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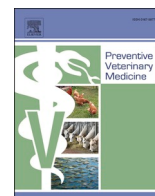
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Detection of foot-and-mouth disease viruses from the A/AFRICA/G-I genotype in the Sultanate of Oman

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ABSTRACT

Rapid identification and characterization of circulating foot-and-mouth disease virus (FMDV) strains is crucial for effective disease control. In Oman, a few serological and molecular studies have been conducted to identify the strains of FMDV responsible for the outbreaks that have been occurring within the country. In this study, 13 oral epithelial tissue samples from cattle were collected from suspected cases of FMD in Ash Sharqiyah North, Al Batinah North, Dhofar and Ad Dhakhyilia governorates of Oman between 2018 and 2021. FMDV RNA was detected in all samples by real-time RT-PCR and viruses were isolated after one- or two-blind passages in the porcine Instituto Biologico-Rim Suino-2 cell line. Antigen capture ELISA characterized all isolates as serotype A and VP1 phylogenetic analysis placed all sequences within a single clade of the G-I genotype within the A/AFRICA toptotype. These sequences shared the closest nucleotide identities to viruses circulating in Bahrain in 2021 (93.5% to 99.5%) and Kenya in 2017 (93.4% to 99.1%). To the best of our knowledge, this is the first time that A/AFRICA/G-I viruses have been detected in Oman. Together with the closely related viruses detected recently in Bahrain, these findings reinforce the importance of deploying effective quarantine control measures to minimize the risks of transboundary transmission of FMD associated with the importation of cattle from East Africa.

1. Introduction

Foot-and-mouth disease (FMD) is a highly infectious and contagious vesicular viral disease of cloven-hoofed animal species (Alexandersen and Mowat, 2005). It causes enormous economic losses to the animal production sectors in endemic countries resulting from trade restriction of livestock commodities, reduction of animal productivity, a high mortality rate of young animals, as well as costs associated with control and surveillance (Knight-Jones and Rushton, 2013). The disease is caused by FMD virus (FMDV), which belongs to the genus *Aphthovirus* within the family *Picornaviridae* (Grubman and Baxt, 2004). The viral genome is a positive-sense, single-stranded RNA of around 8.4 kb in

length that is enclosed in an icosahedral capsid composed of 60 copies of structural proteins, namely VP1, 2, 3, and 4 (Belsham and Martinez-Salas, 2019). In FMDV, a highly variable structural protein of VP1 contains two immunogenic sites: the G-H loop of the highly conserved tripeptide RGD motif and the C-terminus, which are involved in the virus attachment and entry, immune protection, and serotype specificity (Carrillo et al., 2005; Fox et al., 1989; Jackson et al., 1997). FMDV, as an RNA virus, is prone to evolving rapidly, leading to a genetic variation on an epidemiological time scale. This evolution is influenced by complex and multifactorial epidemiological and ecological processes, including virus-host interaction, the immunity profile of the host species, the virus fitness process and environmental factors, resulting in

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competitive interaction among the existing viral strains (Di Nardo et al., 2021). By modelling the evolution of nucleotide sequences obtained from the VP1 coding region, the FMDV serotypes, topotypes (~15% nt divergence), lineages (~10% nt divergence), and sublineages (~5% nt divergence) can be determined based on the genetic similarity thresholds (Samuel and Knowles, 2001). This method provides a powerful tool for tracking the spread and emergence of new FMDV lineages (Knowles et al., 2016). Globally, seven immunologically and genetically distinct FMDV serotypes have been described, namely O, A, C, Asia 1, South African Territories (SAT) 1, SAT 2 and SAT3. Three topotypes within FMDV serotype A has been identified: ASIA, AFRICA and EURO-SA (Knowles et al., 2016).

Most FMDV-infected animals are found in Asia, Africa, Middle East and Venezuela in South America (World Reference Laboratory for Foot-and-Mouth Disease (WRLFMD), 2021). The distribution of FMDV is characterized by seven geographical endemic pools that maintain discrete viral lineages comprising, Pool 1: East Asia, Pool 2: South Asia, Pool 3: West Eurasia, Pool 4: East Africa, Pool 5: West Africa, Pool 6: Southern Africa and Pool 7: South America. In fact, these endemic pools have dynamic boundaries that have not been precisely mapped depending on various ecological and environmental similarities (Brito et al., 2017; Paton et al., 2009; World Organization for Animal Health (WOAH), 2023b).

