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Epizootiological study of foot and mouth disease in the Sudan: the situation after two decades

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ABSTRACT

In order to update information on the situation regarding foot and mouth disease (FMD) in the Sudan, a serosurvey and disease survey were conducted. Recently collected data on FMD in the Sudan showed that FMD is a major constraint to animal production in the country. It presents no threat nor does it cause mild disease in sheep and goats. The disease, with obvious clinical signs, has been detected in cattle only, and is caused by serotype O and SAT 2. Seasonal occurrence of the disease in the cold, dry season has been observed and animal movement seems to play a major role in virus dissemination. A total of 1,069 sera were collected from cattle, sheep, goats, and camel, from seven states in the Sudan, for the detection of antibodies to FMDV. Application of liquid phase blocking (LPB) ELISA revealed that antibodies to four serotypes were present in ruminants; namely O, A, SAT 1 and SAT 2. No antibodies to FMDV were detected in camel sera. The results differed from early reports regarding the prevalence of serotype specific antibodies in different species; for instance, in cattle, the antibodies to type A (78.13%) surpassed that of type O (69.39%) and the antibodies to type SAT 2 (44%) surpassed that of type SAT 1 (20.2%). This work elucidates the current epidemiology of FMD in some parts of the Sudan.

Key words: foot and mouth disease, epizootiology, Sudan

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Introduction

Foot and mouth disease (FMD) (Family *Picornaviridae*, Genus *Aphthovirus*) is a highly contagious, vesicular disease of cloven-footed animals. Infection with FMD has been reported in cattle, sheep, goats, swine, and antelopes, as well as many wild animal species. FMDV has seven distinct serotypes O, A, C, South African Territories 1-3 (SAT 1, SAT 2 and SAT 3) and Asia 1 (MURPHY et al., 1999). In most of sub-Saharan Africa, serotypes O, A, SAT 1 and SAT 2 are predominant (RWEYEMAMU et al., 2000).

In the Sudan, FMD is endemic and FMD outbreaks occur annually; the first record of the disease in the Sudan was in 1903 (EISA and RWEYEMAMU, 1977), and four FMD serotypes out of the seven have been reported in the country. These are O, A, SAT 1 and SAT 2 (ABU ELZEIN, 1983). Serotype O was isolated first, then serotype SAT 1 before 1952, serotype A in 1957, and lastly, serotype SAT 2 in 1977 (ABU ELZEIN and CROWTHER 1979). Antibodies to these four FMDV serotypes were detected in cattle, sheep and goat sera, but their prevalence rate was quite different from species to species (ABU ELZEIN et al., 1987). Camel sera were screened by the agar gel immunodiffusion test (AGID) for the presence of antibodies against FMD virus infection associated (VIA) antigen and proved to be negative (ABU ELZEIN et al., 1984). Since 1987, no study on FMD in the Sudan has been carried out and the type and subtype situation needs to be updated. However, efforts have been recently renewed; a serosurvey has been conducted in Khartoum state (RAOUF et al., 2008) and samples of suspected FMD outbreaks have been sent more regularly to the World Reference Laboratory (WRL) at Pirbright in the UK (ANONYM., 2007).

A detailed survey of FMD in different animal species in the Sudan is insufficient. In this study, an attempt has been made to update and evaluate the FMD situation in some states of the Sudan; questionnaires and interviews with herdsmen and veterinarians were carried out, as well as a serosurvey and disease survey.

Materials and methods

Questionnaire and serosurvey. The survey was conducted between 2006 and 2008. A simple standardized questionnaire was compiled and used to collect information from herdsmen, emphasizing data on hosts and environment. Data was collected on blood-sampled animals (species, age, sex, and breed) alongside the history of FMD in the herd. A total of 1,069 sera were randomly collected from cattle (469), sheep (319), goats (88) and dromedary camel (193) from seven states in the Sudan; namely, Gezira (Wad madani), Northern Kordofan (El Obied), Southern Kordofan (El Deling), White Nile (Rabak), Gedarif (Gedarif), River Nile (Atbarah) and El-Shemalyah (Dongola) (Fig. 1). These localities were selected according to disease history and animal population density.

