Sero-epidemiology of Foot-and-Mouth Disease in Darfur area, Western Sudan

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Research Article

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Abstract

A total of 367 bovine sera positive to antibodies against non-structural proteins (NSPs) of foot-and-mouth disease (FMD) virus were screened for serotype O, A and SAT2 antibodies using the virus neutralization test (VNT). Sera had been collected in 2016 from North (228) and South (139) Darfur States in Western Sudan, where high and low circulation of FMD virus, respectively, prevailed. Tested sera represented the positive-NSPs lot in a random sample of 669 sera collected from both States.

According to standard statistical methods, calculations for serial testing (NSPs ELISA and VNT) were applied to estimate prevalence rates of serotype specific antibodies in the two States. In each State, around 20% of NSPs positive sera failed typing. Prevalence's detected were 49%±5% (O), 27%±5% (A) and 14%±4% (SAT2) in North Darfur State and 27%±5% (O), 17%±4% (A) and 8.0%±3% (SAT2) in South Darfur State. In both States, prevalence rates were significantly higher for serotype O, followed by A then SAT2; the same order that was known in most parts of Sudan. Consistently, estimated prevalence's were statistically significantly higher ($P < 0.05$) in North Darfur than in South Darfur State. Apart from serotype SAT2, detected prevalence rates were lower or similar to those inside the country in previous occasions.

Frequency and pattern of distribution of serotype O prevalence were consistent with its suggested pattern of circulation from the Nile valley to other parts in Sudan and significant within the country's circulation. Alternatively, serotype SAT2 prevalence and distribution in Darfur area were suggestive of sporadic occurrence. However, slightly higher prevalence rates of SAT2 antibodies in Darfur than in neighbouring Kordofan areas in 2013 reflected the wide dissemination of SAT2 (http://www.wrlfmd.org) in Sudan in early 2014. Risk of FMD in Darfur seemed to be associated with the movement of animals to the North in the wet season as part of the pastoral system, and with movement related to trade into urban centers more than with pastoralism across the Western borders. Generally, the result presented little evidence to suggest presence of FMD primary endemic foci in Darfur area.

Introduction

Foot and mouth disease (FMD) is, perhaps, an important transboundary animal disease. It is caused by an *Aphthovirus* of the family *Picornaviridae* of seven immunologically distinct serotypes; O, A, C, Asia1, SAT1, SAT2 and SAT3 that, apart from serotype C, show sustained activity (OIE Manual 2018; Paton et al. 2021). It affects a wide range of cloven-footed animals including domestic and wild ruminants and pigs (Thomson et al. 2003; Alexandersen and Mowat 2005). Of domestic ruminants, cattle are the main target species while small ruminants display mild symptoms or the infection is unapparent (Thomson et al. 2003; Alexandersen and Mowat 2005). The wide range of animal species that contract the disease, the multiple serotypes and subtypes of the virus, the widespread distribution in three continents; Africa, Asia and Latin America and the rapidity with which FMD can spread between animals herds, countries and continents severely complicates its epidemiology. However, it is agreed upon that national and regional control measures would unlikely be successful unless clear insight in its epidemiology is acquired.

East Africa, one of the most FMD active foci in Africa, includes East Africa includes Sudan, South Sudan, Eritrea, Ethiopia, Somalia, Djibouti, Kenya, Tanzania, Uganda, Burundi, Rwanda and Democratic Republic of Congo, and 3 island nations; Seychelles, Comoros and Mayotte. The five serotypes of FMD virus, known to be circulating in the continent in the last decade; O, A, SAT1, 2 and 3, had all been detected in East Africa (http://www.wrlfmd.org; last accessed 30 May 2021). East Africa encompasses two countries known for the highest cattle population in the continent; Sudan and Ethiopia (Rweyemamu et al. 2008). Sudan has experienced FMD since 1902-1903 (Eisa and Rweyemamu 1977; http://www.wrlfmd.org). Recently, based on prevalence data, serotype and topotype distribution

