Veterinary and Animal Science 13 (2021) 100188

Contents lists available at ScienceDirect

Veterinary and Animal Science

journal homepage: www.elsevier.com/locate/vas

Serological detection and genetic characterization of foot-and-mouth disease virus from cattle in northern sudan, 2016-2018

Nussiba H Ahmed [a,b], Nussieba A Osman [b,*], Wefag Alfouz [a,b], Haitham M. Saeed [c], Yazeed A/Raouf [a]

[a] Foot-and-Mouth-Disease Department, Central Veterinary Research Laboratory (CVRL), Soba, P.O. Box 8067, Al Amarat, Khartoum, Sudan
[b] Department of Pathology, Parasitology and Microbiology, College of Veterinary Medicine, Sudan University of Science and Technology, P.O. Box 204 Kuku, Khartoum-North, Sudan
[c] Dongola Veterinary Research Laboratory, Northern State, Sudan

ARTICLE INFO

Keywords:
FMD infection
Sudan
Northern sudan
Serotyping
Genotyping
Virus neutralization test
Structural proteins (SPs) serology

ABSTRACT

Northern Sudan is an important corridor cluster between pools of foot-and-mouth disease virus (FMDV) in East and North Africa. It involves almost the whole border area with Egypt and represents a considerable part of a projected disease-free zone in Sudan. The study monitored FMD infection between 2016 and 2018 in Northern Sudan. Clinical and serological surveillance were carried out. Results largely confirmed previous reports that have described the relatively lower circulation of FMDV in the area than in other parts of the country. Clinical FMD was confirmed, once in the three years period, as serotype O of an unnamed lineage within the topotype East Africa 3 (EA3). Using serial testing (the ID ELISA and virus neutralization test), sero-prevalence estimates of serotype-specific antibodies in the two States of Northern Sudan ranged between 15.4% (serotype A) in the River Nile State to 3.4% (serotype SAT2) in the Northern State. Striking disparities between patterns of FMD in Northern Sudan and the rest of Sudan were observed. Unlike Western, Eastern, Central and Southern Sudan, no predominance of serotype O antibodies was detected in Northern Sudan. Concurrently, a serotype O isolate from Northern Sudan in 2016 was found to be of transboundary nature circulating in East and North Africa and in the Middle East (nt. id. > 99%); like serotype O that caused the last episode of disease in Northern Sudan in 2012. Molecular findings were compatible with the inferred low circulation of FMDV in Northern Sudan. Elsewhere in Sudan, endogenous serotype O viruses seemed to be circulating more unabated. It was concluded that low animal density and limited animal movement in Northern Sudan together with the high antibody levels against serotype O in immediately neighbouring States (Khartoum and Kassala) effectively decreased infiltration of endogenous O viruses.

Introduction

Foot-and-mouth disease (FMD) is an important transboundary and an economically significant viral infection of domestic and wild ruminants. It reduces animal productivity and forces severe restrictions on trade of animals and animal by products (Alexandersen, Zhang, Donaldson & Garland, 2003). It is No. 1 in the World Organization for Animal Health (OIE) list of infectious diseases and ranked by some workers (Domenech, Lubroth, Eddi, Martin & Roger, 2006) as the first and foremost priority animal disease. Foot-and-mouth disease virus (FMDV), a member of the *Aphthovirus* genus of the *Picornaviridae* family, with seven immunologically distinct serotypes; O, A, SAT1–3, C and Asia 1 (Murphy, Gibbs, Horzinek, & Studdert, 1999). All the seven serotypes cause clinically similar diseases characterized by fever and vesicular lesions mainly in the mouth, snout, udder and feet (MacLachlan & Dubovi, 2011).

In Sudan, the first record of FMD was in 1903 (Eisa & Rweyemamu, 1977). The disease in Sudan remained largely without control and is expected to be endemic, at least, in some parts (Abu Elzein, 1983; Habiela, Alamin, Raouf & Ali, 2010a; 2010b; Raouf et al., 2016). Historically four serotypes of FMDV had been reported in the country: O, A, SAT1 and SAT2 (Abu Elzein, 1983). Currently, the maintained activity of

* Corresponding author: Department of Pathology, Parasitology and Microbiology, College of Veterinary Medicine, Sudan University of Science and Technology, P.O. Box 204 Kuku, Khartoum-North, Sudan, ORCID ID: 0000-0001-6224-8376.
E-mail address: nussieba@yahoo.com (N.A. Osman).

https://doi.org/10.1016/j.vas.2021.100188
Received 1 April 2021; Received in revised form 26 June 2021; Accepted 28 June 2021
Available online 29 June 2021

three serotypes; O, A, and SAT2 has been repeatedly confirmed by disease and serological surveillances (Habiela et al., 2010a; 2010b; Raouf, Ali, Khair, & Amin, 2009; Raouf, Ali, El Amin, & Al Shallali, 2010; Raouf et al., 2016; http://www.wrlfmd.org/). Clinical FMD in Sudan is seen in cattle only, while domestic small ruminants undergo largely silent infection (Habiela, Raouf, & Nur Eldin, 2009; Habiela et al., 2010a; Raouf, Ali, El Amin, & Al Shallali, 2010; Raouf, Tamador, Nahid, & Shaza, 2012; Raouf et al., 2017; http://www.wrlfmd.org/).

In spite of the long history of FMD in Sudan and the little efforts of control practiced, different levels of FMD infection were recognized in different geographical areas of the country (Anon, 2016; Raouf, Ali, El Amin, & Abd Alla, 2011; Raouf et al., 2016; Raouf et al., 2017; Saeed, 2019; Saeed & Raouf, 2020). In geography, apart from Northern Sudan which forms one cluster, three geographical clusters include Western, Eastern and the South Eastern cluster (Fig. 1), were described in Sudan (Raouf et al., 2016). Northern Sudan cluster includes the Nile valley North to Khartoum enclosed in two administrative States, the River Nile and the Northern States. Northern Sudan is distinguished by an exclusive desert and semi-desert ecosystem unlike all other three clusters which are traversed by the low rainfall savannah belt. The geographical distribution of FMD was described as penetrating along the South Eastern cluster up to Khartoum State but less prevailing in Eastern, Western and Northern Sudan (Raouf et al., 2016). The Southern regions of the Nile Valley together with Western and Eastern Sudan are mainly animal breeding areas while Central and Northern parts of the Nile valley are animal marketing or trade routes areas. In general, the relatively lower levels of FMD infection are important and encouraging for control efforts, yet FMD infection in Northern Sudan, in particular, though low, could be crucial for virus spill from the country. Northern Sudan is part of a projected disease-free area broadly demarcated by the government of Sudan since 1970s. Additionally, Northern Sudan involves almost the whole border area with Egypt where cross-border trade of livestock through official and unofficial channels is known. Northern Sudan with the River Nile crossing it to Southern Egypt is a rare junction between sub-Saharan and North Africa. Cross-border trade at this junction represents an extra-regional trade i.e. that involved two epidemiological clusters as described by Di Nardo, Knowles, & Paton, 2011. Increasingly, viruses belonging to pool 4 of FMDV, known in the epidemiological cluster of East Africa (Di Nardo, Knowles, & Paton, 2011), were revealed in Egypt in North Africa (Jamal & Belsham, 2013).

Indices of FMD infection in Northern Sudan were described as low in more than one occasion (Anon, 2016; Saeed, 2019; Saeed & Raouf, 2020). Sero-prevalence of neutralizing antibodies against serotypes O, A and SAT2 in cattle in the Northern State in 2012 were 12.9% (9.05%–16.83% C.I. 95%) ($n = 286$), 9.0% (5.03%–12.97% C.I. 95%) ($n = 200$) and 2.4% (2.35%–2.43% C.I. 95%) ($n = 126$), respectively (Saeed & Raouf, 2020). Clinical investigation carried out between 2012 and 2014 in the Northern State, confirmed serotype O clinical disease once early in 2012 (Saeed, 2019). At the same time, a structured-based questionnaire revealed that 44/82 (53.66%) of the cattle owners there had no or little

