Genotyping of foot-and-mouth disease viruses collected in Sudan between 2009 and 2018

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Abstract
Foot-and-mouth disease (FMD) is widely distributed in Sudan where outbreaks occur on an annual basis especially during the winter months (December-February). This study aimed to increase our understanding of the epidemiological patterns of FMD in Sudan and connections to neighbouring countries by characterizing the genetic sequences of FMD viruses (FMDV) collected from samples collected in 10 Sudanese states over a 10-year period (between 2009 and 2018). FMDV was detected in 91 of the 265 samples using an antigen-detection ELISA. Three serotypes were detected: O (46.2%), A (34.0%), and SAT 2 (19.8%). Fifty-two of these samples were submitted for sequence analyses, generating sequences that were characterized as belonging to O/EA-3 (n = 17), A/AFRICA/G-IV (n = 23) and SAT 2/VII/Alx-12 (n = 12) viral lineages. Phylogenetic analyses provided evidence that FMDV lineages were maintained within Sudan, and also highlighted epidemiological connections to FMD outbreaks reported in neighbouring countries in East and North Africa (such as Ethiopia and Egypt). This study motivates continued FMD surveillance in Sudan to monitor the circulating viral lineages and broader initiatives to improve our understanding of the epidemiological risks in the region.

KEYWORDS
Aphthovirus, epidemiology, foot-and-mouth disease, nucleotide sequence, Sudan

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Foot-and-mouth disease (FMD) remains a worldwide major constraint to animal production and international trade. The causative agent, FMD virus (FMDV; genus *Aphthovirus*, family *Picornaviridae*), exists as seven immunologically distinct serotypes (O, A, C, Asia 1, SAT 1, SAT 2 and SAT 3) and the virus is known to infect up to 70 cloven-hoofed animal species (Grubman & Baxt, 2004). The epidemiology of FMD in Africa is complex. This is partly because the continent is resident to three out of the seven endemic FMDV pools (Pools 4, 5 and 6; Paton et al., 2009), and six out of the seven FMDV serotypes have been recorded (Rweyemamu et al., 2008), although serotype C has not been detected since 2004 (Paton et al., 2021). Sudan is one of the largest African countries (nearly 1.9 million km²) which has a resident population of more than 100 million FMD-susceptible animals. It has 30–40 million cattle, 70 million small ruminants as well as a diverse range of FMD susceptible wildlife species that are located mostly in the Southern areas or in the Dinder National Park in Eastern Sudan (Anon, 2009). Sudan is recognized as an important crossroads between sub-Saharan and Northern Africa, and between East and West Africa.

FMD has been known to be present in Sudan since 1903 (Abu Elzein, 1983) and previous studies have highlighted the contribution of FMD circulation in the country to the wider epidemiology of the disease in Africa (Bronsvoorst et al., 2004; Hall et al., 2013; Rweyemamu et al., 2008; Ularamu et al., 2017). In sub-Saharan African countries, FMD is endemic but clinical signs are often mild or inapparent particularly in sheep and goats and outbreaks are inconsistently reported to veterinary services. Together with the costs and logistics associated with the shipment of suspected FMDV samples, these issues result in infrequent submissions to international reference centres [such as the World Reference Laboratory for FMD (WRLFMD), Pirbright, United Kingdom] and consequent potential for biases in epidemiological inferences (Bronsvoorst et al., 2004). To address these sampling and reporting issues, the Government of Sudan together with the Food and Agriculture Organization of the United Nations (FAO) and the European Commission for the Control of FMD (EuFMD) joined efforts in a Technical Corporation Programme (TCP/SUD/3401) to promote active surveillance of FMD in Sudan which was undertaken between 2012 and 2014. In this project extensive serosurveillance to detect antibodies against FMDV in cattle and small ruminants was carried out and the data has been reported elsewhere (Raouf et al., 2016, 2017). Furthermore, epithelium samples were collected during 2009–2018 from suspected disease events in 10 Sudanese states for virus detection, serotyping and molecular characterization. Four different FMDV serotypes have been reported to circulate in Sudan (O, A, SAT 1 and SAT 2) with SAT 1 last detected in 1976 (Habiela et al., 2010a). Vaccination against FMD has not been practiced to any appreciable extent in Sudan up to 2016 when vaccination of cattle in the Northern state was carried out but discontinued thereafter. In previous reports, type O has been detected more frequently while serotypes SAT 2 and A are found more sporadically (Abu Elzein, 1983; Abu Elzein et al., 1987; Habiela et al., 2010b; Raouf et al., 2016). The aim of this current study is to improve our understanding of the epidemiological patterns of FMD in Sudan and connections to neighbouring countries through characterizing FMDV in clinical samples.

