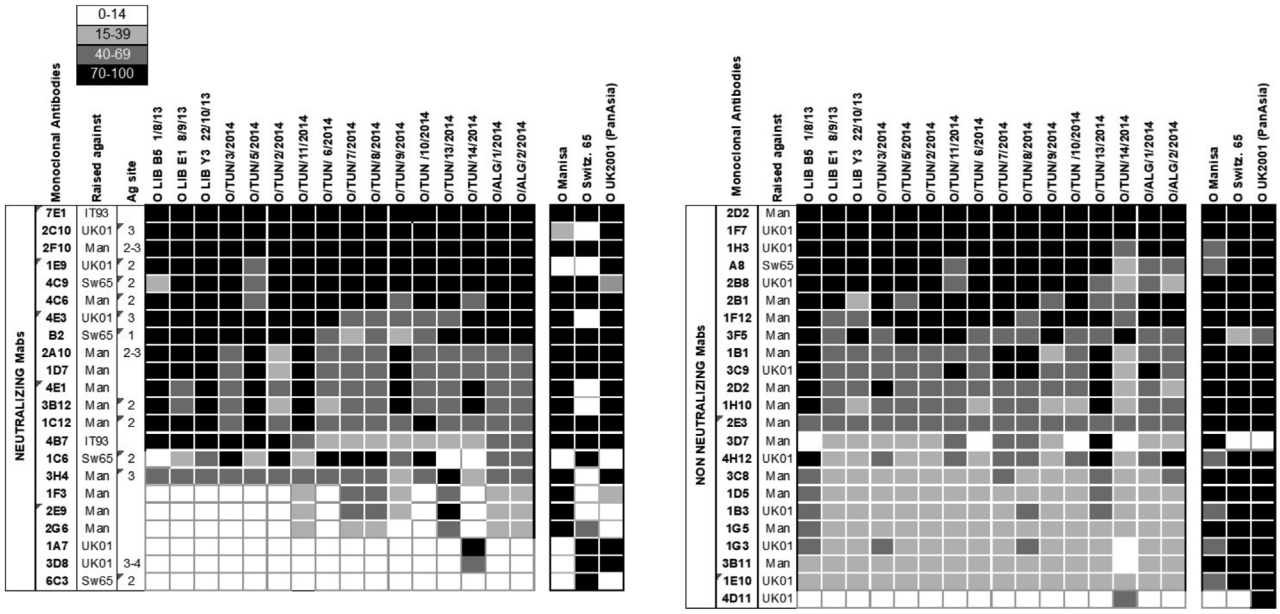

FIGURE 4 Reactivity of 16 FMDV isolates with FMDV type O-specific MAbs. The ELISA results obtained using two panels of MAbs (neutralizing and non-neutralizing), raised against O Manisa/Turkey/69, O1 Brent/Switzerland/65, O Italy 1993 and O UK 31/01 are depicted in greyscale expressing the percentage of reactivity in respect to the reaction of the MAbs with the homologous strain, which was assumed to be 100%. MAbs are tentatively ordered according decreasing reactivity

In Algeria, FMD infections were reported starting from the 23 July 2014, and by the 22 October 2014, a total of 419 outbreaks were reported to the OIE (OIE-WAHIS, Algeria 2nd semester report, 2014). Those cases were, for the majority, in the north region of the country, with the overall morbidity in cattle estimated at about 40% (Bouguedour & Ripani, 2016). No cases were observed in small ruminant populations. In 2015, when Algeria experienced a new wave of FMDV O/Ind-2001d outbreaks, infections were mainly detected in small ruminants (OIE-WAHIS, Algeria 1st semester report, 2015). Phylogenetic analysis, performed using both sequences encoding the VP1/1D gene and those of the polyprotein coding region, supported two different and independent virus introductions in Algeria, with their MRCAs estimated in March and December of 2014, respectively. The phylogenetic clade derived from the first MRCA node (i.e. first introduction) includes viruses isolated from outbreaks reported in northern Algeria and closely related to those occurring in Tunisia along the Mediterranean coast towards the north of the country, where the majority of the cattle population is distributed. This first FMD epidemic phase started with an outbreak in Setif, which is located in the northeast of the country close to the Tunisian border; where trade with Algeria is largely present (Zrelli et al., 2018).