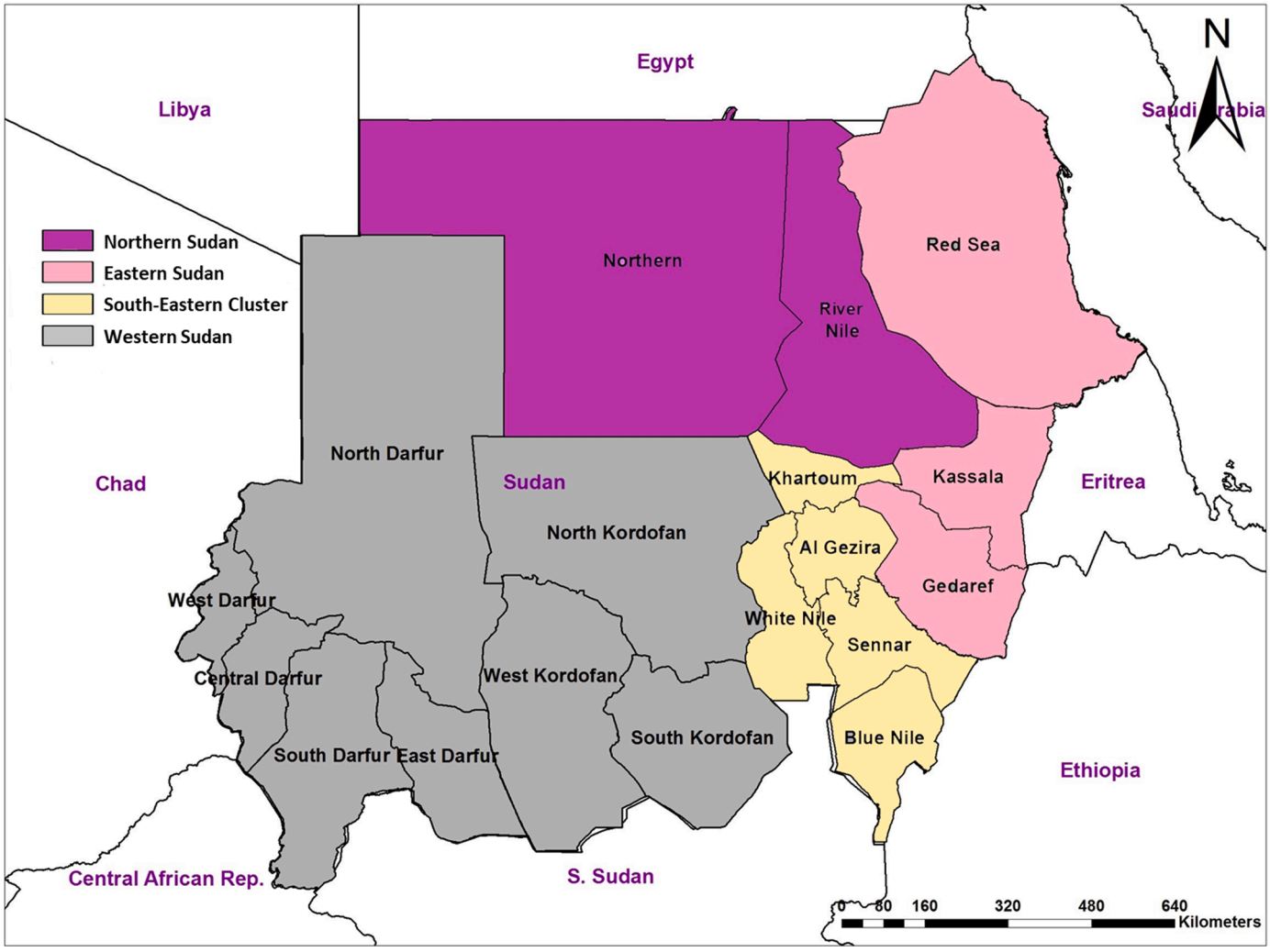

Fig. 1. Map of Sudan showing the study area "Northern and River Nile States". The four geographical clusters of the country, the Northern Cluster (Violet), Eastern Sudan (Pink), South-Eastern Cluster (Yellow) and Western Sudan (grey), were presented.

knowledge of FMD. A country-wide study of Non-Structural Proteins (NSPs) antibodies in cattle during the program Surveillance of Trade Sensitive Diseases (STSD) in 2016 revealed 15.5% [12%−19% C.I. 95%] activity in the Northern State (n = 343) and 39.4% [35%−44% C.I. 95%] in the River Nile State (n = 409). The current work monitored FMD infection in the whole of Northern Sudan (the Northern and the River Nile States) between 2016 and 2018. Clinical investigation, serotyping and genotyping of FMD outbreaks as well as structural proteins (SPs) serology of cattle sera were all carried out. It aims to expand the geographical and 90-time scale of the study of FMD infection in Northern Sudan to avoid biased impressions on disease situation and epidemiology.

Materials and methods

Study area

Northern Sudan falls between 16-22°N and 22–32°E and covers an area of around 458,697 Km^2 of a desert and semi-desert traversed by the River Nile. Fig. 1 shows the map of Sudan and the geographical clusters of the country. Animal density usually reaches 5 cattle/sq km in the desert and semi-desert ecosystem (FAO, 2005) but it is higher beside the River Nile and irrigation canals. The prevailing animal production systems are the urban and the peri-urban production systems. No or very little pastoralism is practiced in Northern Sudan and animal movement is limited to that related to trade. The River Nile State is crossed by a national road from Central Sudan to the country seaport, Port-Sudan, which intensified livestock movement related to international trade. Foot-and-mouth disease susceptible species ranges from 2429,144 in the River Nile State (105,148 head of cattle and 2323,996 head of small ruminants) to 2473,964 in the Northern State (262,871 head of cattle and 2211,093 head of small ruminants) according to the Data centre of the Ministry of Animal Resources, Sudan. Reared cattle are usually cross breeds or milking cows of local breeds (Butana and Kenana).

Suspected FMD outbreaks and disease surveillance

Between 2016 and 2018, suspicion of FMD had arisen at first by end of 2016 (November and December). Vesicular lesions were seen in cattle smuggled to Egypt and in resident cattle in Dongola district in the Northern State. The smuggled cattle were in confinement by the Border Control Department at Dongola. The veterinary authority was notified. Six epithelium samples were collected from resident cattle and five from the smuggled animals. Epithelium samples were collected in transport medium composed 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 till received by the laboratory where they were kept at –20°C.

Active disease surveillance was carried out in the River Nile State. In March 2018, a team was assembled and visited (7–17/3/2018) four districts in the River Nile State: Shendi, Ad-Damar, Atbara and Berber. Visited animal holdings included small dairy holdings (15) and small dairy farms (4). In the small holdings herd size was around 20, and between 40 and 60 in the dairy farms. Case definition is ''an animal possessing vesicular lesions (oral or foot)''. No affected animals were seen.

Serotyping of FMDV

Detection and serotyping of FMDV was carried out on the epithelial samples. The glycerinated epithelium samples were blotted dry on absorbent filter paper. A 10% suspension (w/v) was prepared in Glasgow minimum essential medium (GMEM) (containing double-fold of antibiotics and antimycotics) using pestle, mortar and sterile sand. The suspension was clarified by centrifugation at 2000 rpm for 10 min, divided into two aliquots and stored in liquid nitrogen vapor till use. An antigen ELISA kit developed and distributed by the Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna (IZSLER), Italy, was used for serotyping of FMDV according to the manufacturer instructions. The assay is a sandwich ELISA performed with selected combinations of anti-FMDV monoclonal antibodies (MAbs), used as coated and conjugated antibodies (Grazioli, Ferris, Dho, Spagnoli, & Brocchi, 2012).

Serotyping of FMDV at the world reference laboratory (WRL) for FMD

Five samples out of the 11 collected samples were dispatched, under dry ice, to the World Reference Laboratory for foot-and-mouth disease (WRLFMD) as dangerous biological substance category B UN 3373. Samples were originated from the smuggled (1) and the resident (4) cattle and kept unprocessed in the described transport medium at –20°C till dispatched to the WRL for FMD (July 2018). More information about the dispatched samples is available in Table 3. At the WRL for FMD, samples were passaged once or twice into IB-RS-2 and thyroid cell culture then subjected to antigen detection and serotyping by ELISA assay using the indirect sandwich ELISA kit (WRL for FMD) for detection of FMDV antigen (Roeder & Le Blanc Smith, 1987).

Molecular and genetic characterization of FMD viruses

For determining the serotype and prototype of Sudanese FMD viruses, molecular and genetic characterization including amplification of the partial FMDV serotype-O VP1 (1D) coding region using reverse-transcription polymerase chain reaction (RT-PCR) followed by gene sequencing were performed at the WRL for FMD, the Pirbright Institute, UK, following the protocol described previously by Knowles, Wadsworth, Bachanek-Bankowska, & King, 2016.

RNA extraction

Total RNA was extracted from the epithelial samples using the RNeasy kit (Qiagen, Crawley, West Sussex, UK) according to the manufacturer's instructions as described by Knowles, Wadsworth, Bachanek-Bankowska, & King, 2016. Briefly, total RNA was extracted from 460 µl of the epithelial sample as described by the manufacturer. The purified RNA was eluted in 50 µl of nuclease-free water and placed on ice to perform the RT-PCR immediately, otherwise stored at –20°C.