and after evaluation of epidemiological factors such as animal movement patterns in 2008, experts proposed to include Sudan in two epidemiological clusters “i.e. the Horn of Africa/IGAD Cluster and the Soudan/Sahel Cluster” (Rweyemamu et al. 2008). The first cluster comprises member states of the Inter-governmental Authority on Development (IGAD) which include Sudan together with other countries from East Africa whereas the second cluster includes Western Sudan, and countries from Central and Western Africa. Both clusters contain major FMD primary endemic foci. In the last 15 years, in Sudan, serotype data confirmed the maintained activity of three serotypes; O, A and SAT2. Between 2005 and 2018, Serotype O, A and SAT2 were serotyped 51, 33 and 23 times respectively; 26, 25 and 15 times, in that order, were confirmed at the WRL for FMD. All samples, locally and abroad, were from cattle tissues (Raouf et al. 2009; 2010; Habiela et al. 2010a; 2010b; http://www.wrlfmd.org). Moreover, genotyping data (Habiela et al. 2010b) suggested that, both, within-country circulation and long-distance animal movement across the international border are important mechanisms for maintenance of FMD infections in Sudan. Non-structural proteins (NSPs) (Anon 2016) and structural proteins (SPs) (Raouf et al. 2016) serology in Sudan indicated high circulation of FMD infections along the Nile valley up to Khartoum State and less so to the West, East and North of the country. Such results suggested higher significance for within-country circulation compared to circulation across the international border. However, while that was only true for serotype O, prevalences of serotype A and SAT2 antibodies in some Border States in the South-East and East were higher than or similar to that inside the country which highlighted circulation across the international border (Raouf et al. 2016). Alternatively, predominance of serotype O infection inside the country was reflected in many instances by a good correlation between prevalence of antibodies to NSPs and prevalence of antibodies to serotype O (Anon 2016).

Darfur area in Western Sudan has drawn particular attention. Earlier, Abu Elzien (1983) suspected FMD persistently infected cattle, stressed by the long journey from breeding areas in Darfur to animal markets in the Nile valley, might initiate fresh FMD outbreaks at their destination. More recently, Rweyemamu et al. (2008) considered Darfur area as a part of the aforementioned two epidemiological clusters. Many factors accumulate in Darfur area to raise such concerns. Darfur area represents a major animal breeding area and a significant part of the pastoral production system in the country (Ibrahim, 1999). It comprises the whole Western and South Western border areas of Sudan with four different countries. Nonetheless, paradoxically, information about FMD infection in Darfur area is limited. Only recently during the programme Surveillance of Trade Sensitive Diseases (STSD), FMD activity was investigated in the area using NSPs serology and a simple random sampling (SRS) method. Intriguingly, results showed relatively low prevalence indices of around 40% (n = 948) in South Western Darfur breeding areas similar to those detected in Northern Sudan where animal density is low and no pastoralism is practiced (Anon 2016). Additionally, Northern areas in Darfur showed higher prevalence indices of anti-NSPs antibodies than Southern areas; similar to many other parts in the country but dissimilar to Northern Sudan where Southern areas showed higher prevalence indices. The objective of this work was to investigate further FMD infection in Darfur by determining the serotype specificity of the circulating antibodies against FMD virus in cattle in North and South Darfur States.

Materials And Methods

Study area

Darfur covers an area of 493,180 square kilometers (190,420 sq mi) in Western Sudan between 9-20 ºN and 24-25 ºE (Fig. 1). Administratively, it is divided into 5 States: North Darfur State in the North and West Darfur, South Darfur, Central Darfur and East Darfur States in the South. Darfur area constitutes the international borders with four countries (Fig. 1). It includes many urban centers: the largest are El Fashir (North Darfur) and Nyala (South Darfur). Darfur area keeps ie 9 million head of cattle and 11 million of sheep and 10 million of goats. Animal distribution follows, to large extent, the distribution of the ecological zones in the area. In the semi-desert zone, in the North, <5

cattle and <10 small ruminants per square Km are expected (FAO 2005). To the South, cattle density is generally between 10, in the Northern areas of the savannah belt, and 30 head or more per square Km in the Southern areas. Higher density of small ruminants between 25 and 100 head per square Km is expected in the savannah. Animals are mostly reared in the savannah free rangeland under nomadic or transhumant pastoralist systems of production. The latter pastoralists, unlike nomads, move their animals within limited diameters around tribal homeland "dar". Cattle owners in Darfur area are known for the large size of their cattle (200) and goats (200) herds compared to a smaller size of sheep (70) herds (Ibrahim, 1999). In large urban centers like El Fashir and Nyala, nuclei of the improved modernized systems of cattle rearing are represented by some individuals that own high producing milking cows. These cattle owners' rear mainly cross breeds of cattle while pastoralists own local cattle breeds; mostly Baggara.

