

Foot-and-mouth disease outbreaks in captive scimitar-horned oryx (*Oryx dammah*)

Louis Lignereux^{1,2}  | Anne-Lise Chaber^{1,2}  | Claude Saegerman¹  | Livio Heath³ | Nick J. Knowles⁴  | Jemma Wadsworth⁴  | Valérie Mioulet⁴ | Donald P. King⁴ 

¹Faculty of Veterinary Medicine, Research Unit for Epidemiology and Risk Analysis Applied to Veterinary Sciences (UREAR-ULiège), Centre of Fundamental and Applied Research for Animals and Health (FARAH), University of Liège, Liège, Belgium

²School of Animal and Veterinary Sciences, University of Adelaide, Adelaide, SA, Australia

³Transboundary Animal Disease Program, Onderstepoort Veterinary Institute, Pretoria, South Africa

⁴FAO World Reference Laboratory for Foot-and-Mouth Disease, The Pirbright Institute, Pirbright, UK

Correspondence

Louis Lignereux, Leske building, School of Animal and Veterinary Sciences, University of Adelaide, Roseworthy 5371, South Australia.

Email: l_louis77@hotmail.com

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Abstract

This paper describes three episodes of foot-and-mouth disease (FMD) that were detected during 2013–2015 in scimitar-horned oryx (*Oryx dammah*) (SHO), a large Sahelo-Saharan antelope extinct in the wild housed in a wild ungulate breeding facility located 50 km east of Abu Dhabi, United Arab Emirates. While no mortality attributable to FMD was noted in the population of nearly 4,000 SHO during two of the three outbreaks, the morbidity varied according to the circulating strains and seroconversion reached a plateau of 78.0% within two weeks and remained at this level for at least nine months. Partial or complete sequencing of the VP1 encoding region demonstrated that the three outbreaks were caused by three different FMDV lineages (O/ME-SA/PanAsia-2, A/ASIA/Iran-05 and O/ME-SA/Ind-2001), consistent with FMD viruses that are circulating elsewhere in the region. These findings demonstrate that SHO are susceptible to FMD and highlight the risks of virus incursion into zoos and captive facilities in the Arabian Peninsula.

KEYWORDS

epidemiology, foot-and-mouth disease, Scimitar-horned Oryx (*Oryx dammah*), United Arab Emirates

1 | INTRODUCTION

The scimitar-horned oryx (SHO) (*Oryx dammah*) is a large antelope that along with three other species belongs to the *Oryx* genus within the Hippotraginae subfamily. The SHO had a distribution range across the Sahelian countries, from Mauritania to the Nile river in Egypt and Sudan, and it has been suggested that its population reached one million in the early Holocene period (9500–4500 BC) (Iyengar et al., 2007). Despite being previously widely distributed in large numbers, the twentieth century brought the species to extinction in the wild due to a combination of rangeland degradation, competition with livestock, uncontrolled hunting and civil unrest. Today, survival of this species relies on captive breeding (East, 1999)

and the entire global population is estimated between 15,000 and 19,000 head (Woodfine & Gilbert, 2016), distributed in 444 institutions across 48 countries. Trophy hunting ranches in Texas, United States of America (USA) account for about 11,000 individuals and the United Arab Emirates (UAE) for another 4,000. To counter the disappearance of this species from the wild, conservation organizations have initiated several reintroduction projects and the most important of these in terms of number of reintroduced animals is the one currently underway between the UAE and Chad (Newby, 2016).

Foot-and-mouth disease (FMD) is a highly contagious viral disease affecting all cloven-hoofed animals that is caused by a member of the *Aphthovirus* genus belonging to the *Picornaviridae* family. The virus has a positive-sense single-stranded RNA genome (Flather &

	Dates	Pens	FMDV lineage	Percentage of SHO presenting FMD lesions
Outbreak 1	Jan 13	B1 and A	O/ME-SA/PanAsia-2 ^{ANT-10}	0.6% (0.1%*)
Outbreak 2	Dec 13	B1	A/ASIA/Iran-05 ^{FAR-11}	0.2%*
Outbreak 3	Mar 15	C and D	O/ME-SA/Ind-2001d	28.5%