Reverse-transcription polymerase chain reaction (RT-PCR)

Based on serotyping results confirmed by antigen-detection ELISA, FMDV serotype-O specific primer sets [O-1C244F (5' GCAGCAAAACACATGTCAAACACCTT 3') and O-1C272F (5' TBGCRGGNCTYGCCCAGTACTAC 3') forward primers to anneal with the VP3 and EUR-2B52R reverse primer (5' GACATGTCCTCCTGCATCTGGTTGAT 3') to anneal with the 2B coding region] were used for amplification of the full length FMDV VP1 coding sequence as described previously by Knowles, Wadsworth, Bachanek-Bankowska, & King, 2016. A one-step reverse--transcription polymerase chain reaction (RT-PCR) was carried out using QIAGEN One-Step RT-PCR kit (Qiagen, Germany) following the standard protocol and cycling conditions for RT-PCR amplification of the VP1 region of FMDV as described by Knowles, Wadsworth, Bachanek-Bankowska, & King, 2016. The correct size of the amplicon was determined by analyzing the PCR product on 1.5% agarose-Tris-borate-EDTA gel containing 1 × GelRed nucleic acid stain (Biotium Inc., USA) using a DNA size markers (GeneRuler 100 bp DNA Ladder Plus, Fermentas Inc., USA).

Sequencing of FMDV, sequence and phylogenetic analysis of FMDV VP1

Determining sequencing of the partial FMDV serotype-O VP1 (1D) coding region [639 nt] from the PCR product was performed using the BigDye® Terminator v3.1 Cycle Sequencing Kit (Life Technologies) and FMDV serotype-O specific primer sets [FMD-3161F (5' TCGCVCAGTACTACRCACAGT 3') and FMD-4303R (5'

TGACGTCRGAGAAGAAGAARGG 3')] (Dill, Beer, & Hoffmann, 2017) were used for amplification of the FMDV VP1 coding sequence as described by Knowles, Wadsworth, Bachanek-Bankowska, & King, 2016. To determine the serotype and prototype of FMD viruses from Sudan, the yielded VP1 nucleotide sequences were assembled from multiple reads using SeqMan Pro (Lasergene package, DNAstar Inc., Madison, Wisconsin, USA). To determine the identity of the Sudanese FMDV isolates, the FMDV VP1 obtained sequence was compared with the respective gene sequences of other FMD virus isolates using BLAST Nucleotide (https://blast.ncbi.nlm.nih.gov/). Accordingly, alignment of FMDV VP1 nucleotide sequences (633 nt) of FMDV serotype-O retrieved from NCBI GenBank database was performed using BioEdit v7.2.5, which uses ClustalW multiple alignment program (http://www.ebi.ac.uk/Tools/msa/clust alw2/). Neighbor-joining phylogenetic tree, employing the Kimura 2-parameter nucleotide substitution model for FMDV serotype-O with 1000 bootstrap replicates, was constructed using the MEGA7.0.26 (Molecular Evolutionary Genetics Analysis) program (http://www.megasoftware.net/mega.html).

Serological study

Serum samples

Serum samples were collected in 2016 from apparently healthy cattle, from the Northern and the River Nile States, one year old or above with no history of vaccination against FMD.

Sera were collected from a sampling frame of 6 (River Nile State) and 5 (Northern State) geographical districts (sampling units) (Table 1, Fig. 3 and 4) and five sampling epi-units (herds or collection sites) per each sampling unit. Therefore, a minimal number of 25 epi-units per State was achieved what conform to statistical theory regarding unbiased parameter estimates (Ferrari, Paton, Duffy, Bartels, & Knight-Jones, 2016). A sample size of 70 sera from each sampling unit (district) and 14 sera from each epi-unit (herds or collection sites) was collected using a simple random sampling (SRS) method and standard statistical procedure to determine the sample size (Anon, 2016).

Serum samples were discriminated as positive or negative to anti-NSPs antibodies of FMDV using the ID Screen® FMD NSP Competition ELISA (Roche, Donnet, Malzac, Comtet, & Pourquier, 2014) during the program STSD (Anon, 2016). A total of 184 bovine sera have proven positive to anti-NSPs antibodies of FMDV; 143 sera from the River Nile State and 41 sera from the Northern State. Relevant data of NSPs serology and the origin of positive sera within each State are shown in Table 1.

Virus neutralization test (VNT)

Sera were tested using a screening format (Raouf, Tamador, Nahid & Shaza, 2012) of virus neutralization test (VNT) against serotype O, A and SAT2. The test was carried out into Baby Hamster Kidney-21 (BHK-21) clone 13 cell line originated from Foot-and-Mouth Disease Research Institute (ŞAP Enstitüsü Müdürlüğü), Ankara, Turkey, and employed locally isolated FMD viruses. The Sudanese viral materials were adapted (through 16–22 passages) to grow into BHK cells, typed and retyped several times using reference ELISAs (Pirbright and IZSLER) and designated according to their serotype, geographical origin within Sudan, year and order of isolation from that origin. Four isolates were used in this work; two of serotype SAT2 isolated from Khartoum in 2008 (SAT2-Kh 1/08) (Raouf, Ali, El Amin, & Al Shallali, 2010), and from North Kordofan in 2010 (SAT2-NK 1/010); one of serotype A isolated from Khartoum in 2011 (A-Kh 2/011) (Raouf et al., 2016); and one of serotype O isolated from Khartoum in 2015 (O-Kh 1/015). Virus stocks were prepared, pre-titrated and used at 100 $TCID_{50}$ per 50 μl. Control sera were known positive bovine field sera for either O, A and SAT2 serotypes (Raouf et al., 2016) and fetal calf serum (FCS) (Sigma) free from antibodies against FMDV was used as the negative control sera.

The procedure of the screening format was similar to the standard procedure of VNT (OIE Manual, 2018) except that sera were tested at two dilutions; 1/32 and 1/64, rather than several dilutions to decrease the test workload and span the standard cut-off of 1/45 ($10^{1.65}$) described for the purpose of serosurveillances by the OIE Manual, 2018. To increase further the sensitivity of the assay, the cut-off is lowered to 1/32 ($10^{1.5}$) which is usually considered retest (doubtful) in case of individual serum screening (OIE Manual, 2018).

Statistical analysis

The serological study employed serial testing approach i.e. only sera positive in two test systems were considered positive (Fletcher & Fletcher, 2005). Calculations for serial testing were performed according to the standard procedure (Thrusfield, 2007). Prevalence was calculated as proportion positive to both tests; test A and test B. Test A is the ID Screen® FMD NSP Competition ELISA and test B is the VNT. Accordingly, prevalence = proportion positive detected by test B x proportion positive detected by test A x 100. Proportions positive by test A were provided by the STSD (Table 1). Proportions positive by test B (VNT) in each sub-population were determined by dividing the number of positive reactors identified by the VNT by the number of sera tested in that sub-population.

Prevalence rates were compared by deriving the 95% C. I. derived from a simple random sample, based on the Normal approximation to

Table 1
Numbers and origin of anti-NSPs positive sera.

States data of NSPs serology				Districts data of NSPs serology				
State	No.* of sera tested	No of positive sera	Sero-prevalence	District	No.** of sera tested	No.** of positive sera	Sero-prevalence	No.***of sera tested by VNT
Northern State	343	53	15.45%	Marawi	66	17	25.76%	15
				Dongola	65	21	32.31%	16
				Al-Dabbah	70	8	11.43%	6
				Al Goled	64	0	Nil	–
				Al Burgaig	63	5	7.94%	4
Totals					328	51		41
River Nile State	409	161	39.36%	Shendi and El Matamma	137	58	42.34%	53
				Ed-Damar	69	31	44.93%	27
				Atbara	68	31	45.59%	28
				Berber	67	22	32.84%	20
				Abu Hamad	68	19	27.94%	15
Totals					409	161		143

* Out of 350 collected sera in the Northern State 7 sera were lost.
** Fifteen sera from the Northern State were with unidentified district origin including two +ve sera to NSPs serology.
*** 10 (Northern State) and 18 (River Nile State) sera +ve for NSPs serology were lost before performing the VNT.

the binomial distribution, using the formula: $P \pm 1.96\sqrt{p(1-p)/n}$ (Thrusfield, 2007). Where P is the estimated prevalence, n is the number of samples tested and 1.96 is the appropriate multiplier for the selected level of confidence. When C. I. values did not overlap then the statistics will always be statistically significantly different (Knezevic, 2008). For overlapping C.I. values, *p-values* were calculated using chi-squared and Fisher Exact test available at the Statistical Packages for Social Sciences (SPSS) (www.sociostatistics.com); results were significantly different, if $p < 0.05$. The Fisher Exact test was used for smaller sample sizes.