FMD is endemic in the Middle East region where outbreaks are caused by O, A and Asia 1 serotypes (Di Nardo et al., 2021). Within the Middle East, the trade of live animals occurs in both Asia and Africa. The movement of livestock with Africa inland by Egypt or through the sea from Eritrea, Djibouti, Sudan and Somalia highly contributes to the dynamics of FMDV exchange in the area (Rweyemamu et al., 2008). The Sultanate of Oman is considered an FMD-endemic country (World Reference Laboratory for Foot-and-Mouth Disease (WRLFMD), 2021; Hussain et al., 2019). However, few data on the circulating strains are available. FMDV serotype O was recorded for the first time in the Dhofar region of Oman in 1976 (Hedger et al., 1980). These authors also reported the isolation of serotype O from clinical cases in 1978 and serotype A and Asia 1 from subclinical animals in the same year. The FAO World Reference Laboratory for FMD (WRLFMD; Pirbright Institute, UK) isolated serotype O from samples collected during 1978–1985, 1988–1991 and 2001. FMDV Asia 1 was also recorded in the Dhofar governorate in 1980, 1981 and 1989 (World Reference Laboratory for Foot-and-Mouth Disease (WRLFMD), 2001; Hedger et al., 1980). A serological study conducted between 2003 and 2004 found that 77.7% of the seropositivity of FMDV was due to serotype O and 22.3% to serotype A (Al Busaidi et al., 2013). Another study by Hussain et al. (2019) demonstrated a herd-level seroprevalence of NSP antibodies against FMDV as 41.4%. The highest seroprevalence was observed in cattle (55.2%), followed by goats (38.2%) and sheep (37.7%). Serotype SAT 2 (topotype VII, lineage Alx-12) was reported from samples collected in the Muscat region in April 2015. The VP1 sequence of the SAT2 isolate was most closely related to the strain circulating in Ethiopia in 2014 (World Reference Laboratory for Foot and Mouth Disease (WRLFMD), 2015). The analysis of genetic sequences of Oman FMDV isolates is crucial tool for identifying the virus's genotypes, transmission pathways and its relationship with other strains in the region. In this study, we describe the characterization of FMDV viruses responsible for outbreaks that occurred in the Sultanate of Oman and isolated between 2018 and 2021.

2. Materials and methods

2.1. Study area, sample collection and processing

Between 2018 and 2021, 13 oral epithelial tissue samples were collected from suspected FMD cases in cattle aged between 6 months to 6 years, including 7 females and 6 males in four governorates: Ash Sharqiyah North, Al Batinah North, Dhofar and Ad Dakhiliyah. Animals

that showed typical clinical signs of fever, excessive salivation and blister-like formation on the mouth and feet were subjected to sampling. The samples were collected from lesions in the oral cavity. The collection process involved scraping freshly opened vesicles using sterile blunt-edged forceps. Approximately 0.14–0.73 g of collected samples were preserved in a viral transport medium and a cold chain was maintained throughout the process. The sampled animals' data was collected including species, age, sex, breed and vaccination records as detailed in Table 1. The number of samples collected during outbreaks reported in the governorates in different years was as follows: Dhofar in 2018 (one sample) and in 2021 (three samples), Ad Dakhiliyah in 2020 (seven samples), Ash Sharqiyah North in 2018 (one sample), Al Batinah North in 2018 (one sample) as shown in Fig. 1. The samples collected from Dhofar in 2021 were from two outbreaks in Arsaot and Hijayf. Moreover, the samples collected from Ad Dakhiliyah (Nizwa) in 2020 were from two outbreaks in Al Gubrah.

The samples were placed in a transport medium containing an equal amount of phosphate-buffered saline (PBS) and glycerol, pH 7.2–7.6 with the antibiotics Mycostatin and Neomycin (Sigma-Aldrich) and shipped in a cold box to the Central Laboratory for Animal Health in Oman for storage at -80°C before being shipped to the FMD Reference Laboratory in France (Animal Health Laboratory-ANSES). The samples were treated by washing twice in a cell culture medium (MEM) (GIBCO) with 2% of antibiotics (Ab) i.e. penicillin G sodium (10,000 U/mL) and streptomycin (10 mg/mL) (GIBCO) to remove any remaining transport media. 0.1 g of each sample was placed in a ribolyser tube containing 1 mL of MEM-2% Ab and lysed in MP Biomedicals™ FastPrep-24™ 5 G Instrument for two cycles of 20 s. The samples were centrifuged at 3000 x g for 10 min at 4°C and supernatants were stored at -80°C until further analyses (World Organization for Animal Health (WOAH), 2022).

2.2. RNA extraction and duplex real-time reverse transcriptase polymerase chain reaction (rtRT-PCR)

The viral RNA was extracted from samples according to the manufacturer's protocol (ID Gene Mag Universal Isolation kit, Innovative Diagnostics). Real-time RT-PCR was performed by using the AgPath-IDTM One-Step RT-PCR Kit (Life Technologies) as previously described by Gorna et al. (2016). Briefly, a master mix containing 1 x RT-PCR buffer (including dNTPs and ROX reference dye), primers and probes, 1 x RT-PCR enzyme mix (including Array Script™ Reverse Transcriptase and Ampli Taq Gold® polymerase and nuclease-free water mix was prepared for both 3D/ β -actin and IRES/ β -actin detection. The real-time RT-PCR reactions were performed in a total volume of 25 μl by adding 5 μl of the extracted RNA to 20 μl of the master mix. The following thermocycler program was performed: one cycle of reverse transcription at 45°C for 10 min, RT inactivation, and DNA polymerase activation at 95°C for 10 min followed by 45 cycles of denaturation at 95°C for 15 s and hybridization, elongation, and reading at 60°C for 1 min. The reaction was stopped by a cooling step at 20°C for 2 min. The amplification plots were analyzed and the cycle threshold (Ct) values were determined. A sample was considered negative if the Ct value was undetermined and it was reported as "FMDV genome undetected". Conversely, a sample was considered positive if the Ct value was obtained with a characterized amplification curve "sigmoidal" and it was reported as "FMDV genome detected".