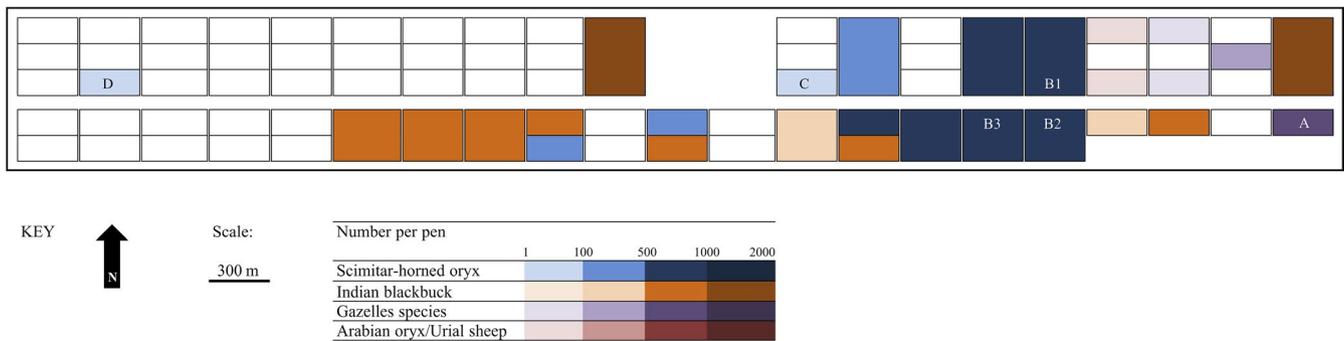


FIGURE 1 Outbreak chronology and schematic representation of the compound. A simplified layout of the animal facility: black lines represent single mesh fence. Empty pens as well as access corridors and perimeter buffer zone appear in white while Pens with scimitar-horned oryx (SHO) appear in blue, Indian blackbucks in orange, gazelles species in purple, Arabian oryx and Urial sheep in pink. Darker colour tones represent pens with larger numbers of animals. For clarity, pen subdivisions are not shown. Pens discussed in this paper as well as locations of FMD outbreaks, dates and isolated FMDV are shown. For each outbreak, the percentage of SHO presenting FMD characteristic lesions was calculated amongst the SHO that underwent clinical examination and/or amongst the SHO that were only visually assessed (*) [Colour figure can be viewed at wileyonlinelibrary.com]

Semler, 2015) that is encapsidated within an icosahedral shell comprising four structural proteins (SP) called VP1, 2, 3 and 4. VP1 is responsible for virus attachment and entry, and also contains many of the determinants that confer protective immunity and serotype specificity (Carrillo et al., 2005). The coding sequence of VP1 (1D) is frequently targeted for sequencing for virus typing and tracing. Seven virus pools (1–7) have been proposed to define the geographical circulation of the seven immunologically distinct FMD virus (FMDV) serotypes: O, A, C, Asia 1, Southern African Territories (SAT) 1, SAT 2 and SAT 3 along with their topotypes, genetic lineages and strains. The Arabian Peninsula (including the UAE) located in Pool 3 is home to regional serotype O, A and Asia 1 lineages (O/ME-SA/PanAsia-2, A/ASIA/Iran-05 and Asia-1/Sindh-08 (Brito, Rodriguez, Hammond, Pinto, & Perez, 2017; Knowles et al., 2009) and has also recently experienced incursions of viral lineages (O/ME-SA/Ind-2001 and A/ASIA/G-VII) from Pool 2 (South Asia) (Bachanek-Bankowska, Di Nardo, Wadsworth, Henry, et al., 2018; Bachanek-Bankowska, Di Nardo, Wadsworth, Mioulet, et al., 2018).

This study describes FMD cases that occurred in high-density captive-bred SHO in the UAE, a country where small ruminants predominate: 1,850,462 sheep and 2,082,926 goats were registered in 2014 compared with 50,103 head of cattle (Ministry of Environment & Water, 2015). This animal collection was located inland in close proximity to an intercity highway and a truck road, 45 km east of Abu Dhabi (United Arab Emirates) and more than 100 km from the borders with neighbouring countries. A separate complex with hundreds of 'ezbas', the local traditional farms (Chaber & Saegerman, 2017) was situated 3,900 m to the east. The facility measured over 6,000 m long by 750 m wide. It could be represented as an elongated chessboard (Figure 1) and comprised more than 50 pens ranging in

size from 150 × 150 m to 300 × 450 m. Animals were not present in all pens and an aerial animal-registration survey undertaken at the end of November 2012 indicated there were 7,931 Indian blackbucks (*Antelope cervicapra*) in 13 pens, 3,894 SHO in 11 pens, 1,300 reem gazelles (*Gazella marica*) in four pens, 258 mountain (*Gazella gazella*) and Indian (*Gazella bennetti*) gazelles in five pens, 11 urial sheep (*Ovis orientalis*) in one pen and four Arabian oryx (*Oryx leucoryx*) in one pen. Species were kept separate, but pens contained animals of both sexes and all age categories (apart from one pen with four male Arabian oryx and one pen with 246 male SHO).