Results

Clinical disease investigation

Between 2016 and 2018, clinical signs of FMD were reported only once. Suspicion has arisen in Dongola district in the Northern State. Cattle affected were dairy cattle of local breeds 'Kenana and Butana' and also cross breeds, resident in the area, and fattening calves smuggled to Egypt. Clinical signs included drolling of saliva, ulcerative lesions in the mouth and udder, lameness and drop in milk production. Morbidity reached 100% in affected farms where 27 animals out of 27 showed clinical signs. However, no similar clinical signs were seen in other ruminant species in the area. Active surveillance in four districts in the River Nile State in 2018, also, detected no clinical signs of FMD.

Serotyping and genotyping of FMD outbreak

Serotype O was detected in epithelium samples collected from clinically affected cattle (Table 2). The outbreak serotype was confirmed (Table 3), the VP1 gene sequence of FMD virus - type O isolate O/SUD/1/2016 (GenBank accession number MK422563.1) was determined and the Sudanese FMDV was genotyped as an unnamed lineage within the topotype O-EA3 (Fig. 2). In the generated phylogenetic tree, FMDV O/SUD/1/2016 was clustered in one subcluster under topotype EA-3 cluster and closer to the cluster contains other Sudanese strains from 2017 (O/SUD/4/2017, O/SUD/5/2017, O/SUD/15/2017), other Egyptian strains from 2016-2017, and Ethiopian strains from 2017-2018 (Fig. 2).

The nucleotide sequence of VP1 gene (1D) region (639 nt) of FMDV O/SUD/1/2016 "serotype O, topotype EA-3″ is closely related to FMDV Sudanese strains from 2017 (unpublished data) and shared the highest nucleotide sequence identity of 99.8–99.7% with many Egyptian strains (O/EGY/33/2017, O/Giza 1/Egy/2017, O/EGY/7/2017, O/EGY/9/2017, O/EGY/11/2017, O/EGY/22/2017, O/EGY/26/2017, O/Alexandria 1/Egy/2016, O/Behira 2/Egy/2017). Alternatively, the topotype EA3 sequence of FMDV O/SUD/1/2016 is closely related to FMDV Sudanese strain (O/SUD/2/86), Ethiopian strains (O/ETH/1/2007, O/ETH/3/2004), other African strains from Uganda (O/UKG/35/2001), Tanzania (O/TAN/2/2004) and Mali (O/MAL/1/98) (Fig. 2). Genotyping data is available at https://www.wrlfmd.org/sites/world/files/WRLFMD-2018-00020-SUD-GTR-O-O_001.pdf (WRLFMD, 2018).

Table 2
Detection and serotyping of FMD in Northern Sudan between 2016 and 2018.

Serial No.	Sample identity (CVRL Reference*)	Sample Origin	Description of Sample	Serotyping Result by IZSLER ELISA
1	Ep-/2017 (1)	Northern State (Dongola), resident cattle	Cattle, epithelium, collected on 25/12/2016	O
2	Ep-/2017 (2)			-ve
3	Ep-/2017 (3)			-ve
4	Ep-/2017 (4)			-ve
5	Ep-/2017 (border control-1)	Northern State (Dongola), Department of Border Control	Cattle, epithelium, collected on 25/12/2016	O
6	Ep-/2017 (border control-2)			O
7	Ep-/2017 (border control-3)			O
8	Ep-/2017 (border control-4)			O

CVRL = Central Veterinary Research Laboratory.
Ep = Epithelium.
* Samples were collected late in 2016 and are included in the disease season of the following year.

Table 3
Confirmation of serotype O outbreak in Northern Sudan in 2016–2017 at the WRL for FMD.

Sample identity (WRL Reference)	Sample identity (CVRL Reference)	Description of sample	Serotyping at CVRL	Serotyping at the WRL	
				PCR result	Serotyping result by cell culture/ ELISA
SUD 1/2016	Ep-/2017 (border control-1)	Cattle, epithelium, collected on 25/12/2016	O	FMDV GD	O
SUD 2/2016	Ep-/2017 (2)	Cattle, epithelium, collected on 25/12/2016	-ve	FMDV GD	NVD
SUD 3/2016	Ep-/2017 (3)	Cattle, epithelium, collected on 25/12/2016	-ve	FMDV GD	NVD
SUD 4/2016	Ep-/2017 (7)	Cattle, epithelium, collected on 25/12/2016	N.D.	FMDV GD	NVD
SUD 5/2016	Ep-/2017 (5)	Cattle, epithelium, collected on 25/12/2016	N.D.	NGD	NVD

N.D. = Not detected.
FMDV GD = FMDV genome detected.
NGD = No genome detected.
NVD = No virus detected.

Serological study

Around 70% (130/184) of NSPs antibodies positive bovine sera in Northern Sudan have screened positive to antibodies to one or more of the three serotypes of FMDV; O, A or SAT2 (Table 4). Indices of prevalence of FMD infection in cattle in Northern Sudan as indicated by prevalence of antibodies to structural proteins (SPs) of FMDV was 27.82% in the River Nile State and 10.96% in the Northern State. It was statistically similar to sero-prevalence of NSPs antibodies in the Northern State but significantly lower than estimates of NSPs serology in the River Nile State (Table 5). However, indices by both test systems were statistically significantly higher in the River Nile State than in the Northern State.

No predominance of antibodies to any serotype could be described in Northern Sudan. Sero-prevalence estimates of serotype-specific antibodies to the three serotypes in each State were similar; apart from that of SAT2 in the Northern State which was to some extent lower than that of the other serotypes (Table 6). On the other hand, sero-prevalence estimates were consistently statistically significantly higher in the River Nile than in the Northern State (Table 6).

In the River Nile State, sero-prevalence's of serotype-specific antibodies at different districts have ranges from 5.5% (O at Abu Hamad) to 26.6% (SAT2 at Ad-Damar) (Table 7). Lowest sero-prevalence's were exclusively detected at the most Northern districts of Abu Hamad