## MATERIALS AND METHODS

### 2.1 Clinical samples

During passive surveillance undertaken in the period 2009–2018, epithelium tissues ($n = 265$) were collected from suspected cases of FMD in cattle in 10 Sudanese states: Khartoum, River Nile, Gezira, Al Qadarif, White Nile, Blue Nile, North Kordofan, Kassala, North and South Darfur. Each year between 15 and 30 samples were collected except for 2013 and 2014 when more than 100 samples were collected. Sampled animals (all cattle) were mainly of around 1 to 5 years old, mostly female and occasionally male calves or bulls. Epithelium tissues were usually collected from recent cases in transport media comprised of equal amounts of glycerol and 0.04 M phosphate buffer, 0.001% phenol red, antibiotics and antimycotics (pH 7.2–7.6). Samples were kept refrigerated until reaching the laboratory where they were kept at $–20^\circ$C.

### 2.2 FMDV detection and serotyping

All samples were screened for the presence of FMDV antigen at the Central Veterinary Research Laboratory (CVRL) either using the indirect sandwich ELISA kit (WRLFMD; Roeder & Le Blanc Smith, 1987) or using the monoclonal antibody based antigen detection and serotyping ELISA kit (IZSLER Biotech, Brescia, Italy; Grazioli et al., 2012; Grazioli et al., 2020). For sample testing, a suitable amount of epithelium was used to prepare a 10% suspension (W/V) using Glasgow minimum essential medium (GMEM), sterile sand and a pestle and mortar. Unprocessed epithelium was returned to $–20^\circ$C deep freezer until shipment to the WRLFMD. The indirect sandwich ELISA kit employed polyclonal antibodies as trapping (rabbit antisera) and detecting (guinea pig antiserum) antibodies in addition to antigen in each set of reagents while the IZSLER kit employed selected combinations of anti-FMDV monoclonal antibodies (MAbs) as coated and conjugated antibodies. All ELISA reagents were prepared and supplied by the WRLFMD (the indirect sandwich ELISA kit) or by the Istituto Zooprofilattico Sperimentale della Lombardia e dell’Emilia Romagna (IZSLER kit) and were used according to the supplied protocols.

A subset of these FMDV positive samples ($n = 52$) were submitted to the WRLFMD (at the Pirbright Institute, Surrey, UK) for further study. Unprocessed epithelium samples in the described transport medium were dispatched, under dry ice, as dangerous biological substance category B UN 3373. At the WRLFMD, samples were tested by real time RT-PCR (Callahan et al., 2002; Shaw et al., 2007) and virus isolation using primary bovine thyroid and IB-RS-2 cells as previously described (de Castro, 1964; Snowden, 1966). Samples were considered FMDV negative if no CPE was observed for 48 h following blind passage of the first cell cultures after 48 h incubation at $37^\circ$C. Samples in which
FMDV was identified (n = 91) are listed in Table 1 together with associated metadata; those lacking a WRLFMD reference number (n = 38) were not submitted to the WRLFMD.

2.3 | Sequencing and phylogenetic analysis

VP1 sequences were generated using previously described methods (Knowles et al., 2016). Briefly, for each serotype two independent RT-PCR assays were performed using the following primer pairs: O-1C244F/EUR-2B52R and O-1C272F/EUR-2B52R (type O); A-1C562F/EUR-2B52R and A-1C612F/EUR-2B52R (type A); and SAT2-1C445F/SAT-2B208R and SAT2-P1-1223F/SAT-2B208R (type SAT 2). Sanger sequencing was performed on an ABI 3730xl DNA Analyzer (ABI Biosystems, Waltham, Massachusetts). Complete VP1 nucleotide sequences were aligned using BioEdit 7.0.5.3 (Hall, 1999) and Clustal W 1.83 (Thompson et al., 1994). Optimal nucleotide substitution models were computed for each serotype using MEGA 7 (Kumar et al., 2016). The maximum likelihood algorithm was used to construct phylogenetic trees using MEGA 7. One thousand bootstrap pseudoreplicates were used to assess branching confidence.

3 | RESULTS

3.1 | FMD positive samples

All the 265 samples collected during this study (2009–2018) were of cattle origin. Approximately one-third of these samples (91/265; 34.3%) were successfully serotyped using antigen detection ELISA. Fifty-two of these samples were shipped to the WRLFMD and subjected to further testing. All 52 samples showed CPE within 24–48 h of being inoculated onto primary BTy cells and were subjected to VP1 nucleotide sequencing. Most of the typed samples were collected during the winter months (62/91; 68.1%): comprising 27/91 (29.7%) in December, 14/91 in January (15.4%), and 20/91 in February (22.0%). This compares to lower numbers of FMDV positive samples collected in March (17/91; 18.7%) and April (6/91; 6.6%). Only 6.6% (6/91) of the typed samples were collected between May and October.

