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# The prevalence of antibodies against foot-and-mouth disease in various species of Sudanese livestock following natural infection

ABU ELZEIN (E. M. E.), NEWMAN (B. J.), CROWTHER (J. R.), BARNETT (I. T. R.), McGRANE (J. J.). Fréquence des anticorps contre le virus de la fièvre aphteuse chez plusieurs espèces de bétail soudanais après une infection naturelle. *Rev. Elev. Méd. vét. Pays trop.*, 1987, **40** (1) : 7-12.

**Au Soudan, la fréquence des anticorps contre le virus de la fièvre aphteuse a été déterminée sur 1 611 sérums recueillis en 1979 et 1980 sur des bovins, des moutons et des chèvres, par les techniques ELISA, DID et de séroneutralisation (SN). Le test ELISA s'est révélé plus sensible que les autres. Les anticorps contre l'antigène associé VIA, indicateur d'infection par le virus aphteux (FMD), furent décelés chez 53 p. 100 des bovins, 2 p. 100 des moutons et 4 p. 100 des chèvres examinés. Les anticorps contre le type O prédominent dans 47 p. 100 du total des sérums examinés. Les anticorps contre les types A et SAT 1 sont décelés respectivement chez 28 et 25 p. 100 des animaux examinés. Ceux contre le type SAT 2 ne sont signalés que chez les bovins et une seule fois. La fréquence la plus élevée d'anticorps du type O est observée chez les bovins, tandis que celle du type SAT 1 a été décelée chez les chèvres et celle du type A chez les moutons.** *Mots clés* : Bovin - Ovin - Caprin - Fièvre aphteuse - Anticorps - Technique immunologique - Soudan.

## INTRODUCTION

Sudan has one of the largest domestic animal population in Africa with approximately twenty million cattle and thirthy-three million small ruminants (FAO Animal Health Yearbook 1985). However, the productivity of its livestock has been greatly reduced due to various endemic diseases, including foot-and-mouth disease (FMD). Although this disease is known to have been endemic in the Sudan for many years, no attempt has been made to study the extent of infection in animals. Four FMD virus serotypes have been recorded (O, A, SAT 1 and SAT 2), all isolated from cattle. There are no confirmed reports of overt disease in sheep, goats, camels or wild animals.

The present study was made to determine the prevalence of antibodies against FMD virus in cattle, sheep and goats, to find out whether virus serotypes could have been maintained in the country. Three methods of antibody measurement were used : serum neutralisation tests and ELISA to determine type specific antibodies ; and the DID test to detect antibodies to the virus infection associated antigen (VIA) (3, 5, 7, 8, 10) to indicate previous infection.

## MATERIALS AND METHODS

The solid phase microplates, buffers and washing procedures were as described by ABU ELZEIN and CROWTHER (1).

### Viruses and reference sera

FMD viruses type $O_1$/UK/1860/1967, $A_{22}$ Mam, C Noville, SAT 1 Sudan 9/69 and SAT 2 Sudan 9/77 were grown in monolayer cultures of BHK 21 cells and purified by sucrose density gradient centrifugation, as described by BROWN and CARTWRIGHT (2), using 1 p. 100 Sarkosyl instead of deoxycholate. Purified virus was stored at - 70 °C in siliconised glass vials. Reference post-infection bovine antisera against FMD virus types O, A, SAT 1 and SAT 2 were obtained from the department of Vaccine Research at this Institute.

### Serum samples

Sera were obtained from apparently healthy cattle, sheep and goats from slaughterhouses at Kassala, Omdurman, Sennar, El Obeid, Atbara, Nyala and Jebel Moya, from production units at Roseiris and Jongoli and from dairy farms at Shambat, Atbara, Nishishiba and Um Benein (Fig. 1). The animals were at least one year old. The sera were inactivated at 56 °C for 30 min. before being tested (and stored at - 20 °C).

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*Fig. 1 : Localities from which samples were collected.*

## Serum neutralisation tests

Serum neutralisation tests (SNT) were carried out on cell monolayers in microtitre plates, using the method described by GOLDING, HEDGER and TALBOT (6).

## Double Immunodiffusion (VIA test)

The tests were carried out in 85 mm disposable plastic Petri dishes containing 16 ml of 1 p. 100 Oxoid agar No. 2 in 0.02M tris buffer with the addition of 0.15 NaCl and 0.1 p. 100 sodium azide. The test pattern consisted of a 4 mm well surrounded at a distance of 4 mm by 6 peripheral wells, each 5 mm in diameter. The center well was filled with antigen previously titrated and 2 opposite peripheral wells with a positive reference serum. The remaining wells were filled with test sera such that each test serum was immediately adjacent to the positive reference serum. Plates were then incubated at room temperature in a humid chamber. Plates were examined after 24 hours and were read finally after a week.

Results were recorded according to the criteria of McVICAR and SUTMOLLER (7), where the appearance of a precipitin line was taken as positive if a line of identity with the positive control of serum was observed. A serum was recorded as a weak positive with the end of the positive control line veered away from the test serum well.