2.3. Virus Isolation

Virus isolation was performed on a monolayer culture of porcine Instituto Biologico-Rim Suino-2 (IB-RS-2) cells (De Castro, 1964). The cells were seeded in a 24-well plate at a concentration of 10^5 cell/cm² with MEM containing 2% Ab, 1.5% lactalbumin (SIGMA), 2.5% HEPES (GIBCO) (zwitterionic sulfonic acid buffering agent) and 7% fetal bovine serum (Eurobio). Cell monolayers were inoculated with two dilutions

Table 1

List of the oral epithelial samples collected from cattle from different governorates between 2018 and 2021.

Sample ID	Collection date	Governorate	Provinces	Animal Species	Age	Sex	Type of Breed	Name of breed	Vaccination Record	
									Date	Serotype
A/OMN/22Z2001/2018/ 22	21-Mar-2018	Ash Sharqiyah North	Bidiya	Cattle	2 years	Female	Local	North Omani	No	-
A/OMN/22Z2004/2018/ 25	10-Apr-2018	Dhofar	Salalah	Cattle	6 years	Female	Local	South Omani	No	-
A/OMN/22Z2005/2018/ 26	20-Mar-2018	Al Batinah North	Shinas	Cattle	0.5 year	Female	Local	South Omani	12-Feb-2018	O, A, Asia1
A/OMN/22Z2028/2020/ 63	17-Mar-2020	Ad Dakhiliyah	Nizwa	Cattle	3-4 year	Male	Imported	Jersey	No	-
A/OMN/22Z2030/2020/ 65	17-Mar-2020	Ad Dakhiliyah	Nizwa	Cattle	3-4 year	Male	Imported	Jersey	No	-
A/OMN/22Z2031/2020/ 66	17-Mar-2020	Ad Dakhiliyah	Nizwa	Cattle	3-4 year	Male	Crossbred	Jersey and North Omani	No	-
A/OMN/22Z2032/2020/ 67	17-Mar-2020	Ad Dakhiliyah	Nizwa	Cattle	3-4 year	Male	Crossbred	Jersey and North Omani	No	-
A/OMN/22Z2033/2020/ 69	17-Mar-2020	Ad Dakhiliyah	Nizwa	Cattle	2 years	Male	Crossbred	Jersey and North Omani	No	-
A/OMN/22Z2038/2020/ 74	17-Mar-2020	Ad Dakhiliyah	Nizwa	Cattle	2 years	Male	Crossbred	Jersey and North Omani	No	-
A/OMN/22Z2064/2020/ 106	22-Dec-2020	Ad Dakhiliyah	Samail	Cattle	4 years	Female	Local	North Omani	No	-
A/OMN/22Z2071/2021/ 113	15-Mar-2021	Dhofar	Salalah	Cattle	4-6 year	Female	Local	South Omani	16-Nov-2020	O, A, Asia1
A/OMN/22Z2072/2021/ 114	15-Mar-2021	Dhofar	Salalah	Cattle	4-6 year	Female	Local	South Omani	16-Nov-2020	O, A, Asia1
A/OMN/22Z2073/2021/ 115	30-Mar-2021	Dhofar	Salalah	Cattle	5 years	Female	Local	South Omani	11-Nov-2020	O, A, Asia1

(1:5 and 1:10) of each sample suspension and incubated for one hour at 37 °C under a 5% CO₂ atmosphere. After incubation, the inoculums were discarded from the wells, and 500 µl of complete culture medium containing MEM-2% Ab, 2.5% HEPES, and 1.5% lactalbumin hydrolysate without fetal bovine serum were added to the wells. The plates were incubated at 37 °C under 5% CO₂ and regularly examined under the microscope for the development of cytopathic effects (CPE), inoculated cells were harvested when CPE is observed. If the CPE was not observed, the plates were frozen at – 80 °C and thawed twice then passed for two successive blind passages in the same way.

2.4. Antigen enzyme-linked immune sorbent assay (Ag-ELISA)

The Ag-ELISA was undertaken to detect FMDV from infected cell lines and identify the serotypes responsible for the infection. This indirect sandwich Ag-ELISA for FMDV was performed according to the protocol described by the WOAHP Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (WOAHP, 2022). Given the geographical location of Oman, the test was performed for serotypes O, A, Asia 1 and SAT 2.

2.5. Sequencing and sequence analysis

The choice of primers for the amplification of the VP1 region depends on the results obtained with Ag-ELISA. RT-PCR for amplification of the FMDV VP1 protein-coding sequence was performed using the One-Step RT-PCR kit (Qiagen) according to the manufacturer's

instructions. The serotype A specific primers A-1C612F / EUR-2B52R previously described by Knowles et al. (2016) and the following cycle conditions were used in the RT-PCR: 50 °C for 30 min and 95 °C for 10 min, followed by 35 cycles of denaturation for 1 min at 95 °C, hybridization for 1 min at 55 °C and extensions for 1.5 min at 72 °C. The RT-PCR reaction was terminated by a final extension step of 5 min at 72 °C. The resulting PCR products were analysed on 1.4% agarose gel electrophoresis before submission to Eurofins (Germany) for sequencing.