The phylogenetic reconstruction of the second introduction of FMD in Algeria, obtained using the two data sets of sequences, is less clear in describing the relationship between those Algerian viruses and contemporary ones circulating within the region: both epidemiological scenarios of virus movement from either Tunisia or Morocco are entirely plausible, given the time and spatial proximity of FMD viruses isolated from these countries. Similar results were already obtained in a comprehensive study of the O/ME-SA/Ind-2001 lineage (Bachanek-Bankowska et al., 2018), which suggests a careful use of WGSs when performing reconstruction of geographical transitions of FMDV in complex endemic systems due to the bias introduced by the potential presence of recombinant viruses.

The two independent introduction pathways here described are also consistent with the livestock farming system that is present in Algeria. In fact, in Algeria 20 out of 25 million small ruminants are farmed in the south of the country (bordering the desert area), which is distant from and less epidemiologically linked with the northern area in which the entire cattle population is located (Bouguedour & Ripani, 2016). Therefore, contact between these host species was unlike to have occurred. In addition, during the outbreaks reported in 2014, only on a few farms there was evidence of proximity between cattle and small ruminants and stamping-out of all susceptible species was immediately applied following disease detection.

It is worth mentioning that FMDV annual vaccination campaigns were carried out in both Tunisia and Algeria since 1999. Both cattle and small ruminants were targeted in Tunisia for a vaccination with a vaccine formulated using O1 Manisa, O/Tunisia '99, A22 Iraq and SAT2 FMDV strains for cattle and only O and A strains for small ruminants, while in Algeria the vaccination campaign covered only cattle with O1 Manisa and A vaccine strains. Although there is no information regarding the vaccination status and then the age of the affected animals, the spread of the infection and the small variability within the viruses collected during the epidemic indicate that the susceptible population in the area was not adequately protected. The O/Tunisia '99 vaccine strain belongs to the West Africa (WA) topotype, while the O1 Manisa strain, as well as the O/Ind-2001d, belong to the

FMDV O/ME-SA topotype. However, the antigen-matching studies carried out by the World Reference Laboratory for FMD (WRLFMD) showed that FMDV O/Ind-2001 had a poor antigenic match with the heterologous O1 Manisa vaccine strain (WRLFMD Quarterly Report, 2014) with an r1 < 0.3. Further studies showed that only a high-potency O1 Manisa vaccine would have been effective against the O/ME-SA/Ind-2001 FMDV lineage (Fishbourne et al., 2017).

It is worth reminding that further FMDV epidemics occurred in North Africa following the O/Ind-2001d of 2014–2015: in 2017, caused by the A/AFRICA/G-IV lineage (Pezzoni et al., 2019) and in 2018–2019 and 2022 by O/EA-3 lineage all correlated with virus circulating in the sub-Saharan regions. Moreover, the political instability occurring in Libya hampers the gathering of information about the animal health situation of any infectious disease circulation, causing a further threat to the region.

This increasing and changing epidemiological dynamics of FMD in the Maghreb makes this region both critical and strategic for controlling the risk of FMDV incursions into Europe.

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CONFLICT OF INTEREST

The authors have no affiliations with or involvement in any organization or entity with any financial interest (such as honoraria; educational grants; participation in speakers' bureaus; membership, employment, consultancies, stock ownership, or other equity interest; and expert testimony or patent-licensing arrangements), or non-financial interest (such as personal or professional relationships, affiliations, knowledge or beliefs) in the subject matter or materials discussed in this manuscript.

ETHICS STATEMENT

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to. No ethical approval was required as in this study samples from naturally infected animals have been analysed.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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REFERENCES

Aktas, S., & Samuel, A. R. (2000). Identification of antigenic epitopes on the foot and mouth disease virus isolate O1/Manisa/Turkey/69 using monoclonal antibodies. *Revue Scientifique Et Technique (International Office of Epizootics)*, *19*, 744–753.