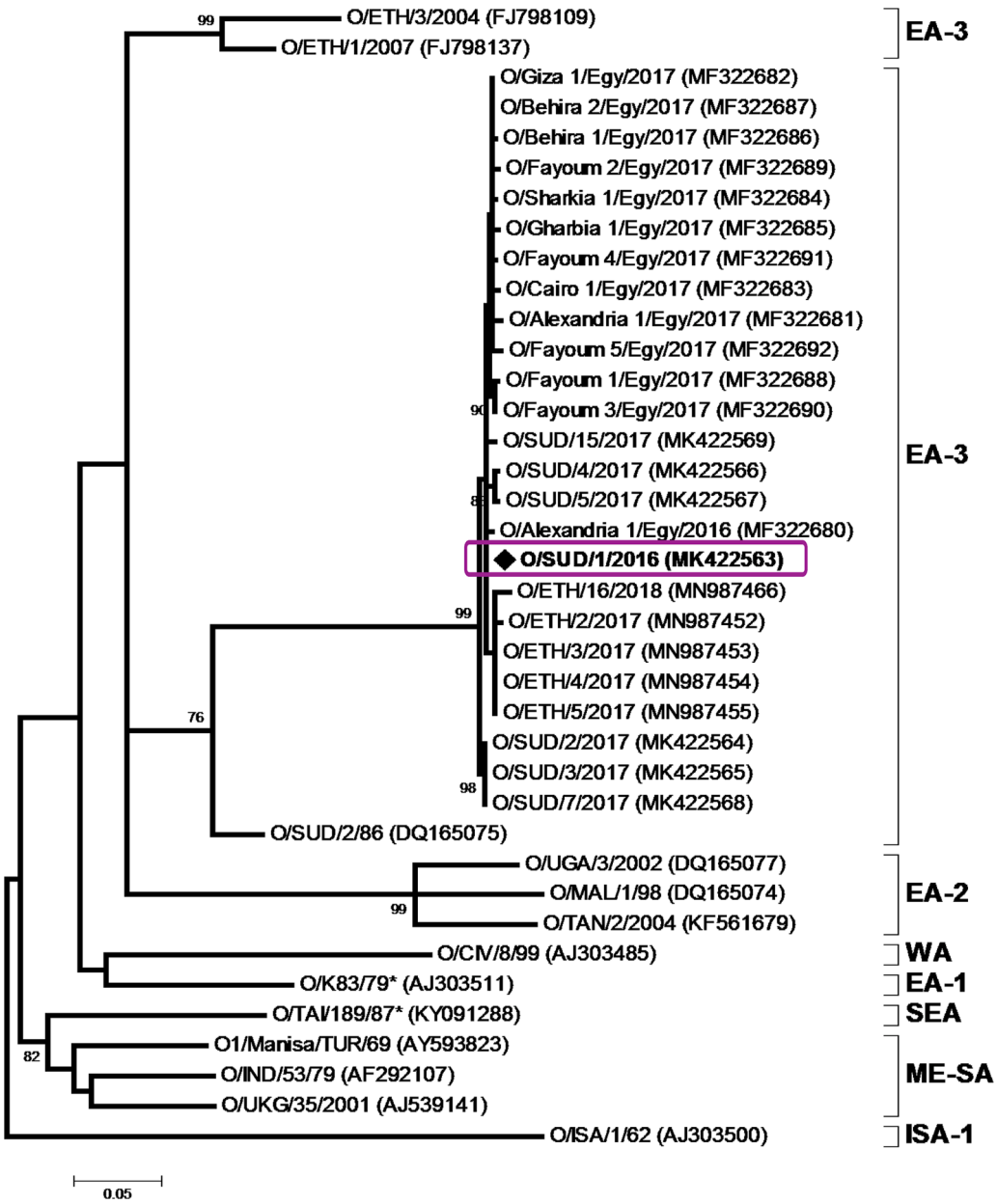

Fig. 2. Neighbor-joining phylogenetic tree generated using nucleotide sequences (633 nt) of the VP1-coding region of serotype-O FMD viruses. The tree was constructed using the MEGA7.0.26 (Molecular Evolutionary Genetics Analysis) program (http://www.megasoftware.net/mega.html) by employing the Kimura 2-parameter nucleotide substitution model for FMDV serotype-O and using the Bootstrap method for test of phylogeny by analyzing 1000 bootstrap replicates.

Table 4
Typing of NSPs antibodies positive bovine sera in Northern Sudan.

State	Sero-prevalence of anti-NSPs antibody	Typing of NSPs antibodies positive sera			Sero-prevalence of anti-SPs antibody (neutralizing antibodies)
		No. tested	No. positive *	% of typed sera	
River Nile State	39.4% (161/409)	143	101	70.62% (101/143)	27.82%
Northern State	15.5% (53/343)	41	29	70.73% (29/41)	10.96%

* Positive to one or more of the three serotypes of FMDV (O, A and SAT2).

Table 5
Comparison between indices of infection of FMD by SPs and NSPs serology in Northern Sudan.

State	Seroprevalence of anti-NSPs antibodies		Seroprevalence of anti-SPs antibodies		*P-value* (Chi squared test)
	Sero-prevalence	95% C.I.	Sero-prevalence	95% C.I.	
River Nile State	39.4%	34.6%–44.1%	27.82%	23.2%–32.4%	0.000725
Northern State	15.5%	11.6%–19.3%	10.96%	7.2%–14.7%	0.106567

(Table 7; Fig. 3). In general terms, the estimated sero-prevalence's could be described as highest at Ad-Damar at the center of the State, consistently relatively high at the Southern districts of Shendi and El

Table 6
Sero-prevalence of FMDV serotype-specific antibodies in cattle in River Nile and Northern States.

Serotype	River Nile State			Northern State			*P-value* (chi-squared test)
	% Positive in test sera	Estimated prevalence	95% C.I.	% Positive in test sera	Estimated prevalence	95% C.I.	
O	32.9% (47/143)	12.9% (47/363)	9.5%–16.4%	41.5% (17/41)	6.4% (17/265)	3.5%–9.3%	0.00753
A	39.2% (56/143)	15.4% (56/363)	11.7%–19.1%	48.8% (20/41)	7.5% (20/265)	4.3%–10.7%	0.002788
SAT2	37.1% (53/143)	14.6% (53/363)	11.0%–18.2%	22.0% (9/41)	3.4% (9/265)	1.2%–5.6%	0.000003
P-value		0.604489			0.10665		

Table 7
Prevalence of FMDV serotype-specific antibodies in cattle sera in different districts in the River Nile State.

District	No. tested	O		A		SAT2	
		Positive (%)	Sero-prevalence estimate	Positive (%)	Sero-prevalence estimate	Positive (%)	Sero-prevalence estimate
El Matamma	27	10/27 (37%)	15.8%	10/27 (37%)	15.8%	8/27 (29.6%)	12.6%
Shendi	26	10/26 (38.5%)	16.2%	8/26 (30.8%)	12.9%	10/26 (38.5%)	16.2%
Ad-Damar	27	12/27 (44.4%)	19.9%	11/27 (40.7%)	18.3%	16/27 (59.3%)	26.6%
Atbara	28	5/28 (17.9%)	8.1%	14/28 (50%)	22.8%	11/28 (39.3%)	17.9%
Berber	20	7/20 (35%)	11.5%	8/20 (40%)	13.1%	4/20 (20%)	6.6%
Abu Hamad	15	3/15 (20%)	5.5%	5/15 (33.3%)	9.2%	4/15 (26.7%)	7.3%

Matamma, variable at Atbara and lowest at Abu Hamad and Berber (Table 7; Fig. 3). Sero-prevalence of serotype O antibodies, unlike that of serotypes A and SAT2, was consistently higher in the three Southern districts (El Matamma, Shendi and Ad-Damar) neighbouring Khartoum and Kassala States (Fig. 3) than the Northern districts (Atbara, Berber and Abu Hamad) neighbouring the Red Sea and Northern State (Table 7 and 8).

Four out of the seven districts in the Northern State were included in this study; Marawi, Dongola, Al-Dabbah and Al Burgaig (Table 9). Two districts in the uppermost North, Halfa and Dalgo, were not studied for anti-NSPs activity and cattle from a third Western district, Al Goled ($n = 64$) were all negative for anti-NSPs activity (Table 1). Sero-prevalences detected to NSPs and SPs (Table 1 and 8) in Al Burgaig, (7.9%, 3.9%, 3.9% and 1.9%) in the North and in Al-Dabbah (11.4%, 5.7%, 3.8% and 0%) in the South West were also insignificant. In spite of the small numbers of reactors (4, 6, 15 and 16) in different districts to NSPs and SPs serology, serotype O and A antibodies were detected in all four surveyed districts while SAT2 antibodies were not. Trends in distribution of serotype-specific antibodies in the Northern State are shown in Fig. 4.

In the Northern State, sero-prevalences of serotype-specific antibodies in different districts could be described as significant at Marawi (in the East) and Dongola (in the Center); insignificant at Al Burgaig (in the North) and Al-Dabbah (in the South West) or nil at Al Goled (in the West). In general terms, observed sero-prevalence's seemed to decrease from East to West and North (Fig. 4).

Discussion

In Northern Sudan, during the 3 years of the study period, FMD outbreak was reported and diagnosed only once, and previously in 2012, only twice; in February 2012 in the Northern State (Saeed, 2019) and December 2012 in the River Nile State (Anon, 2014). In all instances, serotype O was identified which is the known predominant serotype in Sudan (Abu Elzein, 1983; Raouf et al., 2016; http://www.wrlfmd.org/). In Sudan, FMD was usually diagnosed annually and is expected to be endemic, at least, in some parts (Abu Elzein, 1983; Habiela et al., 2010b; Raouf et al., 2016). Similarly, estimated sero-prevalence rates of FMD serotype-specific antibodies in the study area (Table 6) were much lower than the latest estimates reported (Raouf et al., 2016) in other parts of the country. Prevalence of FMD in Northern Sudan ranged from 15.4% (serotype A) to 3.4% (serotype SAT2) compared to a range from 75% (serotype O) to around 5% (serotype SAT2) in other Sudanese States (Raouf et al., 2016). Recently, similar to our finding, levels of NSPs antibody reactivity detected in Northern Sudan, 15.5% in the Northern State and 39.4% in the River Nile State (Anon, 2016), was found to be the lowest in Sudan. In absence of vaccination, SPs serology like NSPs serology is indicative of previous virus exposure. The latter at the herd level is largely accepted as an indication to the degree of FMD virus circulation (Bergmann et al., 2003; Bronsvoort et al., 2004; OIE Manual, 2018).