Following sequencing, VP1 coding sequences obtained were assembled and verified using Geneious Prime software 2022.0.2 (Biomatters Ltd). Multiple sequence alignments including FMDV VP1 sequences from Oman, sequences available at the WRLFMD in addition with sequences retrieved from GenBank were conducted using MEGA7 software (Kumar et al., 2018). The VP1 sequences were aligned using MUSCLE alignment software with default parameters. The phylogenetic relationship between FMDV isolates was inferred using the Maximum Likelihood method based on the Tamura 3-parameter model (Tamura, 1992). Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Joining and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach and then selecting the topology with superior log likelihood value. A discrete Gamma distribution was used to model evolutionary rate differences among sites. There was a total of 639 positions in the final dataset (these data include six nucleotides of the 2 A region).

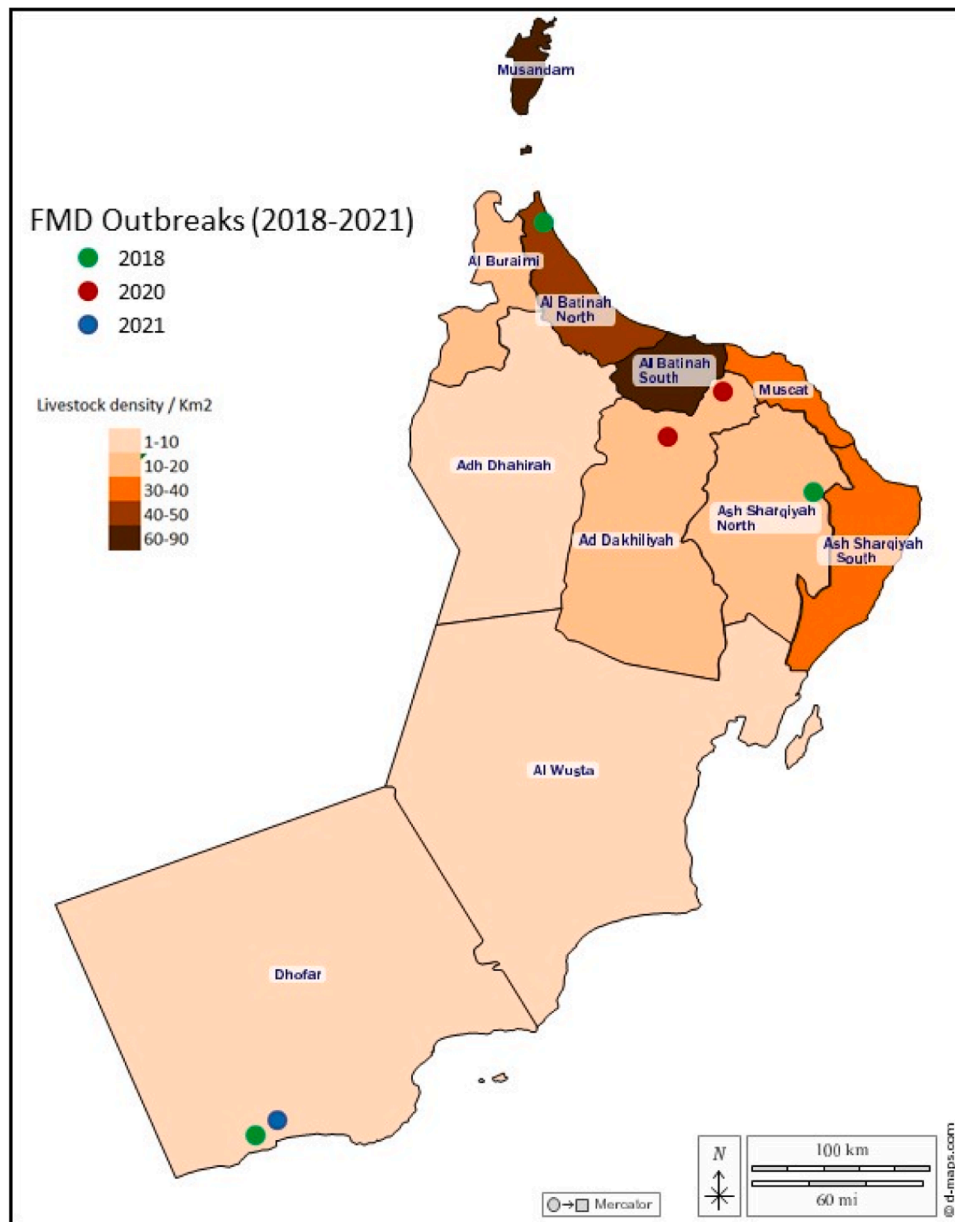


Fig. 1. The geographic location of foot-and-mouth disease virus (serotype A / AFRICA /G-I) outbreaks in Oman between 2018 and 2021 in relation to livestock density in different governorates. Each circle on the map represented the location of a reported outbreak in which green, red and blue circles indicated FMD outbreaks occurred in 2018, 2020 and 2021 respectively.