FMDV antibody detection by LPB ELISA. The LPBE Kits for seven FMDV serotypes O, A, C, SAT 1, SAT 2, SAT 3, and Asia 1, were provided by the Arab Organization for Agricultural Development and the Federal Ministry of Animal Resources and Fisheries. They were obtained from the Institute for Animal Health (IAH), Pirbright Laboratory, UK.

Screening assay for detection of antibodies to four FMDV serotypes present in the Sudan O, A, SAT 1 and SAT 2 (ABU ELZEIN et al., 1987) was carried out according to the instructions of the manufacturer after optimization of the antigen dose for the test.

Collection of FMD virus samples. Virus samples were collected, depending on the history of FMD outbreaks in the area. Probang samples were collected from cattle from past or recent infections and epithelium samples were collected from cattle during an active outbreak.

Collection of oesopharyngeal (OP) fluid (probang samples). Eighteen probang samples and three oral swabs were collected from previously suspected FMD-infected cows. Samples were collected from suspected FMD-carrier animals using a probang cup, according to the method of HEDGER (1968), added to an equal volume of transport media (0.08 M phosphate buffer pH 7.2-7.4) with antibiotics (KITCHING and DONALDSON, 1987), Samples were transported in ice boxes to the Laboratory, and then stored at -70 °C.

Some of the probang samples were submitted to the FAO-OIE World Reference Laboratory (WRL) for FMD at Pirbright, UK.

Collection of epithelial samples. Eight epithelial samples were collected from three outbreaks, one in Gezira State and two outbreaks in White Nile State**.** The epithelium samples were collected during the course of field outbreaks, as described by KITCHING and DONALDSON (1987). Mouth lesions were taken from infected animals, put in transport media (0.04 M phosphate buffer pH 7.2-7.6) with 50% glycerol and antibiotics, kept on ice, transported to the laboratory and stored at -30 to -5 °C.

FMD virus isolation and serotyping. Virus isolation was carried out in bovine thyroid (BTY) cell culture (SNOWDON, 1966) and primary bovine kidney (PBK) cell culture (PATTY et al., 1962) at CVRL. Virus isolates were serotyped using FMD antigen detection ELISA, obtained from the Institute for Animal Health (IAH), Pirbright Laboratory, UK. The ELISA procedures used were similar to those described by ROEDER and SMITH (1987).

Results

Questionnaire and interviews. Of 50 questionnaires distributed, 23 (46%) were returned; eleven questionnaires from Gezira state, four from White Nile state, three from

each North Kordofan and Gedarif states, and one from each South Kordofan and El-Shymaliyah States. The data collected showed that FMD, which is locally known as (*Abu Lisan* = tongue disease), is well known to herdsmen and they are well acquainted with the disease, its clinical signs, seasonality, duration and transmission.

Table 1. Overall positive sera for FMDV antibodies per species

Animal species	Positive sera	Positive%
Cattle	374 [a]/472[b]	79.24%
Sheep	70/305	22.95%
Goat	28/98	28.57%
Camel	0/176	0

a = Number of positive sera, b = Number of tested sample

Table 2. The percentage of positivity of the total sera for each of the four FMDV serotypes using LPBE

SAT2		SAT1		A		O		Serotype
Positive %	Positive sera	Positive %	Positive sera	Positive %	Positive sera	Positive %	Positive sera	Species
44	176 [a]/400 [b]	20.2	60 [a]/297 [b]	78.13	275 [a]/352 [b]	69.39	297 [a]/428 [b]	Cattle
8.99	16/178	5.1	5/98	8.7	8/92	27.51	52/189	Sheep
2.38	2/84	8.51	4/47	15.94	11/69	27.5	22/80	Goats
0	0/193	0	0/193	0	0/193	0	0/193	Camel

a = Number of positive sera, b = Number of tested sample.