Animals displaying only deciduous teeth were considered as juveniles, while those with one or two pairs of adult incisors were categorized as subadults and three or four pairs were defined as adults. Based on those criteria, the subadult category would be estimated between approximately 19 and 27 months old in the closely related Arabian oryx (Ancrenaz & Delhomme, 1997).

There was no history of FMDV vaccination or disease screening for FMD. Typically, no animals went out of the collection but large numbers (hundreds) of sand gazelles were moved in during 2012 and 2013. FMD cases occurred in three distinct episodes (outbreaks) in pens A, B1, 2 and 3, C and D. Pen A was located at one end of the facility, pens B1, 2 and 3, and C were in the middle while pen D was well separated from the high-density pens and located at the other end. In early 2013, a selection process based on morphological criteria and on infectious disease status was initiated to create a breeding herd of SHO that were more intensively managed. This work led to the visual assessment of all 1,952 SHO present in pens B1, B2 and B3. Subsequently between 1 January and 19 March 2013, 351 of these animals were selected and captured; each received an individually numbered ear tag and

a succinct clinical examination including the eyes and oral cavity was performed. Abnormal findings or clinical signs were recorded, and blood was collected from the jugular vein for serological analyses. Sixty-seven passed this first screening test and were moved into pen C. On 5 November 2013, these SHO were blood sampled again and were all re-identified with a subcutaneous microchip because some animals had lost their ear tag, after which they were all moved to pen D, leaving pen C empty. In 2014, the process was repeated in other pens containing SHO resulting in the movement of 41 new SHO into pen C.

Outbreak 1: During the examination that was ongoing between January and March 2013, two juvenile SHO were identified that exhibited lesions compatible with FMD: a 6-month-old male had a 6 mm ulcer on the gum of the upper lip on 28 January 2013 and 8-month-old male displayed ulcers on gum and coronary band the following day. Swab samples from both animals were collected for virology testing. Five days later, on 2 February 2013, four sand gazelles died in pen A, 1,100 m away from pen B1. These animals presented with ulcers in the oral cavity, on the gum or on the tongue (Figure 3). The mortality in that pen during the first quarter of 2013 was very high, with deaths accounting for 663/1095 gazelles (60.7% mortality rate), approximately 10 times the mortality rate observed in a sand gazelle population housed in similar conditions but located in a separate facility that was not affected by FMD (approximately 6% over three months) (Lignereux, Chaber, et al., 2018). It is not possible to determine if FMD accounted for all the recorded mortality, although we speculate it might have played an important role.

After their recapture in pen C in November, the sera from the 67 SHO that met the selection criteria were analysed together with the sera collected from those same animals during Outbreak 1 in pens B1, 2 and 3. Three serum samples collected early 2013 were

not available for these analyses; thus 131 sera were analysed using a commercial indirect (blocking) enzyme-linked immunosorbent assay (ELISA) kit 'FMD 3ABC bo-ov' (Idexx, USA; formerly known as 'Chekit-FMD-3ABC' (Bommeli AG, Switzerland) which specifically measures antibodies directed at FMDV non-structural proteins (NSP). The test was performed and interpreted according to the manufacturer's recommendations; however, since this test has not been validated for the SHO species, results should be interpreted with caution. The results shown in Figure 2 demonstrate that all 17 SHO tested until 21 January 2013 were FMDV NSP seronegative, and the first seropositive case appeared on 28 January 2013. Seroprevalence plateaued at 78.0% amongst the 41 SHO tested (32 positive) between 12 February and 19 March 2013. These results are compatible with an introduction of FMDV during the last week of January 2013 and rapid transmission of the virus within the collection due to its high contagiousness which match the observed clinical cases. On 5 November 2013, the seroprevalence was 77.6%, 95% CI [65.8–86.9] (exact binomial distribution) amongst the 67 SHO tested (52 positive, six doubtful, nine negative).

Outbreak 2: On 16 December 2013 a dead juvenile SHO was found in pen B1 amongst approximately 490 SHO. This animal had gum and coronary band ulcerations that were swabbed for testing (Figure 3).