Alexandersen, S., Zhang, Z., Donaldson, A. I., & Garland, A. J. (2003). The pathogenesis and diagnosis of foot-and-mouth disease. *Journal of Comparative Pathology*, *129*, 1–36.

Bachanek-Bankowska, K., Di Nardo, A., Wadsworth, J., Mioulet, V., Pezzoni, G., Grazioli, S., Brocchi, E., Kafle, S. C., Hettiarachchi, R., Kumarawadu, P. L., Eldaghayes, I. M., Dayhum, A. S., Meenowa, D., Sghaier, S., Madani, H., Abouchoaib, N., Hoang, B. H., Vu, P. P., Dukpa, K., … Knowles, N. J. (2018). Reconstructing the evolutionary history of pandemic foot-and-mouth disease viruses: The impact of recombination within the emerging O/ME-SA/Ind-2001 lineage. *Science Reports*, *8*, 14693–14698.

Bachanek-Bankowska, K., Wadsworth, J., Gray, A., Abouchoaib, N., King, D. P., & Knowles, N. J. (2016). Genome Sequence of foot-and-mouth disease virus serotype O isolated from Morocco in 2015. *Genome Announcements*, 4. https://doi.org/10.1128/genomeA.01746-15

Barnett, P. V., Ouldridge, E. J., Rowlands, D. J., Brown, F., & Parry, N. R. (1989). Neutralizing epitopes of type O foot-and-mouth disease virus. I. Identification and characterization of three functionally independent, conformational sites. *Journal of General Virology*, *70*(Pt 6), 1483–1491.

Baxt, B., Vakharia, V., Moore, D. M., Franke, A. J., & Morgan, D. O. (1989). Analysis of neutralizing antigenic sites on the surface of type A12 foot-and-mouth disease virus. *Journal of Virology*, *63*, 2143–2151.

Bolwell, C., Brown, A. L., Barnett, P. V., Campbell, R. O., Clarke, B. E., Parry, N. R., Ouldridge, E. J., Brown, F., & Rowlands, D. J. (1989). Host cell selection of antigenic variants of foot-and-mouth disease virus. *Journal of General Virology*, *70*(Pt 1), 45–57.

Bouckaert, R. R., & Drummond, A. J. (2017). bModelTest: Bayesian phylogenetic site model averaging and model comparison. *Bmc Evolutionary Biology*, *17*, 42–46.

Bouckaert, R., Vaughan, T. G., Barido-Sottani, J., Duchene, S., Fourment, M., Gavryushkina, A., Heled, J., Jones, G., Kuhnert, D., De Maio, N., Matschiner, M., Mendes, F. K., Muller, N. F., Ogilvie, H. A., du Plessis, L., Popinga, A., Rambaut, A., Rasmussen, D., Siveroni, I., … Drummond, A. J. (2019). BEAST 2.5: An advanced software platform for Bayesian evolutionary analysis. *PLoS Computational Biology*, *15*, e1006650.

Bouguedour, R., & Ripani, A. (2016). Review of the foot and mouth disease situation in North Africa and the risk of introducing the disease into Europe. *Revue Scientifique et Technique (International Office of Epizootics)*, *35*, 757–768.

Carrillo, C., Tulman, E. R., Delhon, G., Lu, Z., Carreno, A., Vagnozzi, A., Kutish, G. F., & Rock, D. L. (2005). Comparative genomics of foot-and-mouth disease virus. *Journal of Virology*, *79*, 6487–6504.

de Castro, M. P. (1964). Behaviour of the foot-and-mouth disease virus in cell cultures: Susceptibility of IB-RS-2 line. *Arquivos do Instituto Biológico, São Paulo*, *31*, 63–78.

Di Nardo, A., Ferretti, L., Wadsworth, J., Mioulet, V., Gelman, B., Karniely, S., Scherbakov, A., Ziay, G., Özyörük, F., Parlak, Ü., Tuncer-Göktuna, P., Hassanzadeh, R., Khalaj, M., Dastoor, S. M., Abdollahi, D., Khan, E., Afzal, M., Hussain, M., Knowles, N. J., & King, D. P. (2021). Evolutionary and ecological drivers shape the emergence and extinction of foot-and-mouth disease virus lineages. *Molecular Biology and Evolution*, *38*, 4346–4361.