Table 3. Results of inoculation of specimens sampled from cattle in different cell cultures

Type of specimen	Number of samples passaged in cell culture	Number of samples caused CPE in cell culture
Probang	21[a]	19
Swabs	2 [a]	2
Epithelium	7 [b]	7

a = Passaged in BTY cells and twice in BK cells, b = At least two passages in BK cells only

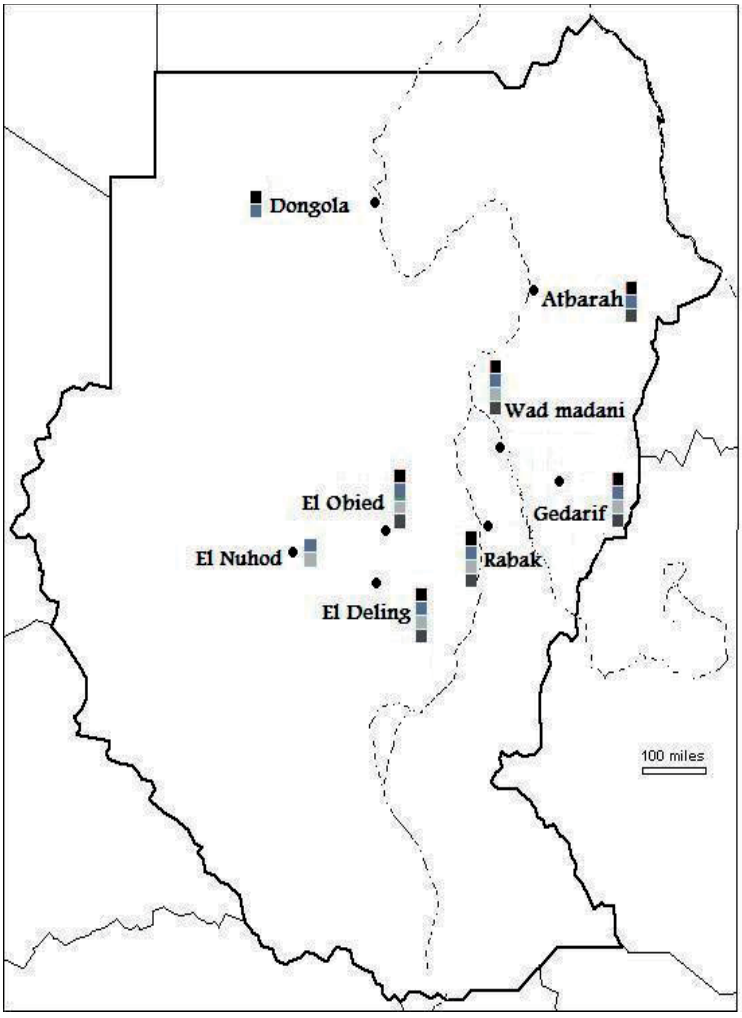

Fig. 1. Map of the Sudan showing the area of the study and locations of sampled livestock between 2006 and 2008.

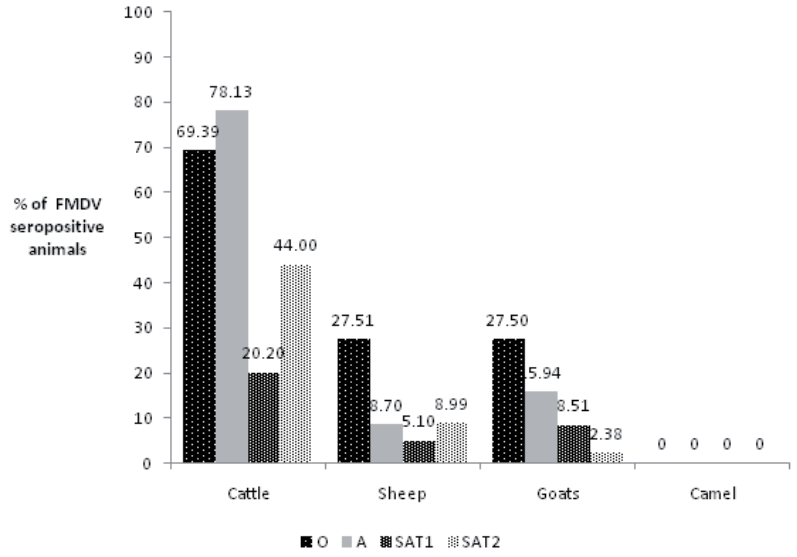

Fig. 2. Overall results of screened sera of different animal species for FMDV antibodies using LPBE 2006-2008