Outbreak 3: On 2 March 2015, 26 out of the 41 SHO in pen C (63.4%) presented with lesions characteristic of FMD infection (Figure 4). The affected animals were predominantly subadults (17 out of 28) but cases also included adults (nine out of 13). Out of 58 lesions seen, the gum was the anatomical region affected the most (39.7% of 58), followed by the dental pad (25.9%), the coronary band (13.8%), the nostril (12.1%), the tongue (6.9%) and the base of the horn (1.7%). In the following 2 weeks, on 9 March and 16 March

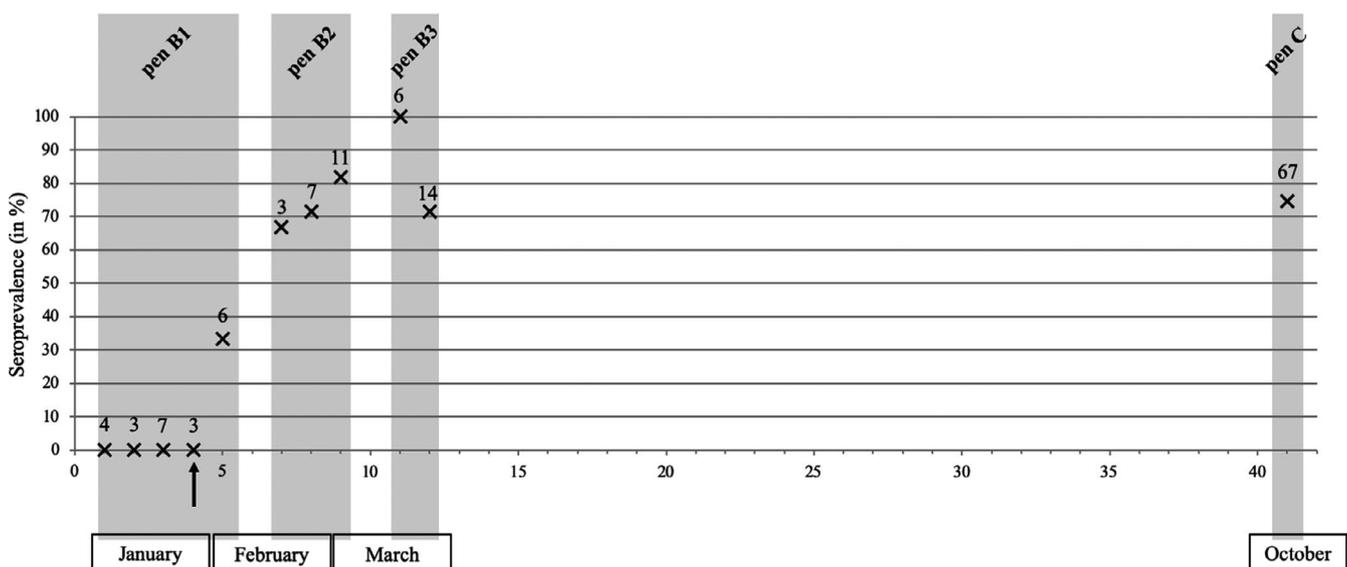


FIGURE 2 FMD seroprevalence in scimitar-horned oryx (SHO) in 2013. Each marker represents the percentage of seropositive results to FMD non-structural protein 3ABC ELISA in SHO tested the same week. The x-axis represents the time, in week, from January to November 2013. The pens where the SHO were blood sampled are indicated by the grey rectangles. The number of SHO tested is given above each marker

FIGURE 3 FMD gross lesions observed during Outbreaks 1 and 2. Picture 1: mucosal ulceration of the tongue observed on a sand gazelle where O/UAE/2/2013 was collected; Picture 2: gingival mucosal ulceration observed on the gum and dental pad of a scimitar-horned oryx (SHO) where A/UAE/1/2013 was collected; Picture 3: perioplic ulceration and Picture 4: circular mucosal erosions of the tongue of the same SHO [Colour figure can be viewed at wileyonlinelibrary.com]



2015, 12 out of the 89 SHO (13.4%) in pen D (formerly in pen C and affected by Outbreak 1) displayed characteristic FMD lesions: cases included two adults out of 54, one subadult out of 17, nine juveniles out of 18. All animals were in good body condition and their feed intake, while not measured, was considered normal at the time. Other parameters, such as fertility, were not assessed.