3. Results

3.1. FMDV detection using rtRT-PCR

FMDV RNA was detected by the duplex rtRT-PCR in all of the thirteen collected samples (Table 1). Both 3D and IRES FMDV genome targets were detected in all samples. The Ct values ranged between 15.90 and 32.77 for the 3D target and between 18.88 and 32.25 for the IRES target.

3.2. Virus isolation and serotype identification

FMDV rtRT-PCR positive samples were tested for virus isolation in the IB-RS-2 cell line. Cytopathic effects were observed as cell rounding and detachment from the cell monolayer at the first passage for four samples and at the second passage for the remaining nine samples. FMDV was confirmed in all infected cells and identified as a serotype A

virus by antigen-capture ELISA.

3.3. Sequencing and genetic characterization

The VP1 sequences were generated from Ag-ELISA positive type A samples (Table 2). They were found to be closely related to type A isolates from the Middle East and Eastern African countries, like Bahrain (BAR/21/2021), Kenya (KEN/9/2017, KEN/17/2017 and KEN/28/2008) and Tanzania (TAN/16/2013 and TAN/18/2013), sharing 92.3 to 99.5% nucleotide (nt) identities. These isolates were characterized within the AFRICA topotype and clustering within the G-I lineage. Table 3.

3.4. Phylogenetic analysis

The phylogenetic analysis reported that serotype A circulating in Oman can be grouped into four clusters, as shown in Fig. 2. Nucleotide

Table 2

Real-time Reverse Transcription-Polymerase Chain Reaction Ct values detected by the 3D and IRES targets for all oral epithelial tissue samples collected from FMD outbreaks in Oman from 2018 to 2021.

Sample number	3D C _t value	β-Actin C _t value	IRES C _t value	β-Actin C _t value
OMN/22Z2001/2018 22	24.55	32.32	24.83	34.24
OMN/22Z2004/2018 25	21.43	25.09	22.39	26.32
OMN/22Z2005/2018 26	21.74	29.04	21.25	30.59
OMN/22Z2028/2020 63	32.77	24.23	25.86	37.82
OMN/22Z2030/2020 65	17.30	21.41	23.44	23.25
OMN/22Z2031/2020 66	21.38	25.32	27.33	27.08
OMN/22Z2032/2020 67	21.81	23.54	25.41	27.40
OMN/22Z2033/2020 69	19.13	20.01	21.77	23.99
OMN/22Z2038/2020 74	20.25	23.50	25.04	25.63
OMN/22Z2064/2020 106	15.90	24.55	27.43	36.12
OMN/22Z2071/2021 113	21.89	42.28	18.88	28.30
OMN/22Z2072/2021 114	18.62	27.29	23.59	31.89
OMN/22Z2073/2021 115	26.57	35.67	32.25	39.97

Table 3

VP1 sequence data of viruses that was obtained or retrieved from GenBank for phylogenetic analysis, along with their details including the collection date, source, origin and their assigned accession numbers.