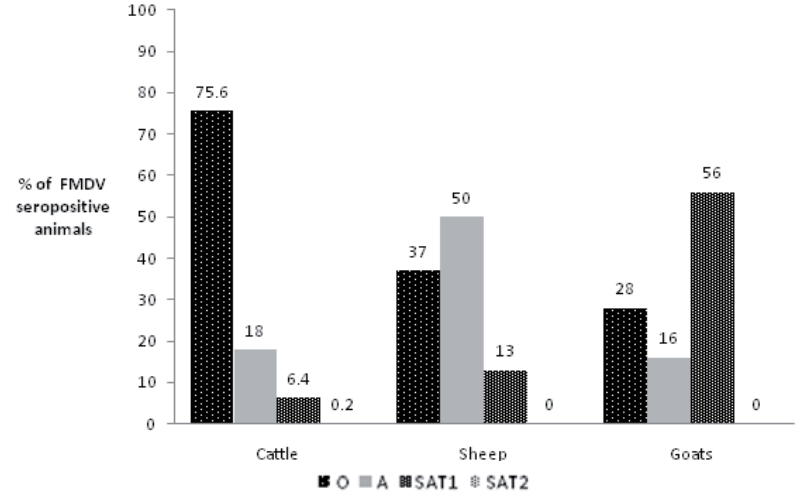

Fig. 3. Prevalence antibodies to FMDV in different animal species sera screened by ABU ELZEIN et al. (1987).