The geographical distribution of FMDV positive samples collected in Sudan between 2009 and 2018 is described in Table 1 and Figure 1. FMDV was detected in 91 samples which were also serotyped. Disease events comprised three serotypes: O (42/91; 46.2%), SAT 2 (18/91; 19.8%) and A (31/91; 34.0%). FMD type O disease events extended over almost the entire reported period (Figure 1), apart from 2018, while serotype A was detected in 5 years (2011, 2013, 2014, 2015 and 2018) and serotype SAT 2 was detected in 4 years (2010, 2013, 2014 and 2017). Serotype A was detected in the Central States of Khartoum and Gezira. In comparison, serotypes O and SAT 2 had a wider geographical distribution (Figure 1). Serotype O was detected along the Nile basin, from the White Nile State in the South to Khartoum and Gezira in Central Sudan and up to the Northern State in North-ern Sudan. Serotype SAT 2 was detected in Central Sudan at Khartoum, Gezira and North Kordofan and in addition in one South Eastern State (Blue Nile) and one Eastern Border State (Al Qadarif).

3.2 | Phylogenetic analysis

All FMDV positive samples generated a single VP1 sequence apart from SUD/7/2017 where a mixture of O and SAT 2 were recovered. Antigen ELISA typing of the SUD/7/2017 BTy1 material showed it to be SAT 2, so sample contamination with FMDV type O cannot be excluded. The most appropriate nucleotide substitution models for each serotype were found to be the Tamura-Nei (TN-93) model, gamma distributed with invariant sites (G+I) (type O and type SAT 2); and the Hasegawa-Kishino-Yano (HKY) model, G+I (type A).

3.3 | Serotype O

All the eighteen serotype O viruses that were genotyped during this study fell within the O/EA-3 topotype (Figure 2). These sequences represent at least four distinct clades (indicated by grey arrows in Figure 2); three of which occurred during the same period (2009–2011), while a fourth cluster contained more recent isolates from 2013, 2016 and 2017. Nucleotide identities within clusters were mostly >95%, in contrast to sequence differences among clusters of between 5% and 7%. The first cluster contained isolate SUD/11/2011 from this study, together with older Sudanese FMDVs collected between 2004 and 2008, with Nigerian viruses collected between 2007 and 2014 and two Cameroon viruses collected in 2010. The second cluster contained three isolates: SUD/1/2009, SUD/1/2010 and SUD/2/2010 related to FMDV sequences from Sudan in 2008, while the third cluster contained five identical sequences for FMDV isolates collected in 2012, one collected a year earlier (O/SUD/9/2011) as well as samples from Eritrea (2011), Ethiopia (O/ETH/59/2011) and Egypt (2012). The final clade comprised seven Sudanese FMDV isolates collected in 2016/17 and an earlier sample from 2013 (O/SUD/4/2013). Viruses in this clade are part of a larger temporal phylogenetic cluster representative of O/EA-3 outbreaks reported in Ethiopia, Egypt, Israel and Palestine in 2017.