Djikeng, A., Halpin, R., Kuzmickas, R., Depasse, J., Feldblyum, J., Sengamalay, N., Afonso, C., Zhang, X., Anderson, N. G., Ghedin, E., & Spiro, D. J. (2008). Viral genome sequencing by random priming methods. *Bmc Genomics [Electronic Resource]*, *9*, 5–5.

Domingo, E., Escarmis, C., Baranowski, E., Ruiz-Jarabo, C. M., Carrillo, E., Nunez, J. I., & Sobrino, F. (2003). Evolution of foot-and-mouth disease virus. *Virus Research*, *91*, 47–63.

Fishbourne, E., Ludi, A. B., Wilsden, G., Hamblin, P., Statham, B., Bin-Tarif, A., Brocchi, E., Grazioli, S., Dekker, A., Eblé, P., & King, D. P. (2017). Efficacy of a high potency O1 Manisa foot-and-mouth disease vaccine in cattle

against heterologous challenge with a field virus from the O/ME-SA/Ind-2001 lineage collected in North Africa. *Vaccine*, *35*(20), 2761–2765. https://doi.org/10.1016/j.vaccine.2017.02.047

Gao, Y., Sun, S. Q., & Guo, H. C. (2016). Biological function of Foot-and-mouth disease virus non-structural proteins and non-coding elements. *Virology Journal*, *13*, 107–z.

Gorna, K., Houndje, E., Romey, A., Relmy, A., Blaise-Boisseau, S., Kpodekon, M., Saegerman, C., Moutou, F., Zientara, S., & Bakkali Kassimi, L. (2014). First isolation and molecular characterization of foot-and-mouth disease virus in Benin. *Veterinary Microbiology*, *171*, 175–181.

Grazioli, S., Fallacara, F., & Brocchi, E. (2013). Mapping of antigenic sites of foot-and-mouth disease virus serotype Asia 1 and relationships with sites described in other serotypes. *Journal of General Virology*, *94*, 559–569.

Grazioli, S., Ferris, N. P., Dho, G., Pezzoni, G., Morris, A. S., Mioulet, V., & Brocchi, E. (2020). Development and validation of a simplified serotyping ELISA based on monoclonal antibodies for the diagnosis of foot-and-mouth disease virus serotypes O, A, C and Asia 1. *Transboundary and Emerging Diseases*, *67*, 3005–3015.

Grubman, M. J., & Baxt, B. (2004). Foot-and-mouth disease. *Clinical Microbiology Reviews*, *17*, 465–493.

Hasegawa, M., Kishino, H., & Yano, T. (1985). Dating of the human-ape splitting by a molecular clock of mitochondrial DNA. *Journal of Molecular Evolution*, *22*, 160–174.

Hemadri, D., Tosh, C., Sanyal, A., & Venkataramanan, R. (2002). Emergence of a new strain of type O foot-and-mouth disease virus: Its phylogenetic and evolutionary relationship with the PanAsia pandemic strain. *Virus Genes*, *25*, 23–34.

Jamal, S. M., & Belsham, G. J. (2018). Molecular epidemiology, evolution and phylogeny of foot-and-mouth disease virus. *Infection, Genetics and Evolution*, *59*, 84–98.

Kitson, J. D., McCahon, D., & Belsham, G. J. (1990). Sequence analysis of monoclonal antibody resistant mutants of type O foot and mouth disease virus: Evidence for the involvement of the three surface exposed capsid proteins in four antigenic sites. *Virology*, *179*, 26–34.

Knight-Jones, T. J., & Rushton, J. (2013). The economic impacts of foot and mouth disease – What are they, how big are they and where do they occur? *Preventive Veterinary Medicine*, *112*, 161–173.