Serotyping and genotyping of epithelium samples from the study area at the WRL for FMD confirmed incidence of serotype O clinical disease and indicated that the isolate, like all other Sudanese isolates, was of an unnamed lineage within the topotype O-EA3. Genotyping data (WRLFMD, 2018) revealed that the serotyped isolate was part of a large temporal cluster (nt. id. > 95%) that involved Sudanese, Egyptian, Ethiopian viruses from 2016, 2017 and 2018 (Fig. 2) in addition to Israeli and Palestinian O viruses from 2017 (WRLFMD, 2018) rather than the Sudanese viruses that had been detected in the Northern State in 2012. It showed phylogenetic identity of above 99% with the Sudanese and the Egyptian member of this cluster which strongly suggests that it was the same virus or the same outbreak. Therefore, it is conceivable that it was introduced to Northern Sudan rather than being circulating in the study area. Interestingly, it was observed that the serotype O virus which had been detected earlier in the study area in 2012 had similarly showed phylogenetic identity of above 99% with Ethiopian and Eritrean viruses but only an identity of 95% with earlier Sudanese viruses (Anon, 2016; Saeed, 2019). The described pattern strongly suggested that in seven years period, between 2012 and 2018, the two episodes of serotype O disease in Northern Sudan were caused by viruses of transboundary nature probably originated from outside the country (phylogenetic identity above 99%) rather than from within the study area. Similarly, Al-Hosary et al. (2019) characterized 2 groups of serotype O viruses from an outbreak of FMD in Southern Egypt in 2015–2016 showing nucleotide identity of 85% and 86% with previously characterized isolates from the area suggesting incursion of new viruses into Egypt. However, the important fact is that the circulating virus was detected in a large geographical area involving two neighbouring countries i.e. its transboundary nature is indisputable and particularly evident. The suggestion suited well the inferred low level of circulation of FMD virus in Northern Sudan.

The serial testing approach which was applied in the serological study is known for increasing specificity but decreasing sensitivity

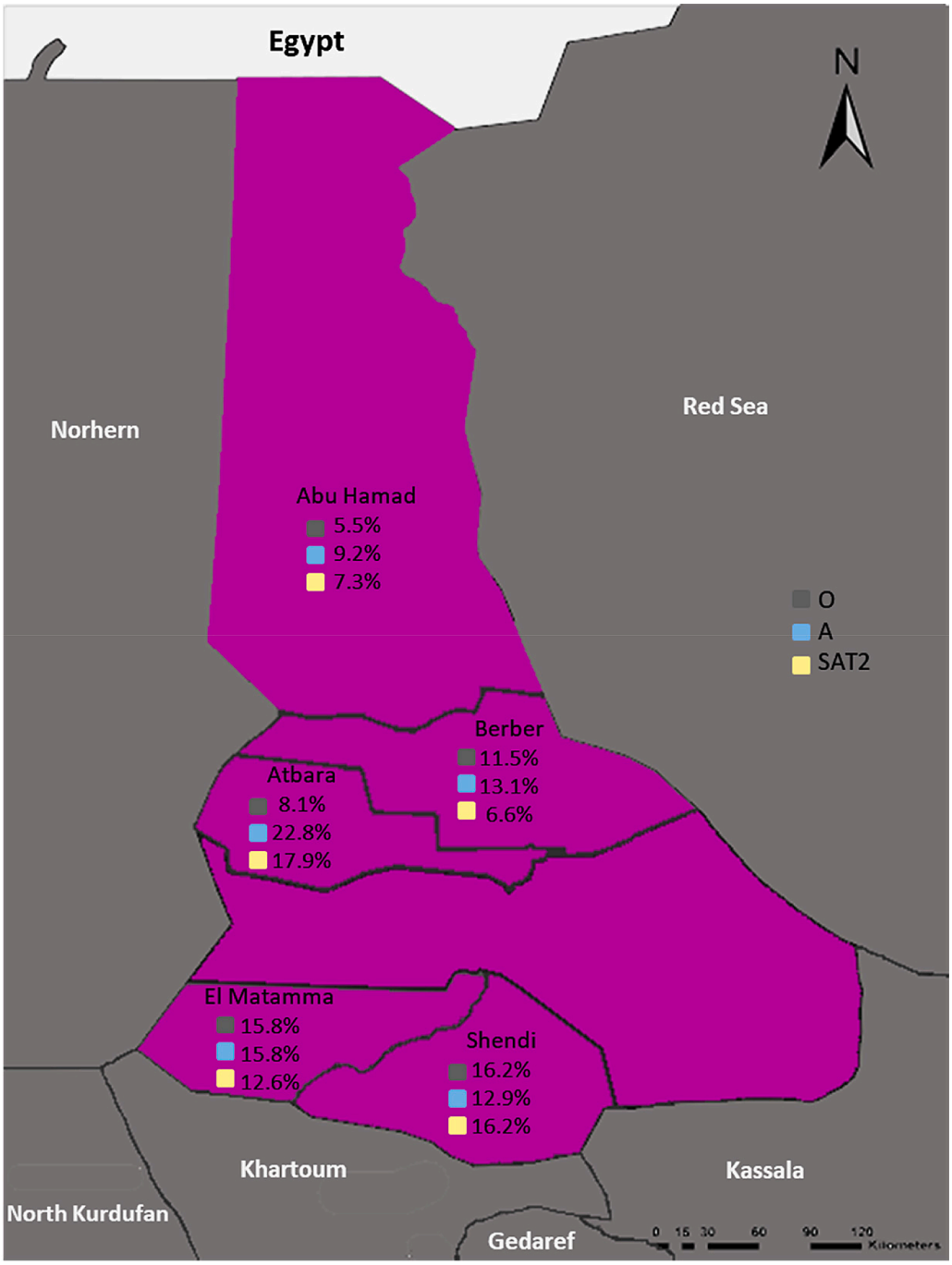

Fig. 3. Prevalence of FMDV serotype-specific antibodies in cattle sera in different localities in the River Nile State.

Table 8
Comparison between sero-prevalence estimates in Southern and Northern districts in the River Nile State.

Districts	O		A		SAT2	
	Positive (%)	Estimated prevalence	Positive (%)	Estimated prevalence	Positive (%)	Estimated prevalence
Southern districts*	32/80 (40%)	17.28%	29/80 (36.25%)	15.66%	34/80 (42.5%)	18.36%
Northern districts**	15/63 (23.8%)	8.4%	27/63 (42.85%)	15.12%	19/63 (30.15%)	10.64%
P-value Fisher exact test	0.0492	0.0126	0.4909	1.0	0.1633	0.0525

* El Matamma, Shendi and Ad-Damar.
** Atbara, Berber and Abu Hamad.

Table 9
Prevalence of FMDV serotype-specific antibodies in cattle sera in different districts in the Northern State.

District	No. tested	O Positive (%)	Sero-prevalence estimate	A Positive (%)	Sero-prevalence estimate	SAT2 Positive (%)	Sero-prevalence estimate
Marawi	15	6/15 (40%)	10.3%	9/15 (60%)	15.45%	4/15 (26.66%)	6.86%
Dongola	16	7/16 (43.75%)	14.13%	7/16 (43.75%)	14.13%	3/16 (18.75%)	6.05%
Al-Dabbah	6	3/6 (50%)	5.71%	2/6 (33.33%)	3.81%	Nil	Nil
Al Burgaig	4	1/4 (25%)	1.98%	2/4 (50%)	3.97%	2/4 (50%)	3.97%