3.4 | Serotype A

Twenty-three serotype A viruses belonging to the A/AFRICA/G-IV lineage were genotyped (Figure 3) during this study. Sudanese FMDVs, collected during 2018, belonged to two genetic clades (12.8–14.6% nt difference), with older ones represented in ancestral clusters. One of these contemporary clusters containing A/SUD/6-9/2018 also contained FMDVs collected during 2015–2017 in Egypt and Ethiopia in 2015 (A/ETH/19/2015). At the common root of both clusters were sequences for FMDVs collected during 2006 from Sudan and Eritrea.
| Disease season | WRLFMD Ref. No. | Collection date | Location | Host | Serotype | Topotype | Lineage | Sequence access no. | Sequence reference |
|----------------|-----------------|-----------------|----------|------|----------|----------|---------|-------------------|-------------------|
| **2009–2010**  | SUD/1/2009      | 12/2009         | Redwan, Omdurman, Khartoum | Cattle | O       | EA-3     |         | KX258033 | Ularamu et al. (2017) |
|                | SUD/1/2010      | 1/2010          | Hilat Kuku, Khartoum | Cattle | O       | EA-3     |         | KX258034 | Ularamu et al. (2017) |
|                | SUD/2/2010      | 5/2010          | Keriab, Khartoum North, Khartoum | Cattle | O       | EA-3     |         | KX258035 | Ularamu et al. (2017) |
|                | SUD/4/2010      | 13B/2010        | Sheikan, Sheikan, North Kordofan | Cattle | SAT 2   | VII      | AIx-12  |KF112968|Hall et al. (2013) |
|                |                 |                 |          |      |          |          |         |       |                   |
| **2010–2011**  | SUD/1/2011      | 1/2011          | Keriab, Khartoum North, Khartoum | Cattle | A       | AFRICA G-IV | MK422575 | This work |
|                | SUD/6/2011      | 14/2011         | Hilat Kuku, Khartoum North, Khartoum | Cattle | A       | AFRICA G-IV | MK422576 | This work |
|                | SUD/7/2011      | 18/2011         | Shigla, Khartoum North, Khartoum | Cattle | A       | AFRICA G-IV | MK422577 | This work |
|                | SUD/12/2011     | 31/2011         | Sarha, Omdurman, Khartoum | Cattle | A       | AFRICA G-IV | MK422579 | This work |
|                | SUD/13/2011     | 38/2011         | Moilah, Khartoum | Cattle | A       | AFRICA G-IV | MK422578 | This work |
|                | SUD/9/2011      | 28/2011         | Moilah, Khartoum | Cattle | O       | EA-3     | MK422556 | This work |
|                | SUD/11/2011     | 31/2011         | Moilah, Khartoum | Cattle | O       | EA-3     | MK422536 | Ularamu et al. (2017) |
|                |                 |                 |          |      |          |          |         |       |                   |
| **2011–2012**  | SUD/1/2012      | Feb/2012-R. Nile 2/5 | 01/02/2012 | River Nile | Cattle | O       | EA-3     | MK422557 | This work |
|                | SUD/2/2012      | Feb/2012-R. Nile 3/5 | 01/02/2012 | River Nile | Cattle | O       | EA-3     | MK422558 | This work |
|                | SUD/3/2012      | Feb/2012-R. Nile 2/7 | 01/02/2012 | River Nile | Cattle | O       | EA-3     | MK422559 | This work |
|                | SUD/5/2012      | Feb/2012-R. Nile 4/7 | 01/02/2012 | River Nile | Cattle | O       | EA-3     | MK422560 | This work |
|                | SUD/6/2012      | Feb/2012-R. Nile 2/11 | 01/02/2012 | River Nile | Cattle | O       | EA-3     | MK422561 | This work |
|                | None            | R. Nile 1/5     | 01/02/2012 | River Nile | Cattle | O       | nd       | MK422562 | This work |
|                | None            | R. Nile 1/7     | 01/02/2012 | River Nile | Cattle | O       | nd       | MK422563 | This work |
|                | None            | Feb/2012-R. Nile (55) | 13/02/2012 | River Nile | Cattle | O       | nd       | MK422564 | This work |
|                | None            | Feb/2012-R. Nile (56) | 13/02/2012 | River Nile | Cattle | O       | nd       | MK422565 | This work |