The tissue collected after swabbing the oral lesions was kept frozen at -20°C in Universal Viral Transport (Beckton and Dickinson, USA) until laboratory analysis could be carried out. Two oral swabs (one from a SHO, one from a sand gazelle) were submitted for Outbreak 1 and following confirmation of the pathogen at WRLFMD (Pirbright, UK) by virus isolation, antigen-detection ELISA and real-time RT-PCR, the VP1 coding region was amplified by RT-PCR and sequenced as previously described (Knowles, Wadsworth, Bachanek-Bankowska, & King, 2016). FMDV was also confirmed with virus isolation and real-time RT-PCR for Outbreaks 2 and 3 at the Onderstepoort Veterinary Research (Pretoria, South Africa) upon receiving four oral swabs, and viral RNA was reverse-transcribed using AMV-Reverse Transcriptase (Promega, USA) and the

partial VP1 gene region was amplified to obtain DNA for sequencing using Go-taq (Promega, USA) combined with the WDA (Beck & Strohmaier, 1987) and VP1O (Rodriguez et al., 1994) Type O-specific or the NK61/A-1C₅₆₂ (Knowles and Samuel, 1998) Type A-specific oligonucleotide sets, respectively.

Sequences were aligned using BioEdit v7.2.5 (Hall, 1999). Maximum-likelihood phylogenetic trees were generated using MEGA7 (Kumar, Stecher, & Tamura, 2016) and were based on the best nucleotide substitution model as implemented in the programme. The Kimura 2-parameter (type O) and Hasegawa-Kishino-Yano (type A) models were chosen. In order to establish the parameters for phylogenetic analyses, a discrete gamma distribution was used to model evolutionary rate differences amongst sites [five categories (+G)] and in the case of the type A tree there was allowance for some sites to be evolutionarily invariable (+I). About 1,000 bootstrap replicates (Felsenstein, 1985) were used to assess branching reliability (only values to 70% and above are shown).

FMD viruses recovered from two samples collected from Outbreak 1 (UAE/1/2013 and UAE/2/2013, GenBank accession