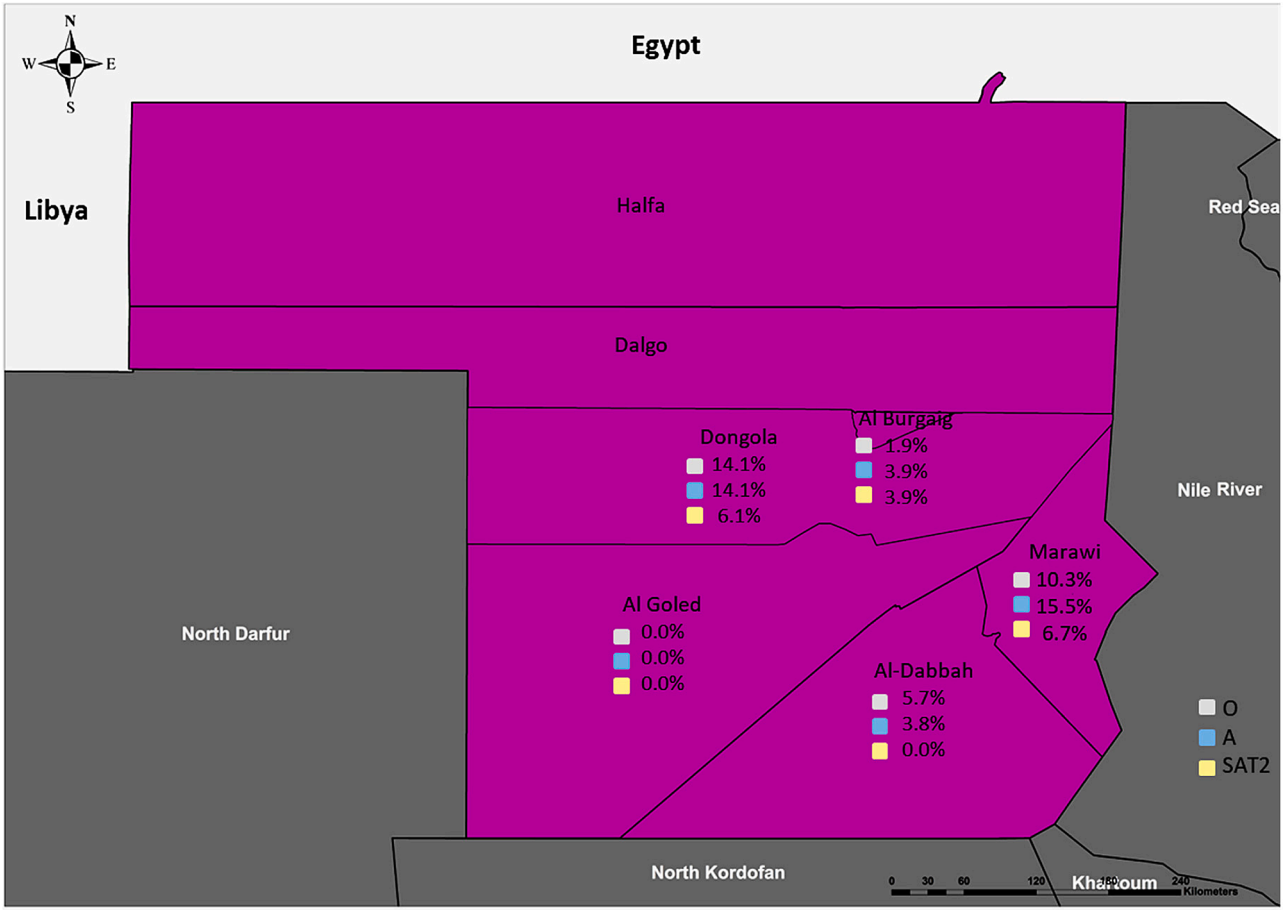

Fig. 4. Prevalence of FMDV serotype-specific antibodies in cattle sera in different localities in the Northern State.

(Fletcher & Fletcher, 2005). In this work, sera negative to NSPs antibodies were not examined for SPs antibodies and around 30% of anti-NSPs positive sera failed to react in SPs serology. Nonetheless, sero-prevalence rates in the Northern State detected in the course of this work were generally similar (overlapping C.I.) to those reported in the Northern State previously using SPs serology (Saeed & Raouf, 2020). The latter workers reported sero-prevalence rates of 9.05% - 16.83% (serotype O), 5.03% - 12.97% (serotype A) and 2.35% - 2.43% (serotype SAT2). Previous studies reported that low sero-prevalence estimates were more associated with NSPs positive SPs negative reactors ($NSP^{+}SP^{-}$) than with NSPs negative SPs positive reactors ($NSP^{-}SP^{+}$) (Bronsvoort et al., 2008; Raouf et al., 2017). Such findings were related to epidemiological factors (Raouf et al., 2017) such as mild repeated exposure to multiple serotypes or single predominant serotype rather than mere sensitivity or specificity of either test. Low sero-prevalence estimates were constantly observed to be associated with higher prevalence rates of NSPs antibodies compared to SPs antibodies (Ranabijuli et al., 2010). However, in this study, sero-prevalence estimates of anti-NSPs and anti-SPs antibodies (Table 3) were not significantly different at the lower level of circulation of FMD viruses in the Northern State ($P = 0.106567$) but rather at the relatively higher level in the River Nile State ($P = 0.000725$).

Patterns of FMD in Northern Sudan showed stark differences from those in other parts of Sudan. Molecular data from Sudan (Habiela et al., 2010b) indicated that within-country circulation is an important mechanism by which serotype O was maintained in the country. Likewise, serological data (Raouf et al., 2016) detected predominance of serotype O antibodies in all studied Sudanese States. Currently, molecular data (http://www.wrlfmd.org/) indicated that recent serotype "O" isolates were likely exotic to Northern Sudan and perhaps to Sudan. Concurrently, serological data in Northern Sudan (Table 6) detected no predominance of serotype O antibodies in the area. Another difference was that; in Northern Sudan, constantly Northern and Western districts showed the lowest seroprevalences (Table 7, 8) while in other parts of

the country Northern region were showing higher seroprevalences than Southern region (Raouf et al., 2016; Anon, 2016). In Northern Sudan, FMD infection is expected to move from South to North while in the other parts of Sudan the case was consistent with the described intense within-country circulation of serotype O. In Northern Sudan, apparently, low animal density and relatively limited animal movements coupled with high levels of antibodies to serotype O in neighbouring States of Khartoum and Kassala (Fig. 1) effectively decrease infiltration of endogenous O strains.

Beside Southern districts in the River Nile State that are neighbouring Khartoum State and Marawi district in the Northern State which is neighbouring the River Nile State, districts containing the State capitals in both States showed high sero-prevalence rates (Fig. 3 and 4). Higher prices of meat and livestock in urban centers, such as States capitals, drive trade animal movements and increase the risk of FMD (Jemberu et al., 2015). State capitals beside Southern district in Northern Sudan were likely the most important portal of entry of FMD viruses into the area.

Conclusion

Differing from other parts of Sudan, low level of FMD infections in Northern Sudan was largely suggested by disease and serological surveillances between 2016 and 2018. Concurrently, unlike other parts of the country, no predominance of serotype O antibodies in bovine sera was detected. Molecular data were also compatible with the inferred low circulation of FMD viruses since a serotype O isolate from Northern Sudan in 2016 was probably originated from outside Sudan rather than being an endogenous strain circulating unabated. It could be concluded that low animal density and limited animal movement in Northern Sudan together with the high antibody levels against serotype O in immediately neighbouring States (Khartoum and Kassala) effectively decreased infiltration of endogenous O viruses.

Conflict of interest statement

None of the authors of this paper has a financial or personal relationship with other people or organizations that could inappropriately influence or bias the content of the paper.

Author contributions

Ahmed N.H. performed sample collection, most of the laboratory work, data analysis, and wrote the drafted manuscript. Alfouz W. and Saeed H.M. contributed to sample collection and laboratory work. Osman N.A. and A/Raouf Y. were responsible for the design and supervision of this research project, data analysis and interpretation, wrote and finalized the manuscript. All authors approved the final manuscript.

Ethical statement

The study used serum samples from cattle collected during STSD program. We assured that the care with cattle completely complied with Sudanese Animal Welfare laws, guidelines and policies.

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.

Acknowledgements

This work was funded by the Government of Sudan (GoS). The Programme "Surveillances of Trade Sensitive Diseases" was funded by the African Union (AU) and GoS. Efforts of the staff of the World Reference Laboratory for FMD (WRLFMD), the Pirbright Institute, UK; and the Department of FMD, CVRL, Sudan, are greatly acknowledged.

References

Abu Elzein, E. M. E. (1983). Foot and mouth disease in the Sudan. *Revue Scientifique et Technique Office International des Epizooties, 2*(1), 177–188.

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

Al-Hosary, A. A., Kandeil, A., El-Taweel, A. N., Nordengrahn, A., Merza, M., Badra, R., … Ali, M. A. (2019). Co-infection with different serotypes of FMDV in vaccinated cattle in Southern Egypt. *Virus Genes, 55*(3), 304–313.

Anon. (2016). *Annual reports of the Central Veterinary Research Laboratory (CVRL)*. Sudan: Khartoum.

Bergmann, I. E., Neitzert, E., Malirat, V., Ortiz, S., Colling, A., Sánchez, C., & Correa Melo, E. (2003). Rapid serological profiling by enzyme-linked immunosorbent assay and its use as an epidemiological indicator of foot-and-mouth disease viral activity. *Archives of Virology, 48*(5), 891–901.

Bronsvoort, B. M., .Parida, S., Handel, I., McFarland, S., Fleming, L., Hamblin, P., & Kock, R. (2008). Serological survey for foot-and-mouth disease virus in wildlife in Eastern Africa and estimation of test parameters of a nonstructural protein enzyme-linked immunosorbent assay for buffalo. *Clinical and Vaccine Immunology, 15*(6), 1003–1011.

Bronsvoort, B. M., Sørensen, K. J., Anderson, J., Corteyn, A., Tanya, V. N., Kitching, R. P., & Morgan,, K. L. (2004). Comparison of two 3ABC enzyme-linked immunosorbent assays for diagnosis of multiple-serotype foot-and-mouth disease in a cattle population in an area of endemicity. *Journal of Clinical Microbiology, 42*(5), 2108–2114.