(Continues)
| Disease season | WRLFMD Ref. No. | CVRL ID | Collection date | Location | Host | Serotype | Topotype | Lineage | Sequence accession no. | Sequence reference |
|----------------|----------------|---------|-----------------|----------|------|----------|----------|--------|----------------------|-----------------|
| None           | R. Nile (60)   | 19/03/2012 | Alkoa, River Nile | Cattle | O    | nd       | MK422580 | This work |
| None           | R. Nile (61)   | 19/03/2012 | River Nile      | Cattle | O    | nd       | MK422580 | This work |
| 2012–2013      | None           | R. Nile 66 | 10/12/2012      | Shendi, River Nile | Cattle | O    | nd       | MK422580 | This work |
| None           | Ep/1/2013      | 13/01/2013 | Jabal Alawila, Khartoum | Cattle | O    | nd       | MK422580 | This work |
| None           | Ep. 5–6/2013   | 21/01/2013 | Al Kamleen, Gezira | Cattle | O    | nd       | MK422580 | This work |
| SUD/1/2013     | Feb/2013-Kh 1/4 | 19/02/2013 | Khartoum State | Cattle | A    | AFRICA G-IV | MK422580 | This work |
| SUD/3/2013     | April/2013-Kh 8 | 16/04/2013 | Soba, Khartoum | Cattle | SAT 2 | VII | Alx-12 | MK422598 | This work |
| 2013–2014      | SUD/4/2013     | Gez 2    | 30/12/2013      | Alzrieiba, Gezira | Cattle | O    | EA-3 | MK422562 | This work |
| SUD/5/2013     | Kh 10          | 31/12/2013 | Mahlab 2, Khartoum | Cattle | SAT 2 | VII | Alx-12 | MK422599 | This work |
| SUD/9/2013     | N. Kordfan 5   | 31/12/2013 | Alhmadia, North Kordfan | Cattle | SAT 2 | VII | Alx-12 | MK422600 | This work |
| SUD/10/2013    | Gez 3          | 31/12/2013 | Alktaiteib, Gezira | Cattle | A    | AFRICA G-IV | MK422581 | This work |
| SUD/11/2013    | Gez 5          | 31/12/2013 | Alktaiteib, Gezira | Cattle | A    | AFRICA G-IV | MK422582 | This work |
| SUD/12/2013    | Gez 7          | 31/12/2013 | Alktaiteib, Gezira | Cattle | A    | AFRICA G-IV | MK422583 | This work |
| SUD/13/2013    | Gez 9          | 31/12/2013 | Alktaiteib, Gezira | Cattle | A    | AFRICA G-IV | MK422584 | This work |
| SUD/4/2014     | Ged 17         | 01/01/2014 | Gedarif State | Cattle | SAT 2 | VII | Alx-12 | MK422601 | This work |
| SUD/7/2014     | B. Nile 11     | 01/01/2014 | Blue Nile State | Cattle | SAT 2 | VII | Alx-12 | MK422602 | This work |
| SUD/11/2014    | Gez 15         | 01/01/2014 | Gezira State | Cattle | SAT 2 | VII | Alx-12 | MK422603 | This work |
| None           | Kh 5           | 31/12/2013 | Mahlab 2, Khartoum | Cattle | SAT 2 | nd       | MK422585 | This work |
| None           | Kh 6           | 31/12/2013 | Mahlab 2, Khartoum | Cattle | O    | nd       | MK422585 | This work |
| None           | N. Kordfan 3   | 31/12/2013 | Alhmadia, North Kordfan | Cattle | SAT 2 | nd       | MK422585 | This work |
| None           | N. Kordfan 6   | 31/12/2013 | Alhmadia, North Kordfan | Cattle | SAT 2 | nd       | MK422585 | This work |
| None           | R. Nile 2      | Jan-2014  | River Nile      | Cattle | O    | nd       | MK422585 | This work |
| None           | Ged 10         | 01/01/2014 | Al Qadarif State | Cattle | SAT 2 | nd       | MK422585 | This work |
| None           | Ged 16         | 01/01/2014 | Al Qadarif State | Cattle | SAT 2 | nd       | MK422585 | This work |
| None           | Gez 13         | 01/01/2014 | Gezira State    | Cattle | SAT 2 | nd       | MK422585 | This work |
| 2014–2015      | SUD/18/2014    | 2/2015   | 24/12/2014      | Al Tibna, Khartoum | Cattle | A    | AFRICA G-IV | MK422585 | This work |
| SUD/20/2014    | 4/2015         | 24/12/2014 | Al Tibna, Khartoum | Cattle | A    | AFRICA G-IV | MK422586 | This work |
| SUD/21/2014    | 5/2015         | 24/12/2014 | Al Tibna, Khartoum | Cattle | A    | AFRICA G-IV | MK422587 | This work |
| SUD/22/2014    | 6/2015         | 24/12/2014 | Al Tibna, Khartoum | Cattle | A    | AFRICA G-IV | MK422588 | This work |
| None           | Ep-1/2015      | Apr-15   | Kuku, Khartoum | Cattle | A    | nd       | MK422585 | This work |
| None           | Ep-3/2015      | Apr-15   | Kuku, Khartoum | Cattle | A    | nd       | MK422585 | This work |
| None           | Ep-6/2015      | Apr-15   | Kuku, Khartoum | Cattle | O    | nd       | MK422585 | This work |