Serum samples and Non-structural protein (NSP) ELISA

Serum samples analyzed in this study have all been shown to contain anti-NSPs antibodies of FMD virus by the ID Screen® FMD NSP Competition ELISA during the programme Surveillance of Trade Sensitive Diseases (STSD). All serum samples had been collected, late in 2015 and early in 2016 from North and South Darfur States, from apparently healthy cattle, older than 1 year with no history of vaccination against FMD (Fig. 1). In each State, sera were collected from an available sampling frame of 5 geographical districts (sampling units) (Table 1) and five sampling epi-units (herds or collection sites) per sampling unit. Accordingly, a minimal number of 25 epi-units per state was achieved what conform to statistical theory regarding unbiased parameter estimates (Ferrari et al. 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. The approximate sample size required to estimate prevalence in an infinite population (large) in each sampling unit was calculated using the formulae (Thrusfield, 2007): $n = 1.645^2 P_{exp} (1- P_{exp})/d^2$. Where n is the required sample size, P_{exp} is the expected prevalence, d is the desired absolute precision and 1.654 is the approximate multiplier for the required level of confidence. The expected prevalence (P) was assumed to be 50%, the least favorable, and the desired absolute precision of 10% was applied under the level of confidence of 90%.

The ID Screen® FMD NSP Competition ELISA was a relatively newly developed competitive ELISA with specificity of 98%-99.9% ($CI_{95\%}$) and sensitivity of 88.88% (Pirbright, UK) and 94.44% (ANSES, France) (Roche et al, 2014). The test procedure, calculation and result interpretation were, all, done according to the manufacturer instructions using the short incubation protocol. Test sera from different states were, always, tested simultaneously in the same ELISA plate. The result of the plate was considered valid if the mean OD value of the negative control was greater than 0.7 and the mean value of the positive control OD was less than 30% of the OD value of the negative control. OD values in test sera wells of less than or equal to 50% of the OD value of the negative control were considered positive.

Numbers and distribution of the positive sera in the different districts in North and South Darfur States were shown in Table 1.

North and South Darfur States were selected to represent the high (North Darfur) and the low (South Darfur) levels of FMD activity reported in Darfur area (Fig. 1) by the programme STSD (Anon 2016). Sero-prevalence rates of anti-NSPs antibodies were 74.0% and 69.0% in North and East Darfur States, respectively, but around 40% in South, West and Central Darfur States. Selection of the sampling units (localities) depended to a large extent on security due to civil unrest in Darfur area. However around 350 bovine sera were collected from each state (Table 1).

Positive and negative control sera

Control sera for virus neutralization test (VNT) were known positive field bovine sera for either O, A and SAT2 serotypes (Raouf et al. 2016) and fetal calf sera (FCS) (Sigma) free from antibodies against FMDV was used as the negative control sera.

Cells, media and FMD viruses

Foot-and-mouth disease viruses used in the virus neutralization test (VNT) were recent local Sudanese isolates, of cattle origin, adapted to grow in cultured cells, typed and retyped using reference antigen detection ELISAs (Pirbright and IZSLER Laboratories). They were designated according to their serotype, geographical origin within Sudan, year of isolation and order of isolation from that origin. Two SAT2 isolates were used, one was isolated from Khartoum in 2008 (SAT2-Kh 1/08), not genotyped at the WRL for FMD yet, however, topotype X111 of SAT2 serotype was circulating in Sudan in 2008 (http://www.wrlfmd.org; Raouf et al. 2010). The other SAT2 isolate was isolated from North Kordfan State in 2010 (SAT2-NK 1/010) of genotype V11 and with the identity of SUD/4/2010 at the WRL for FMD (http://www.wrlfmd.org). Serotype A isolate was isolated from Khartoum in 2011 (A-Kh 2/011), of topotype Africa and with the identity of SUD/7/2011 at the WRL for FMD (http://www.wrlfmd.org; Raouf et al. 2016). Serotype O isolate was isolated from Khartoum in 2015 (O-Kh 1/015) and was not genotyped at the WRL for FMD. To perform the VNT, viruses were grown in BHK-21 cells (clone 13, ŞAP Institute, Turkey), clarified by centrifugation at 2000 rpm for 10 minutes, distributed in 2 ml aliquots and stored in liquid nitrogen vapor. Virus stocks were titrated in the microtitre system (Raouf et al. 2010) using BHK-21 cells. Virus diluent was complete Glasgow minimum essential medium (GMEM) [GMEM containing 10% (V/V) tryptose phosphate broth, 0.0487% (W/V) $NaHCO_3$ and 10% (V/V) tris-buffer (0.05M)]. Growth medium for BHK-21 cells contained in addition 10% (V/V) newborn calf serum (NBCS) (Sigma). Titres were calculated according to the method of Kärber (1931).