Virus name	Accession Number	Date collected	Species	Location	Country
A/BAR/21/2021	OQ214928	02/02/2021	cattle	Buri, Alshamalia	Bahrain
A/KEN/42/66	KF561699	19/01/1966	cattle	Kiphelion, Kericho, Rift Valley	Kenya
A/KEN/28/2008	KF561702	01/08/2008	cattle	Loitokitok, Rift Valley	Kenya
A/K73/08	KJ440870	23/8/2008	cattle	Loitokitok, Rift Valley	Kenya
A/KEN/22/2009	KF561703	01/03/2009	cattle	Olulunga, Narok South, Rift Valley	Kenya
A/K63/09	KJ440871	31/03/2009	cattle	Narok South, Rift Valley	Kenya
A/KEN/9/2017	OQ214929	29/05/2017	bovine	Kasarani, Nairobi	Kenya
A/KEN/17/2017	OQ214930	08/11/2017	bovine	Runyenjes, Embu	Kenya
A/KEN/10/2021	OQ214931	11/03/2021	cattle	Kitui	Kenya
A/OMN/22Z2001/2018 22	OQ240509	21/03/2018	cattle	Bidiya, Ash Sharqiyah North	Oman
A/OMN/22Z2004/2018 25	OQ240510	10/04/2018	cattle	Salalah, Dhofar	Oman
A/OMN/22Z2005/2018 26	OQ240511	20/03/2018	cattle	Shinas, Al Batinah North	Oman
A/OMN/22Z2028/2020 63	OQ240512	17/03/2020	cattle	Nizwa, Ad Dakhiliyah	Oman
A/OMN/22Z2064/2020 106	OQ240505	22/12/2020	cattle	Samail, Ad Dakhiliyah	Oman
A/OMN/22Z2030/2020 65	OQ240513	17/03/2020	cattle	Nizwa, Ad Dakhiliyah	Oman
A/OMN/22Z2031/2020 66	OQ240514	17/03/2020	cattle	Nizwa, Ad Dakhiliyah	Oman
A/OMN/22Z2032/2020 67	OQ240515	17/03/2020	cattle	Nizwa, Ad Dakhiliyah	Oman
A/OMN/22Z2033/2020 69	OQ240516	17/03/2020	cattle	Nizwa, Ad Dakhiliyah	Oman
A/OMN/22Z2038/2020 74	OQ240517	17/03/2020	cattle	Nizwa, Ad Dakhiliyah	Oman
A/OMN/22Z2071/2021 113	OQ240506	16/03/2021	cattle	Salalah, Dhofar	Oman
A/OMN/22Z2072/2021 114	OQ240507	17/03/2021	cattle	Salalah, Dhofar	Oman
A/OMN/22Z2073/2021 115	OQ240508	30/03/2021	cattle	Salalah, Dhofar	Oman
A/TAN/4/2009	KF561692	01/05/2009	cattle	Morogoro Region	Tanzania
A/TAN/11/2009	KF561694	21/06/2009	cattle	Kibaha District, Pwani Region	Tanzania
A/TAN/42/2009	KF561695	07/09/2009	cattle	Mpwapwa, Dodoma Region	Tanzania
A/TAN/45/2009	KF561696	06/11/2009	cattle	Iringa Rural District, Iringa Region	Tanzania
A/TAN/47/2009	KF561697	01/11/2009	cattle	Bagamoyo, Pwani Region	Tanzania
A/TAN-CVL-2009-0052	KJ947812	09/07/2009	bovine	Mbeya	Tanzania
A/TAN-CVL-2009-0042	KJ947814	01/05/2009	cattle	Makongo Juu, Kinondoni, Dar es Salaam	Tanzania
A/TAN-CVL-2009-0052b	KJ947822	09/07/2009	cattle	Kwala, Ilala, Dar es Salaam	Tanzania
A/TAN-CVL-2011-0066	KJ947819	21/09/2011	bovine	Tabora	Tanzania
A/TAN-CVL-2011-0125	KJ947813	21/09/2011	bovine	Mtwara	Tanzania
A/TAN-CVL-2011-0293	KJ947820	13/07/2011	bovine	Kibondo	Tanzania
A/TAN/15/2013	MF592652	20/06/2013	cattle	Bonchugu, Serengeti, Mara	Tanzania
A/TAN/16/2013	MF592653	28/06/2013	cattle	Emboreet, Simanjiro, Manyara	Tanzania
A/TAN/18/2013	MF592654	28/06/2013	cattle	Emboreet, Simanjiro, Manyara	Tanzania
A/T123/2016	MH686525	06/2016	cattle	Kibaha District, Pwani region	Tanzania
A/T141/2016	MH686519	06/2016	cattle	Mkuranga District, Pwani region	Tanzania

sequence analysis of the capsid VP1 gene showed that all thirteen isolates were very similar and grouped into the G-I genotype. The average nucleotide divergence between the study isolates from 2018 to the isolates from 2020 and 2021 was 3.6% (3.6 to 3.8%), whilst the average nucleotide divergence between the isolates from 2020 and 2021 was 6.2% (5.3 to 6.4%). Within 2020, there was a 5.9% (5.8 to 5.9%) average nucleotide divergence between the isolates from Nizwa and Samail.

4. Discussion

Although the Sultanate of Oman undertakes an extensive control program through the vaccination of cattle, sheep and goats, FMD remains endemic in the country (Hussain et al., 2019). Oman uses a trivalent inactivated FMDV vaccine with double emulsion (Water-in-Oil-in-Water, W/O/W) (Brilliant Bio Pharma Private Limited, India), which consisted of type O (strain IND/R2/75), A (strain IND/40/2000), and Asia1 (strain IND/63/72). The national vaccination program plays a pivotal role in reducing FMDV outbreaks. However, the FMDV strains still circulate in the country and cause outbreaks, particularly during the winter and spring seasons (World Organization For Animal Health (WOAH), 2023a; World Reference Laboratory for Foot-and-Mouth Disease (WRLFMD), 2022). Sedentary and transhumant farming systems were the two methods used by the livestock farmers in the study area. Sedentary farming system was practiced in the farms located in northern and interior governorates. While, the transhumance was practiced in Dhofar governorate. Both systems did not practice any biosecurity measure like restricted access and quarantine. The sampled animals