(Continues)
| Disease season | WRLFMD Ref. No. | Collection date | Location | Host | Serotype | Topotype | Lineage | Sequence accession no. | Sequence reference |
|---------------|-----------------|-----------------|----------|------|----------|----------|--------|-----------------------|------------------|
| None<sup>b</sup> | Ep-7/2015 | Apr-15 | Kuku, Khartoum | Cattle | O | nd | | | |
| None<sup>b</sup> | Ep-9/2015 | Apr-15 | Kuku, Khartoum | Cattle | O | nd | | | |
| 2015–2016 | None<sup>b</sup> | Ep-10/2015 | 06/12/2015 | Safola, Khartoum | Cattle | A | nd | | |
| None<sup>b</sup> | Ep-11/2015 | 06/12/2015 | Safola, Khartoum | Cattle | A | nd | | | |
| None<sup>b</sup> | Ep-12/2015 | 06/12/2015 | Safola, Khartoum | Cattle | A | nd | | | |
| None<sup>b</sup> | Ep-13/2015 | 06/12/2015 | Safola, Khartoum | Cattle | A | nd | | | |
| None<sup>b</sup> | Ep-3/2016 | Sep-16 | Algadeada, Khartoum | Cattle | O | nd | | | |
| None<sup>b</sup> | Ep4/2016 | Sep-16 | Algadeada, Khartoum | Cattle | O | nd | | | |
| None<sup>b</sup> | Ep-5/2016 | Sep-16 | Algadeada, Khartoum | Cattle | O | nd | | | |
| None<sup>b</sup> | Ep-6/2016 | Sep-16 | Algadeada, Khartoum | Cattle | O | nd | | | |
| 2016–2017 | None<sup>b</sup> | Ep-9/2016 | Nov-2016 | Dongla, Northern state | Cattle | O | nd | | |
| None<sup>b</sup> | Ep-/2017 (1) (1-5) | 25/12/2016 | border control, Northern state | Cattle | O | nd | | | |
| None<sup>b</sup> | Ep-/2017 (2) (1-5) | 25/12/2016 | border control, Northern state | Cattle | O | nd | | | |
| None<sup>b</sup> | Ep-/2017 (3) (1-5) | 25/12/2016 | border control, Northern state | Cattle | O | nd | | | |
| None<sup>b</sup> | Ep-/2017 (4) (1-5) | 25/12/2016 | border control, Northern state | Cattle | O | nd | | | |
| None<sup>b</sup> | Ep-/2017 (5) (1-5) | 25/12/2016 | border control, Northern state | Cattle | O | nd | | | |
| SUD/1/2016 | 1/2016 | 25/12/2016 | border control, Northern state | Cattle | O | EA-3 | | MK422563 | This work |
| SUD/2/2017 | 1/2017 | 04/01/2017 | Mahlab 3, Kuku, Khartoum | Cattle | O | EA-3 | | MK422564 | This work |
| SUD/3/2017 | 2/2017 | 04/01/2017 | Mahlab 3, Kuku, Khartoum | Cattle | O | EA-3 | | MK422565 | This work |
| SUD/4/2017 | 11/2017 | 09/01/2017 | Al Rdwan, Khartoum | Cattle | O | EA-3 | | MK422566 | This work |
| SUD/5/2017 | 3/2017 | 07/02/2017 | Al Rdwan, Khartoum (No. 1) | Cattle | O | EA-3 | | MK422567 | This work |
| SUD/6/2017 | 4/2017 | 07/02/2017 | Al Rdwan, Khartoum (No. 2) | Cattle | SAT 2 | VII | Alx-12 | MK422604 | This work |

(Continues)
### TABLE 1 (Continued)

| Disease season | WRLFMD Ref. No. | CVRL ID | Collection date | Location | Host | Serotype | Topotype | Lineage | Sequence accession no. | Sequence reference |
|----------------|----------------|---------|-----------------|----------|------|----------|----------|---------|------------------------|------------------|
| SUD/7/2017     | 5/2017         | 07/02/2017 | Al Rdwan, Khartoum (No. 3) | Cattle | O | EA-3 | MK422568 | This work |
| SUD/7/2017     | 5/2017         | 07/02/2017 | Al Rdwan, Khartoum (No. 3) | Cattle | SAT 2 | VII | Alx-12 | MK422605 | This work |
| SUD/9/2017     | 7/2017         | 21/02/2017 | Al Rdwan, Khartoum (No. 4) | Cattle | SAT 2 | VII | Alx-12 | MK422606 | This work |
| SUD/12/2017    | 14/2017        | 21/02/2017 | Al Rdwan, Khartoum (No. 2) | Cattle | SAT 2 | VII | Alx-12 | MK422607 | This work |
| SUD/14/2017    | 8/2017         | 04/06/2017 | Al Aelfon, Khartoum (No. 1) | Cattle | SAT 2 | VII | Alx-12 | MK422608 | This work |
| SUD/15/2017    | 9/2017         | 04/06/2017 | Al Aelfon, Khartoum (No. 2) | Cattle | O | EA-3 | MK422569 | This work |
| 2017–2018      | SUD/3/2018     | 1/2018    | 06/02/2018 | Al Shigla, Khartoum | Cattle | A | AFRICA | G-IV | MK422589 | This work |
| SUD/4/2018     | 2/2018         | 07/02/2018 | Al Shigla, Khartoum | Cattle | A | AFRICA | G-IV | MK422590 | This work |
| SUD/6/2018     | 5/2018         | 20/03/2018 | Mahlab 2, Kuku, Khartoum | Cattle | A | AFRICA | G-IV | MK422591 | This work |
| SUD/7/2018     | 5-a/2018       | 20/03/2018 | Mahlab 2, Kuku, Khartoum | Cattle | A | AFRICA | G-IV | MK422592 | This work |
| SUD/8/2018     | 6/2018         | 20/03/2018 | Mahlab 2, Kuku, Khartoum | Cattle | A | AFRICA | G-IV | MK422593 | This work |
| SUD/9/2018     | 9/2018         | 22/03/2018 | Safola, Khartoum | Cattle | A | AFRICA | G-IV | MK422594 | This work |
| SUD/10/2018    | 11/2018        | 28/03/2018 | Al Shigla, Khartoum | Cattle | A | AFRICA | G-IV | MK422595 | This work |
| SUD/11/2018    | 12/2018        | 28/03/2018 | Al Shigla, Khartoum | Cattle | A | AFRICA | G-IV | MK422596 | This work |
| SUD/12/2018    | 13/2018        | 28/03/2018 | Al Shigla, Khartoum | Cattle | A | AFRICA | G-IV | MK422597 | This work |
| None           | Ep-4/2018      | 20/03/2018 | Mahlab 2, Kuku, Khartoum | Cattle | A | nd | This work |
| None           | Ep-7/2018      | 20/03/2018 | Mahlab 2, Kuku, Khartoum | Cattle | A | nd | This work |