Virus neutralization test (VNT)

All sera were tested for serotype-specific antibodies against FMD virus serotype O, A and SAT2 using standard procedure of VNT (OIE Manual 2018) except that sera were tested at two dilutions; 1/32 and 1/64, rather than several dilutions (Raouf et al. 2012). Test and control sera were inactivated at 56°C for 30 minutes. Sera from different states and from different districts were tested simultaneously. The test was carried out in u-bottomed microtitre plates (Coaster) in equal 50 µl volumes. Serum diluent and virus diluent consisted of complete GMEM containing 10% (V/V) tryptose phosphate broth, 0.0487% (W/V) $NaHCO_3$ and 10% (V/V) tris-buffer (0.05M).

The previously titrated virus preparation was diluted to contain 100 $TCID_{50}$ in 50 µl volume. Virus and serum mixtures were allowed one hour to react at room temperature. After addition of cells, plates were sealed with adhesive tape and incubated at 37°C for 72 hours. Results were read microscopically and thereafter stained with 0.1% crystal violet in 10% formal saline. Positive wells appeared as stained intacted cell monolayers, and get stained while negative wells appeared as empty or with fragmented cell monolayers and patchy stain.

Each serum was tested at 4 wells and each plate tested 20 sera in addition to control serum (positive and negative) and cell control. Adopting the procedure to include only two serum dilutions (1/32 and 1/64) while decreasing the test workload, span the standard cut-off of 1/45 ($10^{6.5}$) described for the purpose of serosurveillance 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). Few sera were found positive at a titre of $10^{1.5}$ but negative at titres of $10^{1.65}$ and $10^{1.8}$ (Raouf et al. 2012; 2016 and 2017). Even fewer sera were found positive at titres of $10^{1.35}$ or $10^{1.2}$ (dilution 1/16) but negative at higher titres (Raouf et al. 2012). Using the

adopted VNT, previous serosurveillance determined seroprevalences as high as 75% (n = 531) (O) and 40% (n = 531) (A and SAT2) and detected subtle differences between States, regions and districts in Sudan (Raouf et al. 2016).

Statistical analysis

Only positive reactors to the NSPs Competition ELISA were tested by the VNT. In effect, both tests, ID Screen® FMD NSPs Competition ELISA (test A) and VNT (test B) were conducted consecutively (Fletcher and Fletcher 2005). Calculations for serial testing were performed according to the standard procedure (Thrusfield 2007). Prevalence was calculated as proportion positive to both tests according to the formula:

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 programme STSD (Table 1) whereas proportions positive by test B (VNT) in each sub-population were determined by dividing the number of positive reactors identified using the VNT by the number of sera tested in that sub-population. Sera eligible for the calculation of prevalence of combined serotype specific antibodies (three serotypes) should be positive to one or more serotypes and/or negative to the three serotypes.

Prevalence rates were compared by driving the 95% C.I. was derived to compare prevalence rates. It was derived from a simple random sample, based on the Normal approximation to 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., *p-values* were calculated using chi-squared test available at the Statistical Packages for Social Sciences (SPSS) at (www.sociostatistics.com); results were significantly different, if $p < 0.05$.

Results

Index of prevalence of FMD infection in North and South Darfur States (serial testing approach)

In each State, around 20% of NSPs positive sera failed typing by the VNT (Table 2). In North Darfur State, index of prevalence of FMD infection decreased from 74.0% by NSPs serology to 61.0% by the serial testing approach (Table 2). Similarly, in South Darfur State, it decreased from 43.0% to 34.0% (Table 2). Nevertheless, indices of FMD infection remained statistically significantly higher (non-overlapping C.I.) in North Darfur than in South Darfur (Table 3).

Sero-prevalence of FMDV serotype-specific antibodies in North and South Darfur States

In both States, approximately two thirds of the typed sera were positive to serotype O, c.a. one third was positive to serotype A and c.a. one fifth was positive to serotype SAT2 (Table 4). Consistently, in both States, sero-prevalence of antibodies to serotype O was statistically significantly higher than sero-prevalence of antibodies to serotype A and that of serotype A was statistically significantly higher than that of serotype SAT2 (Table 4). Also, consistently, the three serotypes showed statistically significantly higher sero-prevalence of serotype specific antibodies in North Darfur than in South Darfur (Table 4).

Sero-prevalence of FMDV serotype-specific antibodies in different localities of North Darfur State

Serotype O and A showed the highest sero-prevalences at El Fasher capital city but the lowest at Um Keddada district which neighbors, mainly, East Darfur State. In contrast, serotype SAT2 showed the highest sero-prevalence at Um Keddada but the lowest at the Northern district of El Kuma where serotype O showed relatively high sero-prevalence. Serotype A showed relatively high sero-prevalence at the Southern locality of El Taweish and El Lait (Table 5; Fig. 2A).