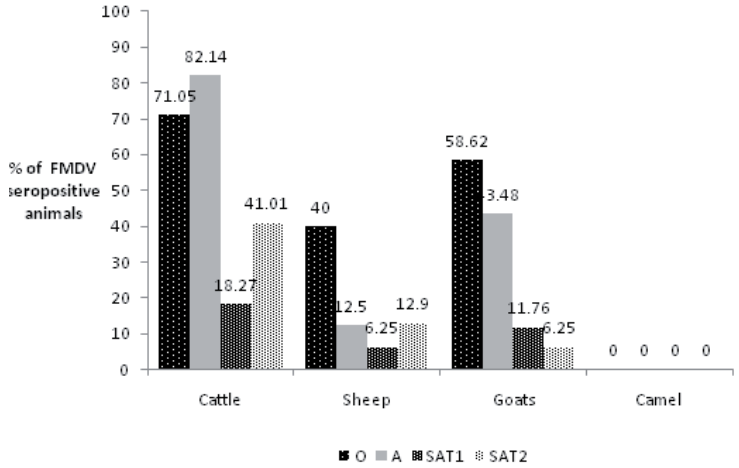

Fig. 4a. Gezira state results

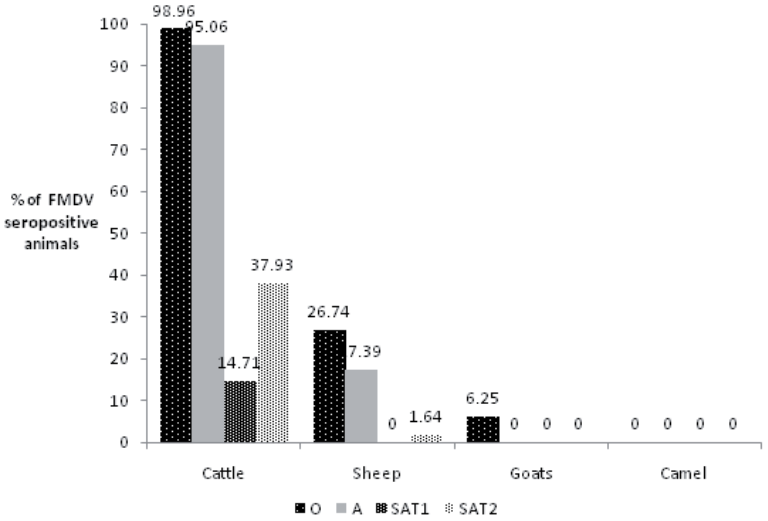

Fig. 4b. Northern Kordofan state results

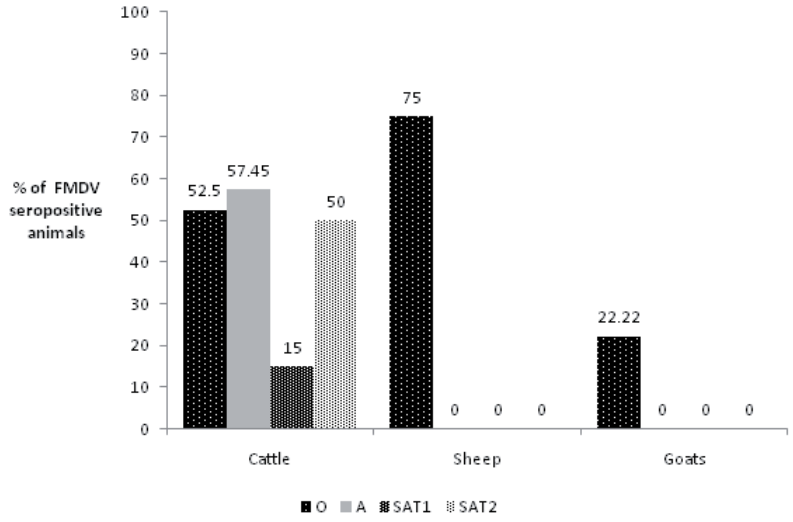

Fig. 4c. White Nile State results

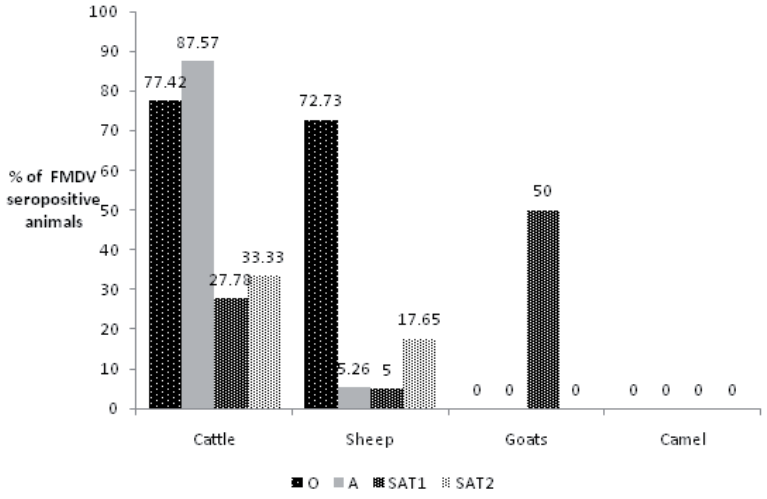

Fig. 4d. Southern Kordofan State results

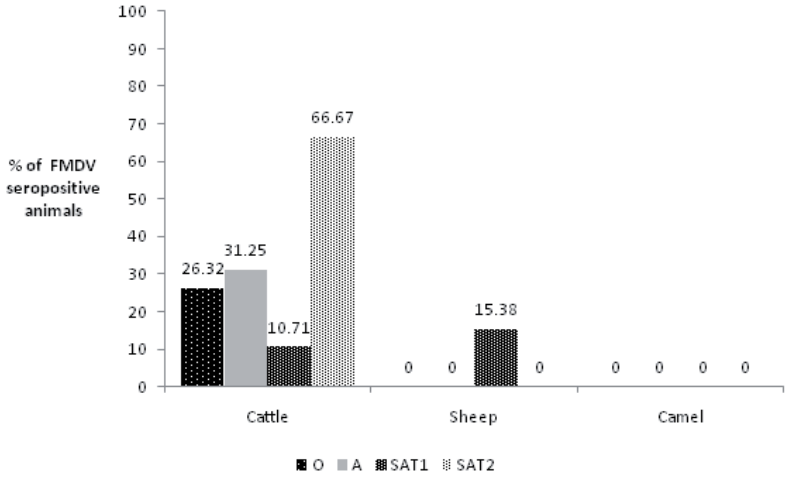

Fig. 4e. River Nile State results

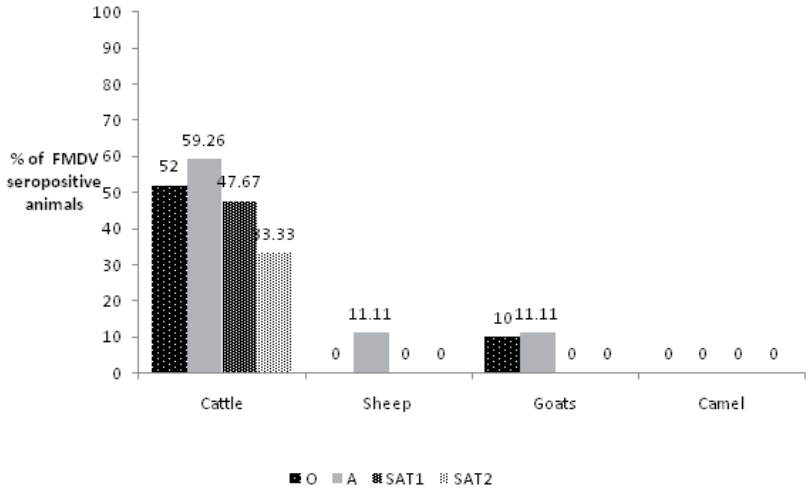

Fig. 4f. Gedarif State results

Figs. 4. Results of screened sera of different animal species for FMDV antibodies using LPBE in some states in the Sudan between 2006-2008

The husbandry systems practiced in the investigated herds were either extensive (59%) or semi-intensive, with free animal movement (41%). The questionnaire data showed that FMD clinical signs were observed only in cattle; and caused mild or no clinical signs in small ruminants, especially those intermingling with cattle. It was predominantly encountered in the cold, dry season (November to March). The morbidity rate may approach 100%, especially in cross-bred cattle. According to the questionnaire, 13.6% of herdsmen mentioned that the morbidity rate reached 90-100%, 45.5% noted 80-90%, 27.3% noted 70-80% and 13.6% noted less than 50% morbidity rate; while the mortality rate was low and only occurred in young animals. Losses were largely due to the death of newborn and suckling calves, loss of weight and milk production, and a decrease in draft power and infertility.

The data revealed that the currently applied control policy comprises the restriction of animal movement and quarantine. To reduce the effects of the disease, herdsmen add the powder obtained from the ground seed pods of *Acacia* trees to the drinking water of infected animals, to cure mouth ulcers. Many species of Acacia trees are found in Sudan, such as *Acacia nilotica* and *Acacia seyal* (AREF et al., 2003), and their seed pods and bark are known to contain a high concentration of tannic acid (tannins), as well as alkaloids and flavanoids (SAINI et al., 2008). Herdsmen also apply glycerine and antibiotics to protect infected animals from secondary bacterial infection.