Knowles, N. J., & Samuel, A. R. (2003). Molecular epidemiology of foot-and-mouth disease virus. *Virus Research*, *91*, 65–80.

Knowles, N. J., Samuel, A. R., Davies, P. R., Midgley, R. J., & Valarcher, J. F. (2005). Pandemic strain of foot-and-mouth disease virus serotype O. *Emerging Infectious Diseases*, *11*, 1887–1893.

Knowles, N. J., Wadsworth, J., Bachanek-Bankowska, K., & King, D. P. (2016). VP1 sequencing protocol for foot and mouth disease virus molecular epidemiology. *Revue Scientifique Et Technique (International Office of Epizootics)*, *35*, 741–755.

Kumar, S., Stecher, G., Li, M., Knyaz, C., & Tamura, K. (2018). MEGA X: Molecular evolutionary genetics analysis across computing platforms. *Molecular Biology and Evolution*, *35*, 1547–1549.

Lawrence, P., & Rieder, E. (2009). Identification of RNA helicase A as a new host factor in the replication cycle of foot-and-mouth disease virus. *Journal of Virology*, *83*, 11356–11366.

Lea, S., Hernández, J., Blakemore, W., Brocchi, E., Curry, S., Domingo, E., Fry, E., Abu-Ghazaleh, R., King, A., Newman, J., Stuart, D., & Mateu, M. G. (1994). The structure and antigenicity of a type C foot-and-mouth disease virus. *Structure*, *2*(2), 123–139. https://doi.org/10.1016/s0969-2126(00)00014-9

Lemey, P., Rambaut, A., Drummond, A. J., & Suchard, M. A. (2009). Bayesian phylogeography finds its roots. *PLoS Computational Biology*, *5*, e1000520.

Mason, P. W., Grubman, M. J., & Baxt, B. (2003). Molecular basis of pathogenesis of FMDV. *Virus Research*, *91*, 9–32.

Mateu, M. G. (1995). Antibody recognition of picornaviruses and escape from neutralization: A structural view. *Virus Research*, *38*, 1–24.

Nunez, J. I., Fusi, P., Borrego, B., Brocchi, E., Pacciarini, M. L., & Sobrino, F. (2006). Genomic and antigenic characterization of viruses from the 1993 Italian foot-and-mouth disease outbreak. *Archives of Virology*, *151*, 127–142.

Paton, D. J., Sumption, K. J., & Charleston, B. (2009). Options for control of foot-and-mouth disease: Knowledge, capability and policy. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, *364*, 2657–2667.

Pezzoni, G., Bregoli, A., Grazioli, S., Barbieri, I., Madani, H., Omani, A., Sadaoui, H., Bouayed, N., Wadsworth, J., Bachanek-Bankowska, K., Knowles, N. J., King, D. P., & Brocchi, E. (2019). Foot-and-mouth disease outbreaks due to an exotic virus serotype A lineage (A/AFRICA/G-IV) in Algeria in 2017. *Transboundary and Emerging Diseases*, *66*(1), 7–13. https://doi.org/10.1111/tbed.13017

Pfaff, E., Thiel, H. J., Beck, E., Strohmaier, K., & Schaller, H. (1988). Analysis of neutralizing epitopes on foot-and-mouth disease virus. *Journal of Virology*, *62*, 2033–2040.

Rambaut, A., Drummond, A. J., Xie, D., Baele, G., & Suchard, M. A. (2018). Posterior summarization in Bayesian phylogenetics using Tracer 1.7. *Systematic Biology*, *67*, 901–904.

Rodrigo, A. G., & Learn, G. H. J. (2001). *Computational and evolutionary analysis of HIV molecular sequences*. Springer US.

Samuel, A. R., Knowles, N. J., & Mackay, D. K. (1999). Genetic analysis of type O viruses responsible for epidemics of foot-and-mouth disease in North Africa. *Epidemiology and Infection*, *122*, 529–538.

Samuel, A. R., Knowles, N. J., Samuel, G. D., & Crowther, J. R. (1991). Evaluation of a trapping ELISA for the differentiation of foot-and-mouth disease virus strains using monoclonal antibodies. *Biologicals*, *19*, 299–310.