Prevalence of FMDV serotype-specific antibodies in different localities of South Darfur State

In South Darfur, similar to North Darfur, serotype O showed relatively high sero-prevalence at Northern district (Niteaga) and at the State capital (Nyala). Also, serotype A showed relatively high sero-prevalence at the capital city of Nyala but relatively low sero-prevalence at the North (Niteaga and Marshang) and at the South (Bielel). Sero-prevalence of serotype SAT2 was relatively low. However, remarkably, it showed the lowest sero-prevalence at the Northern district of Niteaga (Table 6; Fig. 2B).

Discussion

Recently, to study the prevalence of serotype specific antibodies against FMD virus in cattle in Sudan, VNT has been employed extensively (Raouf et al. 2016; Haithum et al. 2019). In this study, to decrease the load of the work, VNT was used simultaneously with NSPs ELISA (ID ELISA); the latter being the primary testing method. This approach is known to increase specificity but decrease sensitivity (Fletcher and Fletcher 2005). Particularly, NSPs-ELISAs are expected to be less sensitive than SPs serology in detecting mild FMD infection after vaccination; due to limited virus multiplication (Brocchi et al. 2006; King et al. 2015). In the field, where no vaccination is practiced, this is comparable to mild repeated infection with the same serotype which is more likely to happen with the predominant serotype than with the subordinate serotypes. Serotype O, the most predominant serotype in many parts of Sudan (Abu Elzein et al. 1987; Raouf et al. 2016). Was also detected as the predominant serotype in this work expressed marked predominance of serotype 'O' whether the levels of FMD infection was high (North Darfur) or low (South Darfur). Another concern was raised due to the known genetic heterogeneity of the 3ABC polypeptide of the SAT serotype (Van Rensburg et al. 2002; Nsamba et al. 2015). It was feared that NSPs-ELISA expressing 3ABC polyprotein derived from the classical "European/South American" types (O, A and C) may be less efficient in detection of NSPs-antibodies from FMD virus SAT infections. However, Chitray et al. (2018) have shown that NSPs-ELISAs irrespective of the origin of the 3ABC antigen, were reliable and accurate for the detection of FMD virus SAT 3ABC antibodies. As far as the specificity of the approach is concerned, some recent reports described cross reactions in the VNT (Tekleghiorghis et al. 2014; 2015). It was observable, at least in one of these two cases, that sera were collected between 2 weeks to 2 months following confirmed FMD outbreaks (Tekleghiorghis et al. 2014). To increase the specificity of the neutralization assay, Tekleghiorghis et al. (2015) used a cut-off value different from the standard cut-off value of 1.65 $\log_{10}$ (OIE Manual 2018). From our experience, in Sudan, although a cut-off of 1.5 $\log_{10}$, around the standard cut-off value or slightly lower, was used, significant differences in the prevalence and distribution of circulating FMD virus serotypes were observed previously (Raouf et al. 2016; 2017) and also in this work.

For optimum sensitivity of the neutralization assay, the virus used in the assay should be closely matched to the strain circulating in the field (OIE Manual 2018). Local FMD virus isolates used in the study were all recent isolates obtained in 2008, 2010, 2011 and 2015. Yet, about 20% of anti-NSPs positive sera in this work failed to show anti-SPs activity. Disease surveillance in Sudan in the last 15 years detected serotype O FMD viruses, mostly, followed by A then SAT2 (Raouf et al. 2009; 2010; Habiela et al. 2010a; 2010b; http://www.wrlfmd.org). Serosurveillance in the country detected activity of these viruses also mostly in that same order (Raouf et al, 2016) what gave credibility to both type of surveillance. Had there been any undetected activity of serotype SAT1 and SAT3 in Sudan, it is fair to expect it to be of minor importance and account for little or insignificant part of the un-typed sera. Alternatively, such reactors (NSPs + ve SP –ve) were also detected following vaccination and experimental challenge (Brocchi et al. 2006). Brocchi et al. (2006) reported that these same experimental sera/reactors were detected repetitively by different NSPs-ELISAs and in different occasions. Therefore, they were unlikely to be non-specific reactors. In the field, in different occasions, studies that used different NSPs-ELISAs and VNT also reported these reactors. Bronsvoort et al. (2008) reported 26/327 (8%) such reactors in buffalo and 7/11 (64%) in non-buffalo wild ungulates, Tekleghiorghis

et al. (2015) reported 190/555 (34%) in cattle and Raouf et al. (2017) reported 49/215 (23%) in small ruminants and 3/66 (5%) in cattle. Bronsvoort et al. (2008) associated these reactors with low seroprevalence estimates whereas Raouf et al. (2017) expected that repeated mild exposure to different serotypes is likely to boost immune response to NSPs but not to SP what result in this type of reactors. In this study, it was remarkable that proportion of such reactors remained similar at two significantly different levels of FMD virus activity in the North and in the South what suggested a likely minor role for the sensitivity of the testing methods.