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### 3.5 Serotype SAT 2

Twelve serotype SAT 2 viruses within the SAT 2 topotype VII, distributed between 2010 and 2017 were characterized (Figure 4). All of these Sudanese viruses belonged to the Alx-12 lineage which also contains sequences from FMDVs collected from other countries including Egypt 2012, 2014 and 2017. Older Sudanese viruses (from 1977 and 2007–2008) belonged to topotype XIII and were related to viruses from Ethiopia.

### 4 DISCUSSION

During the study period (2009–2018), clinical cases in cattle were observed year-round over a wide geographical distribution with...
FIGURE 1  Geographical distribution of FMDV outbreaks in Sudan 2009–2018. Map represents Sudanese provinces where the intensity of the colour reflects on cattle density (FAO). Circles define numbers of samples that were serotyped using antigen detection ELISA (Red: serotype O; blue: serotype A; purple: serotype SAT 2).

![Map of Sudan with outbreaks marked]

| Serotype | 2009 | 2010 | 2011 | 2012 | 2013 | 2014 | 2015 | 2016 | 2017 | 2018 | Totals |
|----------|------|------|------|------|------|------|------|------|------|------|--------|
| O        | 1    | 2    | 2    | 12   | 4    | 1    | 3    | 11   | 6    |      | 42     |
| A        |      | 5    | 5    | 4    | 6    |      | 11   |      |      |      | 31     |
| SAT 2    | 1    | 6    | 6    |      |      | 7    |      |      |      |      | 18     |
| Totals   | 1    | 3    | 7    | 12   | 15   | 11   | 9    | 11   | 11   |      | 91     |

Relatively more cases reported in the winter months (December–February). So far, four FMDV serotypes have been reported in Sudan: O, A, SAT 1 and SAT 2 (Abu Elzein, 1983), although no cases due to the SAT 1 serotype were detected in this study or have been recorded in Sudan since 1976 (Abu Elzein & Crowther, 1979). The low incidence of SAT1 in Sudan is supported by serological data for SAT1-specific antibodies in comparison to serotypes O and A (Habila et al., 2010a; Raouf et al., 2009). Serotype O was the most frequently detected; findings which are also supported by recent serological data (Raouf et al., 2016) showing higher prevalence of serotype O specific antibodies (60.2%) compared to serotype A (30.0%) and serotype SAT 2 (12.3%). Nucleotide distances of more than 15% (for serotypes O and A) or 20% (for serotypes SAT 1 and SAT 2) are used to classify isolates into different topotypes (Knowles & Samuel, 2003; Samuel & Knowles, 2001), while nucleotide differences of between 5% and 15% indicate distinct virus lineages (Bronsvoord et al., 2004). The Sudanese sequences reported here differed from the prototype strains of the relevant topotypes (Knowles et al., 2016) by 7.7–12.1% (O/SUD/2/86), 13.8–16% (O/ETH/3/2004), 15.2–17.8% (O/ETH/2/2006), 12.5–15.5% (O/ETH/1/2007), 13.4–16% (A/SUD/3/77), 7.4–9.9% (SAT2/SAU/6/2000) and 11.9–14.4% (SAT2/CAR/8/2005). These differences indicated the continued divergence of virus lineages within each topotype.