Serology. The overall percentages of positive sera for FMDV in the four tested animal species were 79.24% in cattle, 22.95% in sheep, 28.57% in goats and no positive serum was observed in the camel sera tested (Table 1). Antibodies to the four FMDV serotypes used in the study were observed in the animal sera from all the investigated states; with the highest prevalence in cattle (Table 2).The results obtained by LPB ELISA showed that serotype A (78.1%) was the most prevalent in cattle, followed by serotype O (69.4%), SAT 2 (44%) and SAT 1 (20.2%). In sheep, serotype O (27.5%) was the most prevalent, followed by SAT 2 (9.1%), A (8.7%), and SAT 1 (5.1%). In goats, serotype O (27.5%) was the most prevalent, followed by A (15.9%), SAT 1 (8.5%) and SAT 2 (2.4%) (Table 2, Fig. 2).

FMD virus isolation and serotyping. From the eighteen probang samples and three swabs inoculated in BTY cell culture, seventeen samples caused cytopathogenic effects (CPE) and progressive changes in cell culture within 24-72 hours following inoculation. Eighteen cell culture harvests were re-inoculated in bovine kidney (BK) cell culture; ten samples produced CPE in BK cell culture (Table 3).

A 10% suspension of collected epithelium samples from infected cows during FMD outbreaks was inoculated in BK cell culture and a progressive CPE started within two to three hours following inoculation. After 24 hours, the monolayer was completely destroyed (Table 3).

Identification of virus isolates by antigen detection ELISA. All probang samples of derived cell culture material tested by the antigen detection ELISA showed an optical density of less than 0.1 and were considered negative. A total of 18 probang samples and three fluids tested at the FAO-OIE World Reference Laboratory (WRL) for FMD at Pirbright, United Kingdom (UK) also proved to be negative.

A total of 3 epithelium samples from Gezira state (Al-Kiraiba) were positive for serotype SAT-2, whereas 1 out of 4 epithelium samples from the White Nile state (Jabal Biyout) was positive for serotype O. Epithelium samples from Alkonoz and Omshatain were negative for FMDV.