One of the main objectives of the presented work was to define the extent of infection of different FMD virus serotypes in cattle in Darfur area. In absence of vaccination, prevalence of serotype specific antibodies is indicative of previous infection. Prevalence of serotype specific antibodies in Darfur was found to be highest for serotype O followed by A then SAT2 (Table 4) similar to the order detected previously in other part of the country (Raouf et al. 2016), apart from Northern Sudan (Haithum et al. 2019). In every case, prevalence's detected were higher in North Darfur than in South Darfur State. In South Darfur, cattle graze most time of the year in their Southern grazing fields away from trade route and away from the Eatern areas of Western Sudan which are subjected to FMD virus spill from the Nile valley. On the other hand, in Northern Darfur the FMD-infected Eastern areas of Western Sudan are part of the cattle pastoral system. In general terms, prevalence's of serotype specific antibodies in Darfur compared to prevalence's in other parts of the country in 2013 (Raouf et al. 2016) were found to be lower for serotype O, similar for serotype A and higher for serotype SAT2. In neighbouring Kordofan area, prevalence's detected in 2013 were 67.5% (serotype O), 26.4% (serotype A) and 5.1% (serotype SAT2) in North Kordofan and 46.3% (O), 24.1% (A) and 4.5% (SAT2) in South Kordofan State. In comparison, corresponding figures detected in this work were 48.9%, 26.2% and 13.6% in North Darfur and 27.2%, 16.7% and 8% in South Darfur State. The lower frequency for serotype O antibodies in Darfur area compared to other parts of Sudan was consistent with its suggested pattern of circulation from the Nile valley to other parts in the country (Raouf et al. 2016). The frequency of serotype SAT2 antibodies in Darfur in 2015/2016 is higher than in Kordofan in early 2013 which was consistent with the detected wide dissemination of SAT2 infection in five Sudanese States (http://www.wrlfmd.org), after the surveillance performed late in 2013 and early in 2014.

Not only did the prevalence rates of the three FMD virus serotypes differ considerably but their distribution in different districts in the two States showed different patterns. Serotype O, unlike serotype A and SAT2, consistently showed high prevalence at the capital cities and at the Northern districts but low prevalence at the Southern districts. Serotype A clearly showed high prevalence at the capital cities while no particular pattern could be described for serotype SAT2. Because of the higher prices of meat and livestock in urban centers, capital cities drive trade animal movements and increase the risk of FMD (Jemberu et al. 2015). The described pattern for serotype O was consistent with the indicated spread of serotype O from North to South (Raouf et al. 2016) and significant within the country's circulation while the picture for SAT2 was more suggestive of occasional or sporadic spread. Darfur area comprises the whole Western border of Sudan and represents a major animal breeding area where pastoral system prevails. It is of paramount importance to define the influence of these two factors on the epidemiology of FMD in the area and in Sudan. Non-structural proteins serology showed that level of FMD virus circulation in Southern Darfur area was lower than most parts of the country and that level in Northern Darfur area was similar to neighbouring Kordofan area (Anon 2016). Likewise, structural proteins serology presented no evidence for a particular risk of the circulation of FMD virus through the western border or through pastoralism across these borders. Apart from prevalence of SAT2 antibodies, prevalence rates detected were lower or similar to those inside the country and remained with the same order observed in other parts of Sudan; O, A then SAT2. Therefore, though many border districts escaped examination in this work due to civil unrest, it could be concluded that the load of FMD infections crossing the international border of Darfur was negligible or too weak to impact prevalence data. Animal movement to the North during the wet season

from June to October, as part of the pastoral system, and movement related to trade into urban centers seem to bear the risk of introducing and maintaining FMD infection in Darfur area. Otherwise, results presented little evidence to suggest presence of FMD primary endemic foci in Darfur area.