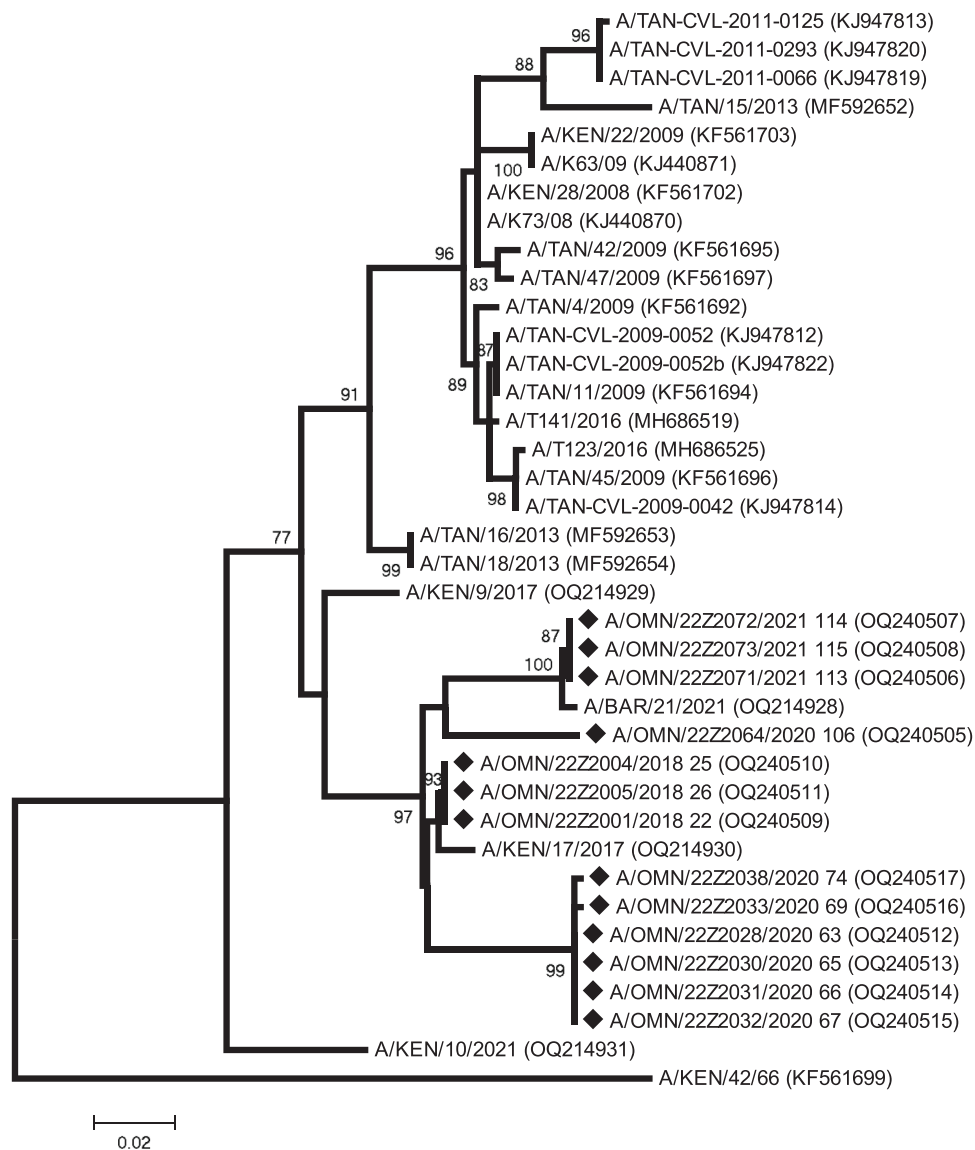


Fig. 2. Phylogenetic tree reconstructed by Maximum Likelihood method based on the Tamura 3-parameter model of nucleotide evolution (5 categories (+G, parameter = 0.3743)), showing the relationship between the serotype A virus isolates from Oman (highlighted with black diamonds) and reference viruses retrieved from GenBank and WRLFMD database of VP1 coding sequences. The percentage of trees (70% and above) in which the associated taxa clustered together is shown next to the branches. Oman viruses obtained from samples collected between 2018 and 2020 were genetically related to viruses from Kenya in 2017. While, 2021 Oman viruses were similar to viruses reported from Bahrain in 2021.

were kept for dual purpose and they were separated from other animal species, however, newly purchased animals from different sources were mixed together. In this case, subclinically infected animals can disseminate the virus to other animals in the herd.

The unique geographic location of Oman, which provides opportunities for live animal trade between the Indian subcontinent, the Arabian Gulf and East Africa, poses a risk for the incursion of FMDV strains from those regions. FMDV serotype A is the most genetically and antigenically diverse serotype compared to the other six serotypes (Mohapatra et al., 2011; Knowles et al., 2009). AFRICA, ASIA, and EUROPE-SOUTH AMERICA are the three topotypes of serotype A (Knowles and Samuel, 2003). The AFRICA topotype is further subdivided into eight genotypes, G-I to G-VIII (Woldemariam et al., 2023; Knowles, 2009).

For genetic characterization, phylogenetic trees based on the VP1 region of FMDV have been extensively used due to the high degree of variation and serotype specificity of this genome region. This study used phylogenetic analysis to define the genetic relationships between Oman FMD viruses and those isolated from other countries. The thirteen

(serotype A) viruses obtained in this study all belonged to the G-I genotype within the AFRICA topotype. Our result is consistent with the recent reports on FMDV from Bahrain and Kenya, which have shown FMDV clustering within the AFRICA topotype (World Reference Laboratory for Foot and Mouth Disease (WRLFMD), 2021; World Reference Laboratory for Foot-and-Mouth Disease WRLFMD, 2018). More specifically, our results support the route of virus transmission from East African countries to Gulf countries through Oman, likely associated with livestock movements. Indeed, FMDV topotypes O/EA-3 and SAT2 XIV, originating from East African countries have been implicated in causing outbreaks in Gulf countries (Canini et al., 2022; World Organization for Animal Health (WOAH), 2023a).