Di Nardo, A., Knowles, N. J., & Paton, D. J. (2011). Combining livestock trade patterns with phylogenetics to help understand the spread of foot and mouth disease in sub-Saharan Africa, the Middle East and Southeast Asia. *Revue scientifique et technique (International Office of Epizootics), 30*(1), 63–85.

Dill, V., Beer, M., & Hoffmann, B. (2017). Simple, quick and cost-efficient: a universal RT-PCR and sequencing strategy for genomic characterisation of foot-and-mouth disease viruses. *Journal of Virological Methods, 246*, 58–64.

Domenech, J., Lubroth, J., Eddi, C., Martin, V., & Roger, F. (2006). Regional and international approaches on prevention and control of animal transboundary and emerging diseases. *Annals of the New York Academy of Sciences, 1081*, 90–107.

Eisa, M., & Rweyemamu, M. M. (1977). A note on the epizootiology of foot and mouth disease in the Sudan. *Bulletin of Animal Health and Production in Africa, 25*, 108–115.

FAO. (2005). *Food and Agriculture Organization of the United Nations*. Sudan, Livestock Information: Sector Analysis and Policy Branch, AGAL.

Ferrari, G., Paton, D., Duffy, S., Bartels, C., & Knight-Jones, T. (2016). Foot and mouth disease vaccination and post-vaccination monitoring: Guidelines. In Samia Metwally, & Susanne Münstermann (Eds.), *published by the food and agriculture organization of the united nations*. Food and Agriculture Organization of the United Nations (FAO) and the World Organisation for Animal Health (OIE). www.fao.org/publications.

Fletcher, R. H., & Fletcher, S. W. (2005). *Clinical Epidemiology: The Essentials, 4th edition*. Philadelphia, Pennsylvania, USA: Lippincott Williams & Wilkins.

Grazioli, S., Ferris, N., Dho, G., Spagnoli, E., & Brocchi, E. (2012). Ready-to-use ELISA kit for FMDV diagnosis and serotyping tailored for Africa. *Session Research Great Saint Technical Committee of the Europe. Comm. Control FMD-Jerez*. Spain, 29 Oct-31 Oct 2012.

Habiela, M., Alamin, M. A. G., Raouf, Y. A., & Ali, Y. H. (2010a). Epizootiological study of foot and mouth disease in the Sudan: The Situation after two decades. *Veterinarski Arhiv, 80*(1), 11–26.

Habiela, M., Ferris, N. P., Hutchings, G. H., Wadsworth, J., Reid, S. M., Madi, M., Ebert,, K., Sumption,, K. J., Knowles,, N. J., King,, D. P., & Paton,, D. J. (2010b). Molecular characterization of foot-and-mouth disease viruses collected from Sudan. *Transboundary Emerging Diseases, 57*(5), 305–314.

Habiela, M., Raouf, Y. A., & Nur Eldin, H. (2009). Sero-survey of anti-foot and mouth disease virus antibodies in sheep and goats in Khartoum State, Sudan. *The Sudan Journal of Veterinary Research, 24*, 61–64.

Jamal, S. M., & Belsham, G. J. (2013). Foot-and-mouth disease: past, present and future *Veterinary Research, 44*(1), 116–129. http://www.wrlfmd.org/(The *World Reference Laboratory for Foot-and-Mouth Disease designed* by the Food and Agriculture Organization (FAO) of the United Nations and the Office International des Epizooties (OIE), The Pirbright Institute, UK).

Jemberu, W. T., Mourits, M. C., Sahle, M., Siraw, B., Vernooij, J. C., & Hogeveen, H. (2015). Epidemiology of foot and mouth disease in Ethiopia: a retrospective analysis of district level outbreaks, 2007-2012. *Transboundary and Emerging Diseases, 63*(6), e246–e259.

Knezevic, A. (2008). StatNews # 73: Cornell University statistical consulting unit. *Overlapping Confidence Intervals and Statistical Significance*. available from: http://www.cscu.cornell.edu/news/statnews/stenews.

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*(3), 741–755.

MacLachlan, N. J., & Dubovi, E. J. (2011). *Picornaviridae. In: Finner`s Veterinary Virology, 4th edition* (pp. 426–440). California, USA: Elsevier Academic Press.

Murphy, F. A., Gibbs, E. P. J., Horzinek, M. C., & Studdert, M. J. (1999). *Foot and mouth disease. In: Veterinary Virology, 3rd edition* (pp. 517–532). San Diego, California, USA: Academic Press.

OIE Manual. (2018). *Manual of Standard for Diagnostic Tests and Vaccines for Terrestrial Animals, Foot and Mouth Disease (Infection with Foot and Mouth Disease Virus), chapter 2.1.8.* Paris, France: Office International des Epizooties, rue de Prony, 75017.

Ranabijuli, S., Mohapatra, J. K., Pandey, L. K., Rout, M., Sanyal, A., Dash, B. B., … Pattnaik,, B. (2010). Serological evidence of foot-and-mouth disease virus infection in randomly surveyed goat population of Orissa, India. *Transboundary and Emerging Diseases, 57*(6), 448–454.

Raouf, Y. A., Ali, B. H., El Amin, M. A., & Abd Alla, S. (2011). Prevalence of antibodies against three active types of foot-and-mouth disease virus in cattle in Khartoum State at central Sudan: epidemiological significance. *Bulletin of Animal Health and Production in Africa, 59*(4), 399–409.

Raouf, Y. A., Ali, B. H., El Amin, M. A. I., & Al Shallali, A. M. (2010). Laboratory investigation of three outbreaks of foot-and-mouth disease at central Sudan and the disease type situation. *Bulletin of Animal Health and Production in Africa, 58*(4), 308–314.

Raouf, Y. A., Ali, B. H., Khair, S. M., & Amin, A. M. (2009). The prevalence of antibodies against SAT1 foot-and-mouth disease virus in cattle in Khartoum State: epidemiological significance. *Bulletin of Animal Health and Production in Africa, 57*(4), 339–347.

Raouf, Y. A., Tamador, M. A. A., Nahid, A. I., & Shaza, M. (2012). A Survey for antibodies against current infection of Foot-and-Mouth disease virus in Sudanese cattle, sheep and goats using neutralization test. *Bulletin of Animal Health and Production in Africa, 60*(3), 351–358.

Raouf, Y. A., Yousif, H., Almutlab, A. A., Hassen, A. A., Al-Majali, A., Ahmed, A., & Tibbo,, M. (2017). Role of small ruminants in the epidemiology of foot-and-mouth disease in Sudan. *Bulletin of Animal Health and Production in Africa, 65*(1), 145–156.

Raouf, Y. A., Yousif, H., Almutlab, A. A., Hassen, A. A., Ibra, A., Tibbo, M., & Al-Majali,, A. (2016). Sero-epidemiology of foot-and-mouth disease in Sudan. *Bulletin of Animal Health and Production in Africa, 64*(4), 443–451.

Roche, M., Donnet, F., Malzac, M., Comtet, L., & Pourquier, P. (2014). *New competitive ELISAs for detection of non-structural or structural FMDV antibodies*. Cavtat, Croatia: Open session of the Standing Technical and Research Committees of the EuFMD, 29-31 October 2014.

Roeder, P. L., & Le Blanc Smith, P. M. (1987). Detection and typing of foot-and-mouth disease virus by enzyme-linked immunosorbent assay: a sensitive, rapid and reliable technique for primary diagnosis. *Research in Veterinary Science, 43*(2), 225–232.

Saeed, H. M. (2019). *Epidemiological study of foot and mouth disease in cattle in Northern State of the Sudan. Master degree Thesis*. Khartoum, Sudan: Sudan Academy of Sciences.

Saeed, H. M., & Raouf, Y. A. (2020). Serological study of foot-and-mouth disease on cattle in Northern Sudan. *Sudan J. of Veterinary Research, 35*, 33–42.

Thrusfield, M. (2007). *Veterinary Epidemiology, 3rd edition*. Oxford, UK: Blackwell Science Ltd.

WRLFMD (2018). OIE and FAO World Reference Laboratory for Foot-and-Mouth Disease (WRLFMD), genotyping report wrlfmd (2018) available from: http://www.wrlfmd.org/sites/world/files/WRLFMD-2018-00020-SUD-GTR-O-O_001.pdf.

SPSS (Statistical Packages for Social Sciences).

The World Reference Laboratory for Foot-and-Mouth Disease designed by the Food and Agriculture Organization (FAO) of the United Nations and the Office International des Epizooties (OIE), The Pirbright Institute, UK.