Abbreviations

FMD, Foot-and-mouth disease; FMDV, Foot-and-mouth disease virus; VNT, Virus neutralization test; NSPs, Non-structural proteins; SPs, Structural proteins; IGAD, Inter-governmental Authority on Development; O, serotype O; A, serotype A; SAT, serotype SAT; STSD, Surveillance of Trade Sensitive Diseases; FAO, Food and Agriculture Organization; Km, Kilometers; ELISA, Enzyme linked immunosorbent assay; Kh, Khartoum; NK, Khartoum-North; BHK-21; Baby Hamster Kidney-21 cells; GMEM, Glasgow minimum essential medium; NBCS, newborn calf serum; OIE, Office International des Epizootics; µl, Microliter; $TCID_{50}$, Tissue Culture Infective $Dose_{50}$

Declarations

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"Declarations" statement

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Compliance with ethical standards

Competing interests

The authors declare no competing interests.

Code availability

'Not applicable'

Author's contributions

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

Ethics approval

No approval of research ethics committees was required for this study because the serum samples used in the study were collected during Surveillance of Trade Sensitive Diseases (STSD) programme.

Consent to participate

'Not applicable'

Consent for publication

'Not applicable'

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Tables

Table 1. Data of NSPs serology in North and South Darfur States: Numbers and origin of anti-NSPs positive sera

States data				Districts data				
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
North Darfur	329	243	74.0%	El Kuma	61	47	77%	43
				Umm Keddada	65	45	69%	44
				El Taweish	66	36	55%	30
				El Lait	67	56	84%	53
				El Fasher	70	59	84%	58
Totals					329	243		228
South Darfur	340	146	43.0%	Niteaga	66	33	50%	29
				Nyala North	68	33	49%	30
				Nyala South	68	30	44%	28
				Marshang	68	26	38%	26
				Bielel	70	24	34%	26
Totals					340	146		139

*Collected sera were 350 per State (according to the list) but some were lost.
**Not all positive sera were tested since some were lost.

Table 2. Typing of anti-NSPs positive cattle sera

State	Indices of prevalence of FMD infection by NSPs serology (Sero-prevalence of anti-NSPs antibodies %)	Typing of NSPs positive sera by cVNT				Indices of prevalence of FMD infection by serial testing (NSPs serology and cVNT)
		No. tested*	No. tested positive to one or more serotype	% of typed sera	% of sera failed typing	
North Darfur	74% (243/329)	228	188	82% (188/228)	18% (40/228)	61%
South Darfur	43% (146/340)	139	110	79% (110/139)	21% (29/139)	34%

Table 3. Comparison between indices of prevalence of FMD infection by NSPs serology and by cVNT-O, A and SAT2 (serial testing approach)

State	Sero-prevalence of anti-NSPs antibodies		Sero-prevalence of anti-O, A and SAT2 antibodies-combined (serial testing approach)		P value (chi-squared test)
	Sero-prevalence%	95% C.I.	Sero-prevalence%	95% C.I.	
North Darfur	74%	69% - 79%	61%	56% - 66%	0.000448
South Darfur	43%	38% - 48%	34%	29% - 39%	0.01735

Table 4. Sero-prevalence of FMD virus serotype-specific antibodies in North and South Darfur States

FMDV Serotype	North Darfur			South Darfur			P value (chi-squared test)
	% positive in NSPs positive sera	Estimated prevalence	95% C. I.	% positive in NSPs positive sera	Estimated prevalences	95% C. I.	
O	66% (151/228)	49%	44%-54%	63% (88/139)	27%	22%-32%	0.00753
A	36% (81/228)	27%	22%-32%	39% (54/139)	17%	13%-21%	0.002788
SAT2	18.4% (42/228)	14%	10%-18%	19% (26/139)	8.0%	5.0%-11%	0.000003

Table 5. Sero-prevalence of FMD virus serotype-specific antibodies in different localities of North Darfur State

Locality	No. of tested sera	O		A		SAT2	
		% +ve	Estimated Prevalence	% +ve	Estimated Prevalence	% +ve	Estimated Prevalence
El Fasher	58	83% (48/58)	70%	50% (29/58)	42%	19% (11/58)	16%
El Kuma	43	70% (30/43)	54%	30% (13/43)	23%	12% (5/43)	9.0%
El Taweish and El Lait	83	60% (50/83)	41%	53% (44/83)	37%	14% (12/83)	10%
Um Keddada	44	52% (23/44)	36%	11% (5/44)	8.0%	32% (14/44)	22%

Table 6. Sero-prevalence of FMD virus serotype-specific antibodies in different localities of South Darfur State

Locality	Total No. tested	O		A		SAT2	
		% +ve	Estimated Prevalence	% +ve	Estimated Prevalence	% +ve	Estimated Prevalence
Niteaga	29	72% (21/29)	36%	28% (8/29)	14%	3.0% (1/29)	2.0%
Nyala North	30	70% (21/30)	34%	47% (14/30)	23%	20% (6/30)	10%
Nyala South	28	64% (18/28)	28%	39% (11/28)	17%	21% (6/28)	9.0%
Marshang	26	62% (16/26)	24%	42% (11/26)	16%	27% (7/26)	10%
Bielel	26	54% (14/26)	18%	31% (8/26)	11%	23% (6/26)	8.0%

Figures

Figure 1

The study area “North Darfur and South Darfur States” in Darfur, Sudan. Two levels of activity against NSPs of FMD virus; a higher level (streaked area) and a lower level (non-streaked area) were shown.