Discussion

In studying the current status of FMD in the Sudan, it is obvious that FMD is still endemic in the country. It occurs mostly in the cold, dry season. The extensive livestock husbandry systems adopted in the Sudan seems to favor conditions for the spread of FMD virus. Cattle reared under nomadic conditions in the Sudan use their feet to wander around for grazing, which may extend for many kilometers and use their tongues while eating grass, but when these functions are impaired by FMD lesions in the feet and mouth, they become recumbent and mostly suffer from starvation (ABU ELZEIN, personal communication, 2008). These observations add a further dimension to the economic significance of FMD in the Sudan and clearly refute the notion in enzootic areas that FMD is not a particularly serious disease and its relevance is only to international trade. DOEL (1999) observed that FMD might have devastating effects on animals and herdsmen, regardless of the animal population.

Most data in the questionnaires returned were similar, but it was recognized that the responses of herdsmen to the questionnaire did not take into account the economic effect of the disease on their animals, as they do not pay great attention to this, or do not record it.

It was realized that the application of tannic acid and glycerine by herdsmen reduces significantly secondary bacterial infections as a consequence of FMD lesions in the mouth or feet of infected cattle. It was found that *Acacia nilotica* has potential antibacterial and antifungal activities (SAINI et al., 2008). WAKASA et al. (1998) showed that tannic acid has an antibacterial effect. It has also been mentioned that glycerine acts as an antibacterial agent to inhibit the growth and toxins of potentially pathogenic bacteria associated with wounds (SCHLIEVERT et al., 1992). Although, no research on the effects of tannic acid on FMDV has been carried out, an early study by SABIN et al. (1936) showed that an instillation of tannic acid applied intranasally in monkeys and mice, by acting on the mucosa or nasal tract, prevents nasal route infection with poliomyelitis virus and

equine encephalomyelitis virus, respectively. Poliomyelitis virus belongs to the same *Picornaviridae* family as FMDV, which may indicate the same manner of tannic acid reducing infection or preventing FMDV infection.

In the present study, besides Gezira, Northern Kordofan and River Nile States, previously screened by ABU ELZEIN et al. (1987), another four states were included in the recent study, i.e. Southern Kordofan, White Nile, Gedarif and El-Shymalyah states. Twenty years after the last study on FMD in the Sudan, and without implementing any vaccination or eradication programmes, the prevalence of antibodies to FMDV serotypes has changed in both magnitude and order. In cattle, the prevalence of antibodies to serotype A surpassed that of serotype O and the prevalence of antibodies to serotype SAT 2 surpassed that of serotype SAT 1**.** In this study, in cattle, antibodies to serotype A and O showed a prevalence rate of 78.1% and 69.4%, compared to 18% and 75.6% respectively in the previous study (ABU ELZEIN et al., 1987) (Fig. 3). Antibodies to serotype SAT 2 and SAT 1 showed a prevalence rate of 44% and 20.2%, compared to 0.2% and 6.4% respectively in the previous study (ABU ELZEIN et al., 1987). These results coincided with the results of the recent serosurveilance of cattle species in Khartoum state (RAOUF et al., 2008). In sheep and goats, the prevalence rate had also changed; serotype O was found to be the most prevalent serotype in sheep and goats (27.5% each) instead of serotype A in sheep and SAT 1 in goats as in previous reports (ABU ELZEIN et al., 1987), moreover, antibodies to SAT 2 were detected for the first time in sheep and goat sera from the states investigated - 8.99% and 2.38% respectively. Similar results were observed in recently surveyed sheep and goat sera in Khartoum state (HABIELA et al., unpublished data). Similar to previously published data (ABU ELZEIN et al., 1987), the prevalence of antibodies to FMDV in sheep and goat sera was much lower than those detected in cattle sera. This could be due to the fact that some of the flocks of sheep and goats screened graze without intermingling with cattle, as in some parts of Northern Kordofan, River Nile and Gedarif states (Fig. 4).

The high prevalence rates of FMDV serotypes A, O and SAT 2 antibodies detected in cattle species were consistent with the isolation of serotype O and SAT2 in this study and with the recent reports of FMDV isolates of serotype A and SAT 2 from the Sudan by Pirbright Laboratory, UK (ANONYM., 2007) .