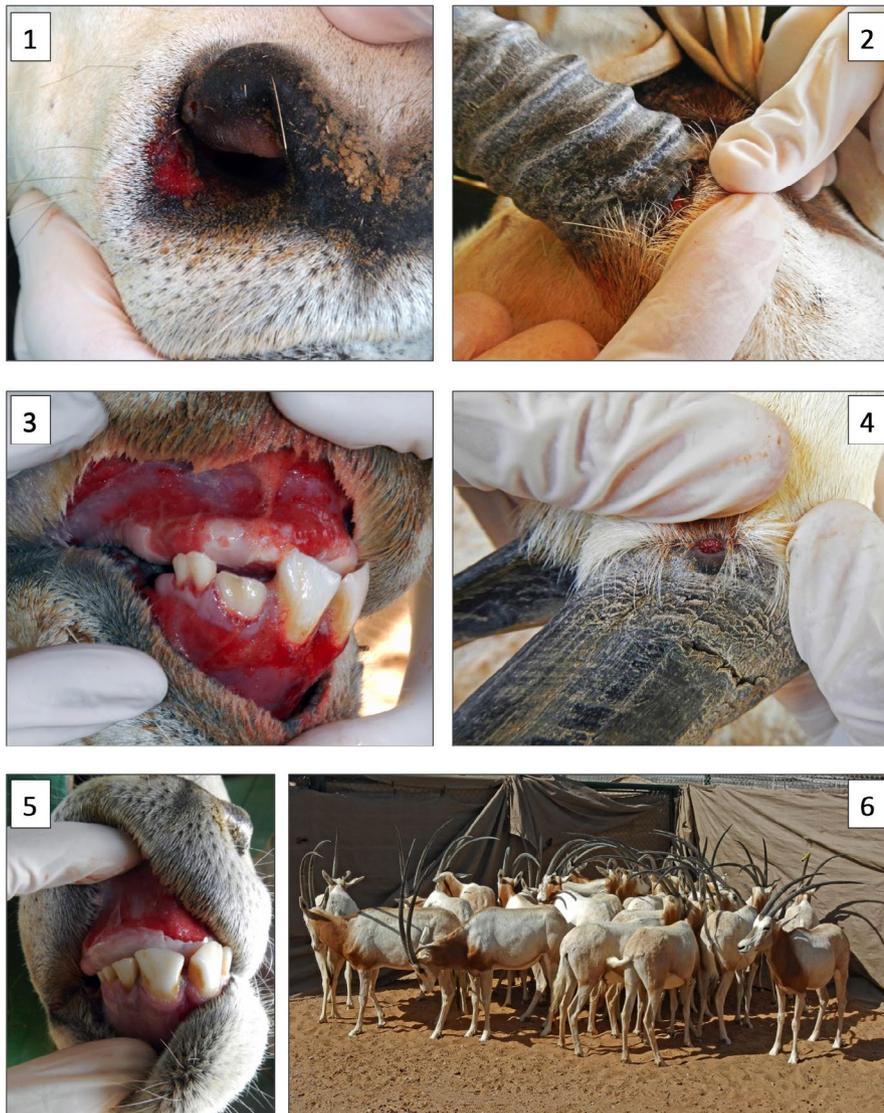


FIGURE 4 External gross lesions observed in a group of scimitar-horned oryx (SHO) during Outbreak 3. Picture 1: mucosal ulceration in a nostril; Picture 2: cutaneous ulceration at the base of a horn; Pictures 3 and 5: gingival mucosal ulceration of the dental pad and the gum; Picture 4: perioplic ulceration; Picture 6: the group of young SHO where the outbreak was described, showing their apparent external good condition [Colour figure can be viewed at wileyonlinelibrary.com]

number MN276040 and MN276041) were characterized as belonging to the O/ME-SA/PanAsia-2^{ANT-10} sub-lineage (Figure 5), sharing closest nucleotide identity (98.8%) with an FMDV isolate collected from Iran (O/IRN/13/2012). The FMDV responsible for Outbreak 2 in December 2013 was a serotype A belonging to the A/ASIA/Iran-05^{FAR-11} sub-lineage (GenBank accession number MN276043) (Figure 6), while partial VP1 sequences (304 nucleotides) recovered from Outbreak 3 were characterized as belonging to the O/ME-SA/Ind-2001d lineage (Figure 5). The sequence for the Outbreak 3 virus (GenBank accession number MN276042) was identical to O/UAE/1/2014 and O/UAE/2/2014, GenBank accession number KM921877 and KM921878, respectively, collected on 8 January 2014 on gazelles in captivity, 40 km north from the collection studied here and is representative of a FMDV lineage that has been recently introduced into the Arabian Peninsula on multiple occasions (Bachanek-Bankowska, Di Nardo, Wadsworth, Mioulet, et al., 2018).

During the 3-year span of this study, no case fatality or clinical signs that could have been attributed to FMD were recorded in the approximately 8,000 Indian blackbucks even in pens contiguous to

SHO affected by FMD. This observation contrasts somewhat with the high mortality following an FMD type O outbreak described by Kar, Hota, and Acharjyo (1983) and relayed by Thomson, Vosloo, and Bastos (2003) and Weaver, Domenech, Thiermann, and Karesh (2013). The high mortality observed in the gazelle population, while speculated and not well recorded in our case is more in agreement with other published data (Bailey, O'Donovan, Kinne, & Wernery, 2009; Shimshony et al., 1986). The number of individuals in the collection belonging to other ruminant species is too low to draw conclusions about their epidemiological role. The Arabian oryx was previously documented as being a spill-over following O serotype outbreak (Ostrowski & Anajariyah, 2003) in (Frölich et al., 2005), and high mortality and morbidity due to FMD were recorded in this species (Lignereux, Alzahlawi, Al Kharusi, & Pesci, 2018).

The three FMD outbreaks that are recorded in this study demonstrate that SHO are susceptible to infection with FMDV and may exhibit variable clinical expression depending on the lineage of FMDV. The sequences represent three viral lineages (from two serotypes) currently circulating in Pool 3 where FMD is endemic. Long-distance