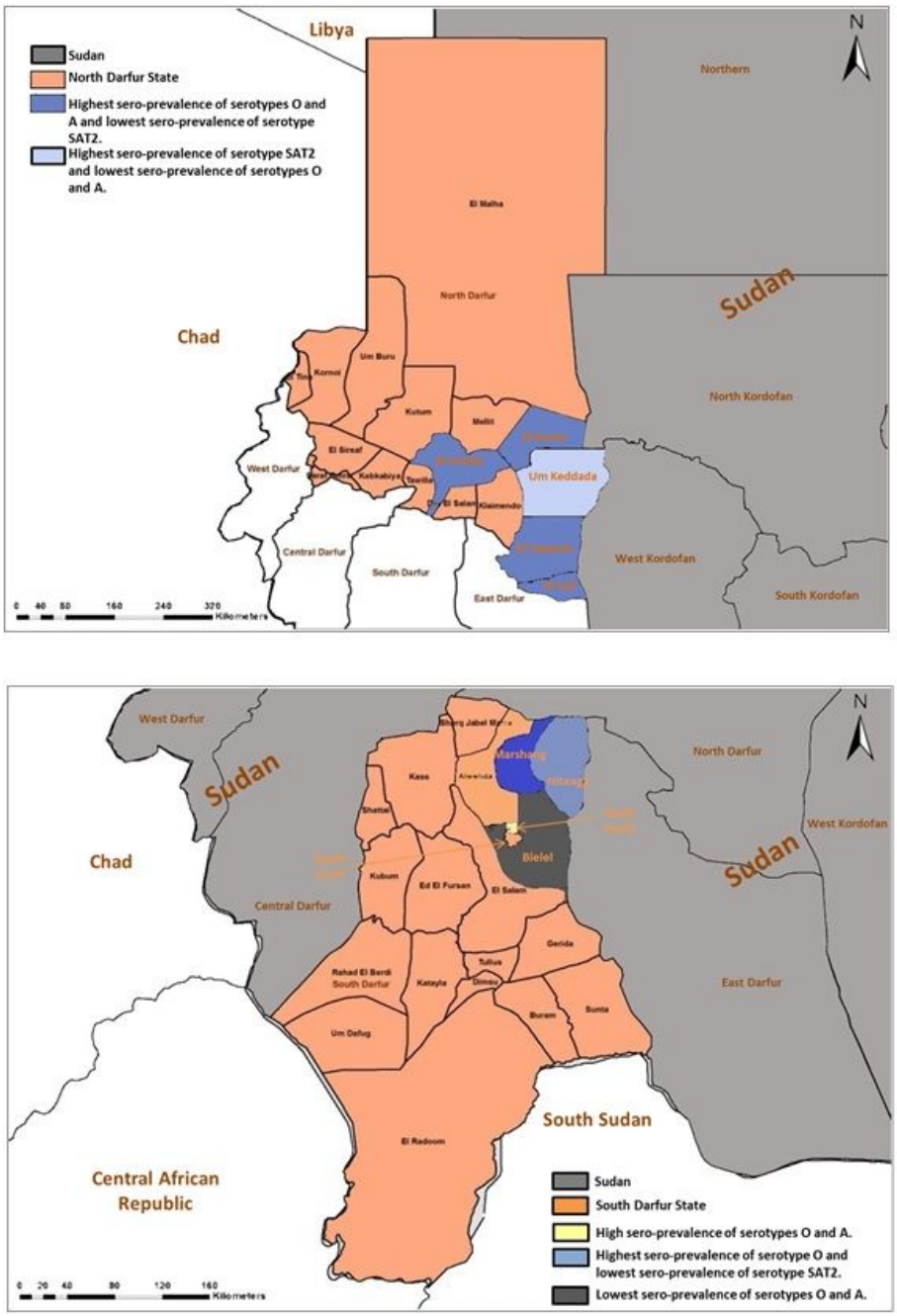

Figure 2

Prevalence of serotype-specific antibodies against FMD virus in cattle sera in different districts in: 2A. North Darfur State. 2B. South Darfur State.