FMDV topotypes originating from the East African countries such as O/EA-3, SAT2, SAT2/VII and SAT2 XIV, caused outbreaks in the Gulf countries. These circulating FMDV serotype A isolates are genetically different to the sequence of the vaccine strain A/IND/40/2000 (Accession number: AF390646) used in the country, which belongs to genotype G-VII of the ASIA topotype. Given these differences, further studies are

warranted to assess the heterologous responses of post-vaccination sera against representative A/AFRICA/G-I isolates to confirm that this vaccine strain elicits adequate cross-neutralization responses.

It is difficult to determine the precise origin of the A/AFRICA/G-I isolates in Oman as the country imports livestock from different African countries, such as Somalia, Sudan and Kenya, to fill the gaps of increasing demand for animal products with low domestic production. In East Africa, FMDV-A/AFRICA/G-I has been found in, Kenya (1966, 1971, 1973–74, 1978, 1980–81, 1983–85, 1991–95, 2003, 2005–06, 2008–09, 2012–2017 and 2021), Democratic Republic of the Congo (2011), and Tanzania (1967–68, 1980, 2008–09, 2011–13 and 2016–17) (Casey-Bryars et al., 2018; Kasanga et al., 2015; Sallu et al., 2014; Wekesa et al., 2014).

The emergence and geographical distribution of A/AFRICA/G-I lineage in the Sultanate of Oman was found to extend from the northern governorates (Al Batinah North and Ash Sharqiyah North) to the southern governorate (Dhofar) in 2018. As in other endemic settings, FMDV is predominantly transmitted through unrestricted transboundary and local trade of livestock in Oman. A greater risk of FMDV incursion is associated with the import of live animals from Somalia, Sudan, and Kenya to the Raysut seaport during major Muslim festivals such as Ramadan, Eid al-Fitr, and Eid al-Adha. Additionally, other potential factors, such as mixing animals on pastures and in live animal markets, mixed farming of cattle and small ruminants, as well as the lack of biosecurity within farms, could induce a significant risk of disease transmission and maintenance in a susceptible population (Hussain et al., 2019). The outbreaks were recorded in 2020 in Al Batinah North, Sohar and in Ad Dhakhlyia regions in two provinces, Nizwa and Samail. In 2021, the virus emerged again in Dhofar. It seems that the free movement of the infected animals among the different provinces to meet the market demands along with the likely vaccine failure due to a miss-match with the circulating FMDV serotype A could play a critical role in FMD dissemination in Oman. The study molecular epidemiology approach provides a more detailed and precise understanding of the FMDV strains circulating in Oman. It helps in identifying the potential source of outbreaks, track the movement of the virus, and contribute to the development of effective control and prevention strategies. Since that the study output represented FMD viruses isolated from reported field outbreaks by the farmers to the veterinarians, it did not reflect the real epidemiological situation. It is more likely that some outbreaks were underreported and the sampling was not accessible.

5. Conclusion

To the best of our knowledge, the findings of the current study demonstrate the circulation of the genotype G-I within the AFRICA topotype of serotype A FMDV in the Sultanate of Oman from 2018 to 2021. The transboundary spread of FMDV serotype A from East African countries to the Arabian Peninsula is mainly due to animal mobility. Therefore, regular surveillance, genetic and antigenic characterization of the circulating FMDVs are critical to understanding the dynamics of virus movements, along with the relevance and effectiveness of the vaccine being used. Moreover, there is a need to strengthen the veterinary quarantine capacities to avoid further FMDV incursions.

CRedit authorship contribution statement

Elshafie Elshafie: Supervision, Writing – review & editing. **Hicks Hayley:** Writing – review & editing. **Wadsworth Jemma:** Formal analysis, Software, Visualization, Writing – review & editing. **Al-Ansari Aliya:** Supervision, Writing – review & editing. **Baqir Senan:** Supervision. **Zientara Stephan:** Funding acquisition, Project administration, Writing – review & editing. **King Donald:** Conceptualization, Project administration, Supervision, Writing – review & editing. **Di Nardo Antonello:** Data curation, Visualization, Writing – review & editing. **Knowles Nick:** Conceptualization, Formal analysis, Investigation,

Writing – review & editing. **Bakkali-Kassimi Labib:** Conceptualization, Funding acquisition, Methodology, Project administration, Resources, Software, Supervision, Validation. **Bernelin-Cottet Cindy:** Conceptualization, Data curation, Formal analysis, Methodology, Project administration, Validation, Visualization. **Sangula Abraham:** Resources, Writing – review & editing. **Al Riyami Bahja:** Funding acquisition, Project administration, Supervision, Writing – review & editing. **Al Salloom Fajer:** Resources. **Al-Rawahi Wafa:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Resources, Visualization, Writing – original draft, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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