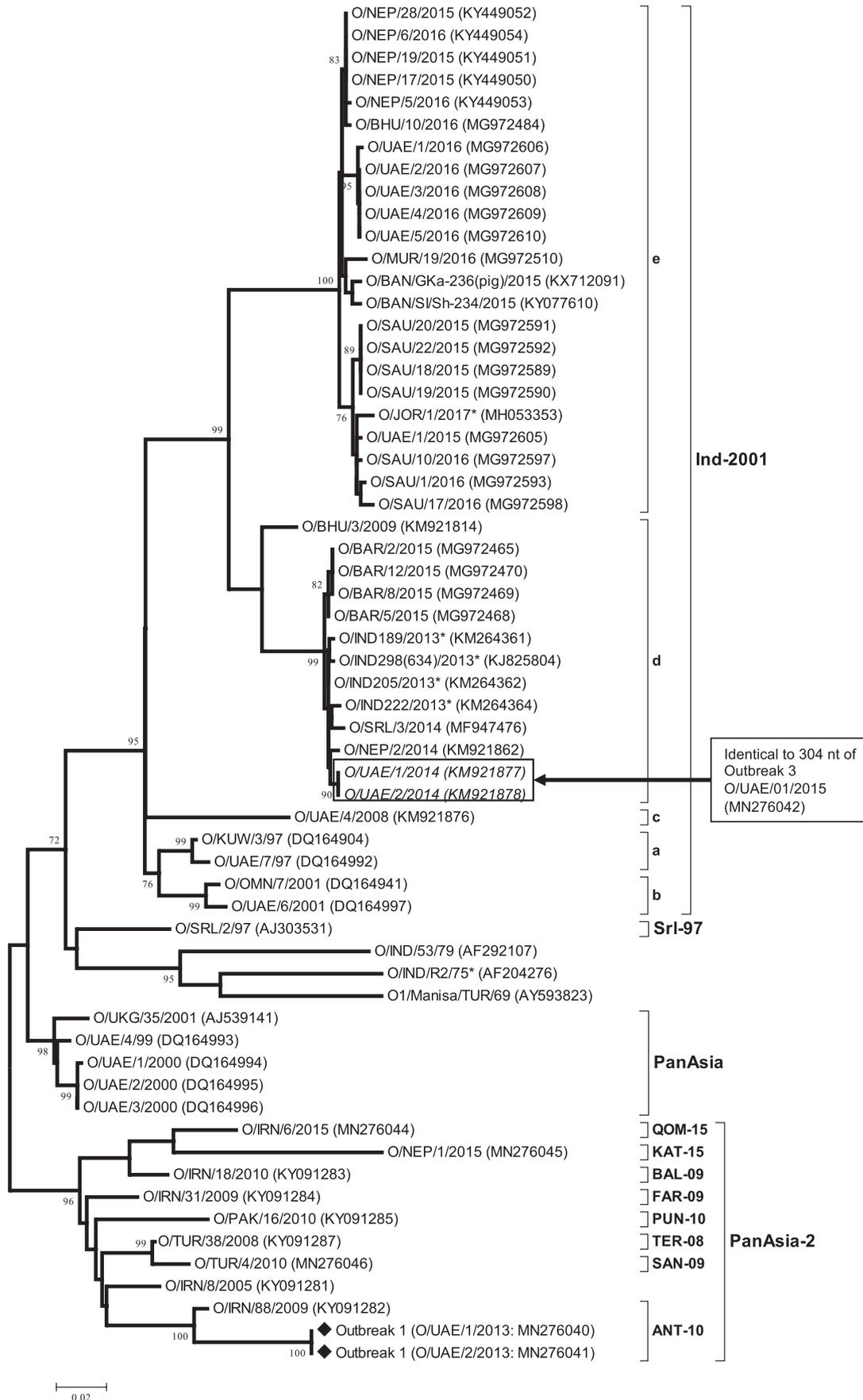


FIGURE 5 Phylogenetic tree based on viral VP1 sequences showing the relationship between the serotype O sequences recovered from Outbreak 1 and Outbreak 3 (shown with a diamond symbol [♦]) * denotes FMD virus sequences that are not WRLFMD codes