In the Sudan, camels frequently browse in contact with other ruminants under free range conditions and at watering points. Screening of camel sera by LPB ELISA, which was used for the first time in the Sudan, revealed that no antibodies to any of the four FMDV serotypes used in the present study were detected. This result is in agreement with the findings of ABU ELZEIN et al. (1984) who reported that Sudanese camels were seronegative to FMDV antibodies. Moreover, these findings are consistent with recent reports showing that dromedary camel are not susceptible to FMDV and do not show

a detectable serological response to it, even under experimental conditions (WERNERY and KAADEN 2004; LARSKA et al., 2008). Though antibodies to FMDV serotype O were detected in experimentally infected dromedary camel, using a high infection dose of FMDV, 10 to 14 days after infection, the camel remained negative for infectious virus (ALEXANDERSEN et al., 2008). It was suggested that dromedary camel are not susceptible to FMDV and do not get infected naturally and when they do react with antibodies, it is at a level that can only be picked up by sensitive techniques and which last for only a very short time following FMD virus exposure (ALEXANDERSEN personal contact, 2009).

Theoretically, FMDV carrier animals might harbor the infectious virus in their esopharyngeal region (SUTMOLLER et al., 2003; ALEXANDERSEN et al., 2002), in which case the risk of transmitting the virus to other susceptible animals cannot be ruled out. Collection of OP samples from previously infected animals revealed that no FMDV was recovered or detected. In spite of this, 81% of the probang samples caused CPE in BTY cell culture and 56% of the re-inoculated probang samples in BK caused CPE. Normally, it is difficult to isolate FMDV from probang samples, so most probably another virus or toxicity of the samples caused this CPE.

SAT 2 is the serotype most recently introduced in the Sudan, in the 1970's (ABU ELZEIN, 1979); this serotype is often associated with outbreaks in sub-Saharan Africa (BASTOS et al., 2003). It shows a wide range of spread, as can be deduced from the results of our recent serosurvey (RAOUF et al., 2008) and SAT 2 isolates from Sudan by WRL for FMD (ANONYM., 2007). Serotype SAT2 antibodies were detected in all the states investigated, and this virus serotype was isolated in one outbreak during the course of this study.

In conclusion, Sudan is the largest country on the African continent, covering 2.5 million square kilometers, with different ecosystems and a massive diversity of animal species. Our results indicated that FMD was detected in all the seven states investigated, reflecting the contagiousness of the disease. Since the last serosurveillance two decades ago, the antibody prevalence of the virus serotypes has changed. For instance, the SAT 2 serotype has spread and was involved in one of the recent FMD outbreaks in the country; this coincided with the results obtained from the Khartoum state survey (RAOUF et al., 2008).

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SAŽETAK

Radi pružanja informacija o sadašnjem stanju slinavke i šapa u Sudanu provedena su serološka istraživanja te je prikazana njezina pojavnost. Svježe prikupljeni podatci o pojavi slinavke i šapa u Sudanu pokazali su da ona predstavlja veliku prepreku životinjskoj proizvodnji u toj zemlji. U ovaca i koza javlja se kao blaga bolest i ne predstavlja veliku prijetnju, dok se u goveda javlja s očitim kliničkim znakovima, a uzrokovana je serotipovima

O i SAT 2. Bolest se javlja sezonski u hladnoj i suhoj sezoni, a promet životinjama ima glavnu ulogu u širenju virusa. Ukupno je bilo prikupljeno 1069 uzoraka seruma goveda, ovaca, koza i deva podrijetlom iz sedam država u Sudanu radi pretrage na prisutnost protutijela za virus slinavke i šapa. Blokirajućim imunonenzimnim testom dokazana su protutijela za četiri serotipa virusa: O, A, SAT 1 i SAT 2. Protutijela za virus slinavke i šapa nisu bila dokazana u uzorcima seruma deva. Rezultati se razlikuju od ranijih izvješća s obzirom na prevalenciju specifičnih protutijela u različitih vrsta. Npr., specifična protutijela za serotip A dokazana su u 78,13% goveda, za serotip O u 69,39%, serotip SAT 2 u 44% te serotip SAT 1 u 20,2% goveda. U radu je prikazano sadašnje epizootiološko stanje slinavke i šapa u nekim dijelovima Sudana.

Ključne riječi: slinavka i šap, epizootiologija, Sudan