Serological findings from 2013 (Raouf et al., 2016) demonstrate that serotype O is present along the Nile basin up to Khartoum and in Western, Eastern and Northern Sudan. A previous study
**Figure 2** Midpoint-rooted maximum likelihood tree showing the relationships between the VP1 sequences of the 2009–2017 serotype O viruses from Sudan (indicated with red diamonds) and other contemporary and reference viruses. Four distinct clades which contained Sudanese sequences are indicated by grey arrows. Bootstrap values of 70% and above are shown. *Reference number not assigned by the WRLFMD.*
FIGURE 3  Midpoint-rooted maximum likelihood tree showing the relationships between the VP1 sequences of the 2011–2018 serotype A viruses from Sudan (indicated with blue diamonds) and other contemporary and reference viruses. Bootstrap values of 70% and above are shown. *Reference number not assigned by the WRLFMD.
Figure 4. Midpoint-rooted maximum likelihood tree showing the relationships between the VP1 sequences of the 2010–2017 serotype SAT2 viruses from Sudan (indicated with purple diamonds) and other contemporary and reference viruses. Bootstrap values of 70% and above are shown. *Reference number not assigned by the WRLFMD.
Data for serotype A sequence also support the concept that FMDV lineages are maintained solely within Sudan. The phylogenetic tree contains two lineages that comprise sequences only from Sudan collected from different years (2013–2014 and 2018). In common with the serotype O data, there was also evidence for the northerly spread of A/AFRICA/G-IV into Egypt (during 2011–2012). Epidemiological links to FMD cases in Ethiopia are highlighted in a second clade that contained Egyptian sequences (from 2015 to 2017) and more recent sequences collected from Sudan (from 2018), where the Ethiopian sequence (A/ETH/19/2015) pre-dated those for the Sudanese samples. More broadly, the serotype A (and serotype O) phylogenetic trees highlight the relationship between recent sequences for FMDVs collected in Ethiopia (Gizaw et al., 2020) and contemporary data from Sudan. For both serotypes, there are examples where Sudanese sequences have a more basal ancestral location in the tree, for example, the relationships between O/Sudan/2012 and O/Ethiopia/2018–2019 and A/Sudan/2013–2014 and A/Ethiopia/2018–2019, respectively. These results suggest that these FMDVs could have spread from Sudan into Ethiopia; however, this interpretation of the data should be treated cautiously in view of the likely high number of unsampled FMD cases in both countries where the sampling density is very low and susceptible livestock populations are high.

Serotype SAT 2 was the last FMDV serotype to be detected in Sudan in 1976 (Abu Elzein & Crowther, 1979) and 18 Sudanese isolates of serotype SAT 2 were detected during four of the years during this study. All of these sequences from 2010 to 2017 were characterized as belonging to the SAT 2/VII/Alex-12 lineage that has spread widely in the region to cause FMD outbreaks in countries such as Egypt, Palestine and Israel. In common with serotypes O and A, sequences for viruses from this lineage collected from Sudan are earlier compared to those for viruses sampled in Ethiopia. The SAT 2/VII/Alex-12 lineage is distinct to earlier viral clades detected in Sudan such as those that have spread previously from East Africa into West Africa (Habiela et al., 2010b; Ularamu et al., 2017; highlighted by presence of SAT2/SUD/1/2007 in the phylogenetic tree).

No previous reports have described clinical FMD in Sudan in species other than cattle (Abu Elzein, 1983; Habiela et al., 2010a; Raouf et al., 2010). Recent serological studies suggested a relatively low seroprevalence of non-structural protein antibodies among small ruminants at around 14%, except in Khartoum (Habiela et al., 2009) and Blue Nile State (Raouf, 2015; Raouf et al., 2017). This may indicate a varied role of sheep in the epidemiology of FMD. FMD infection in wild ruminants in Sudan has not been investigated.

In summary, FMD infection in Sudan remains regionally significant and this study highlights the epidemiological connections between FMDV sequences collected in Sudan and neighbouring countries such as Ethiopia and Egypt. Due to incomplete and convenience-biased sampling, it should be recognized that there are limitations in our understanding of the transboundary connectivity in the region and the sequences reported here provide only a crude snapshot survey of the underlying FMD transmission events. Recognition of border areas as particularly risky hotspots for the introduction of FMDVs is of high importance in developing a risk-based control strategy especially when resources are limited. In this context, continued studies are warranted to improve our understanding of FMD epidemiology in Sudan and risk-pathways in the region.

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CONFLICT OF INTEREST

The authors declare no commercial or financial conflict of interest.

ETHICAL 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 this study did not involve any experimental animal protocols.

DATA AVAILABILITY STATEMENT

All nucleotide sequences generated were submitted to GenBank and accession numbers can be found in Table 1.

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