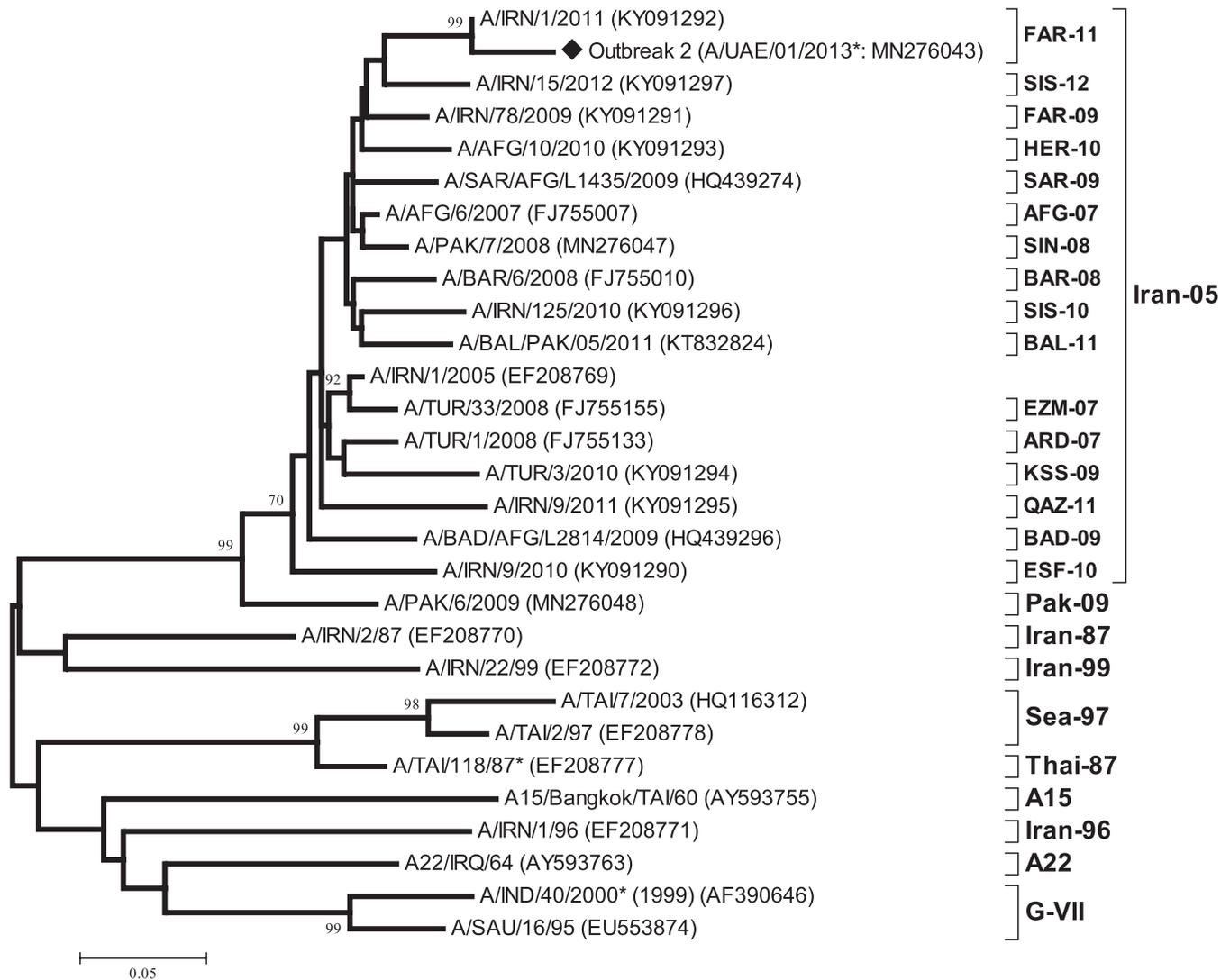


FIGURE 6 Phylogenetic tree based on viral VP1 sequences showing the relationship between the serotype A sequences recovered from Outbreak 2 (shown with a diamond symbol [♦]). * denotes FMD virus sequences that are not WRLFMD codes

FMD transmission has been documented (Gloster, Sellers, & Donaldson, 1982) but vicinity to roadways, where livestock transit and to traditional farms compounds could be seen as potential risk factors for FMD infection for wildlife collection. However, the precise transmission routes by which these viruses have entered the facility are not known. Further studies should focus on evaluating the cost-benefit ratio of vaccination programmes in those wildlife collections as well as systematic investigation of the unlikely maintenance host status of the affected species. Within the UAE, FMD outbreaks in wildlife collections are frequently reported, and results from this study indicate that it would be beneficial to integrate the large wildlife collections present in the UAE as sentinels to provide valuable epidemiological data in the framework of an FMD control programme.

How these different FMD viruses entered and spread unnoticed on at least three occasions remains unanswered. The importance of contributing factors that might help explain these patterns is not well understood, such as the presence of large numbers of small ruminants, known to harbour and spread FMDV

with mild to inapparent clinical signs (Geering, 1967; Hughes et al., 2002; Stenfeldt et al., 2015) as well as connections to numerous traditional farms in the region, possibly with poor biosecurity (Chaber & Saegerman, 2017) and inadequate FMD vaccination coverage. In light of these gaps, we recommend that efforts are made to identify and quantify the risk factors for FMD importation to, and transmission within the UAE.

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CONFLICT OF INTEREST

The authors declared that they have no conflict of interest.

ETHICAL APPROVAL

Ethical statement is not applicable to this study as the data were gathered through the everyday animal handling for population management purposes, as part of an ex situ conservation project.

ORCID

Louis Lignereux  <https://orcid.org/0000-0001-6493-9226>

Anne-Lise Chaber  <https://orcid.org/0000-0001-9042-8753>

Claude Saegerman  <https://orcid.org/0000-0001-9087-7436>

Nick J. Knowles  <https://orcid.org/0000-0002-6696-1344>

Jemma Wadsworth  <https://orcid.org/0000-0002-1110-3203>

Donald P. King  <https://orcid.org/0000-0002-6959-2708>

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