Stoker, M., & Macpherson, I. (1964). Syrian hamster fibroblast cell line Bhk21 and its derivatives. *Nature*, *203*, 1355–1357.

Strimmer, K., & von Haeseler, A. (1997). Likelihood-mapping: A simple method to visualize phylogenetic content of a sequence alignment. *PNAS*, *94*, 6815–6819.

Subramaniam, S., Mohapatra, J. K., Sharma, G. K., Biswal, J. K., Ranjan, R., Rout, M., Das, B., Dash, B. B., Sanyal, A., & Pattnaik, B. (2015). Evolutionary dynamics of foot-and-mouth disease virus O/ME-SA/Ind2001 lineage. *Veterinary Microbiology*, *178*, 181–189.

Subramaniam, S., Pattnaik, B., Sanyal, A., Mohapatra, J. K., Pawar, S. S., Sharma, G. K., Das, B., & Dash, B. B. (2013). Status of foot-and-mouth disease in India. *Transboundary and Emerging Diseases*, *60*, 197–203.

Tamura, K., & Nei, M. (1993). Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Molecular Biology and Evolution*, *10*, 512–526.

Thomas, A. A., Woortmeijer, R. J., Puijk, W., & Barteling, S. J. (1988). Antigenic sites on foot-and-mouth disease virus type A10. *Journal of Virology*, *62*, 2782–2789.

Valdazo-González, B., Knowles, N. J., & King, D. P. (2014). Genome sequences of foot-and-mouth disease virus O/ME-SA/Ind-2001 lineage from outbreaks in Libya, Saudi Arabia, and Bhutan during 2013. *Genome Announcements*, *2*(2), e00242–14. https://doi.org/10.1128/genomeA.00242-14

Valdazo-Gonzalez, B., Polihronova, L., Alexandrov, T., Normann, P., Knowles, N. J., Hammond, J. M., Georgiev, G. K., Ozyoruk, F., Sumption, K. J., Belsham, G. J., & King, D. P. (2012). Reconstruction of the transmission history of RNA virus outbreaks using full genome sequences: Foot-and-mouth disease virus in Bulgaria in 2011. *PLoS One*, *7*, e49650.

World Organisation for Animal Health (OIE) – World Animal Health Information System (WAHIS). Algeria half-yearly report, (2014), 2nd semester. https://wahis.oie.int/#/report-smr/view?reportId=25224&period=SEM02&areaId=64&isAquatic=false

World Organisation for Animal Health (OIE) – World Animal Health Information System (WAHIS). Algeria half-yearly report (2015) 1st semester. https://wahis.oie.int/#/report-smr/view?reportId=25430&period=SEM01&areaId=64&isAquatic=false

World Organisation for Animal Health (OIE) – World Animal Health Information System (WAHIS). Tunisia half-yearly report (2014) 1st

semester. https://wahis.oie.int/#/report-smr/view?reportId=25038&period=SEM01&areaId=230&isAquatic=false

World Organisation for Animal Health (OIE) - World Animal Health Information System (WAHIS). Tunisia half-yearly report (2014) 2nd semester. https://wahis.oie.int/#/report-smr/view?reportId=25219&period=SEM02&areaId=230&isAquatic=false

WRLFMD Quarterly Report, July-September (2014). https://www.wrlfmd.org/sites/world/files/quick_media/OIE-FAO%20FMD%20Ref%20Lab%20Report%20Jul-Sep%202014.pdf

Zrelli, M., Kalthoum, S., Baccar, M. N., & Cherni, J. (2018). Animal movements in the run-up to religious festivals: Their scale and the risk for Tunisia. *OIE Bulletin*, 01 1: 6. doi: https://doi.org/10.20506/bull.2018.2.2865

Xie, Q. C., McCahon, D., Crowther, J. R., Belsham, G. J., & McCullough, K. C. (1987). Neutralization of foot-and-mouth disease virus can be mediated through any of at least three separate antigenic sites. *Journal of General Virology*, *68*(Pt 6), 1637–1647.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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