The antigen was prepared from FMD virus type $O_1$ BFS in BHK 21 cells, according to the method previously described by COWAN and GRAVES (3).

## ELISA

Horse radish peroxidase conjugated rabbit anti-bovine, rabbit anti-sheep and rabbit anti-goat IgG were obtained from Nordic Laboratories, U.K. Stock conjugates were stored at - 70 °C and those used frequently were stored at + 4 °C. The working dilution of each conjugate was determined as described earlier (11). A solution of orthophenylene diamine (OPD) was prepared by adding 0.4 gm of OPD to a litre of distilled water containing 5.10 gm citric acid and 9.14 gm $Na_2HPO_4$. Before the solution was used, 40 µl of $H_2O_2$ (30 p.100 v/v) were added per 100 ml. The substrate reaction was stopped by adding 50 µl of an IM solution of $H_2SO_4$.

The indirect ELISA procedure, described earlier (1), was followed, except that 0.1 ml/well of each reagent in the test was used and that sera were incubated overnight at 4 °C. Preliminary experiments involving the testing of full scale dilution series of known positive and negative sera, demonstrated that dilutions of serum of 1/100 or 1/200 were suitable for « spot-testing » of samples in the ELISA. At these dilutions , non-specific serum proteins binding for negative sera was reduced to plate background OD, while post-infected animal sera showed significant colour development. The upper limits of negativity (OD in ELISA) for each species were determined after examination of sero-negative populations taken from British Stock and from VNT negative, VIA antigen negative animals from the Sudan. The distribution of OD readings from such sera diluted to 1/100 of 1/200 was examined. Positivity of antisera against virus was ascribed to test sera which gave values $\geqslant$ 2.5 standard deviations above the negative population means found. In most of the tests a negative serum characterising the mean was used as a control, and the overall negative population standard deviation was used to calculate the upper limit of negativity above the control serum OD found. This exercise was performed for each of the FMD antigens used in the study, namely types 0, A, C, SAT 1 and SAT 2. Sera from the different species following infection with FMD viruses were included as positive control samples for each type. All sera were tested against FMD virus types O, A, SAT 1 and selected sera were tested against types C and SAT 2.

## RESULTS

The relationship between antibodies detected by ELISA and the VIA immunodiffusion test for all the sera examined is shown in Fig. 2. Both tests detected a high percentage of antibody-positive cattle. In sheep and goats, low numbers of animals showed antibody against VIA ; however, higher numbers of ELISA-positive animals were demonstrated.

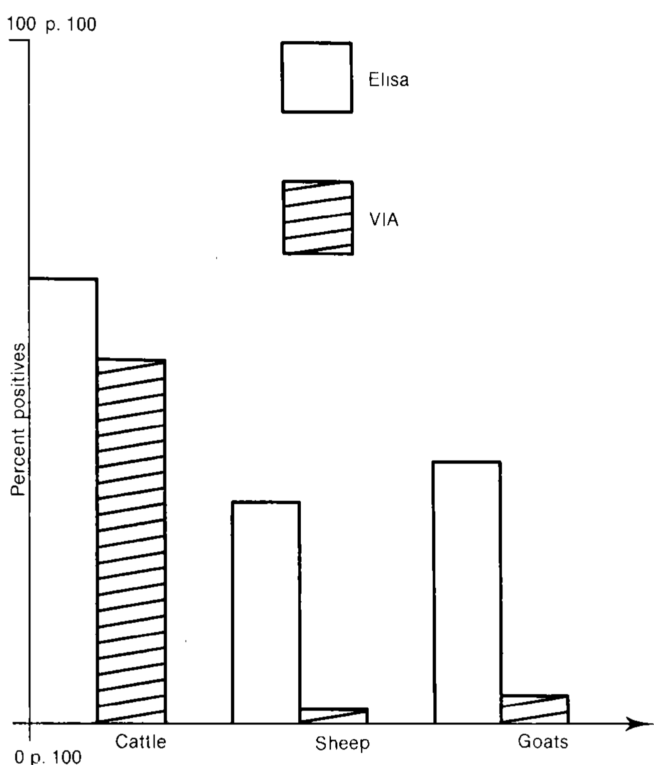


*Fig. 2 : Comparison of ELISA and DID (VIA) tests.*

The type-specific antibody ELISA results for cattle sera collected in different locations are shown in Fig. 3. Antibodies against type O and type A were the most widespread, followed by type SAT 1, with antibodies against type SAT 2 being detected from one area only. Above 95 p. 100 of the sera from the cattle in Nishishiba and Omdurman were positive for type O and in Sennar for type A. Thirty per cent of cattle were positive to SAT 1 in Shambat and Nyala. SAT 2 antibodies were detected only in Jebel Moya district in 20 p. 100 of the cattle tested. No antibodies against type C were detected in cattle.

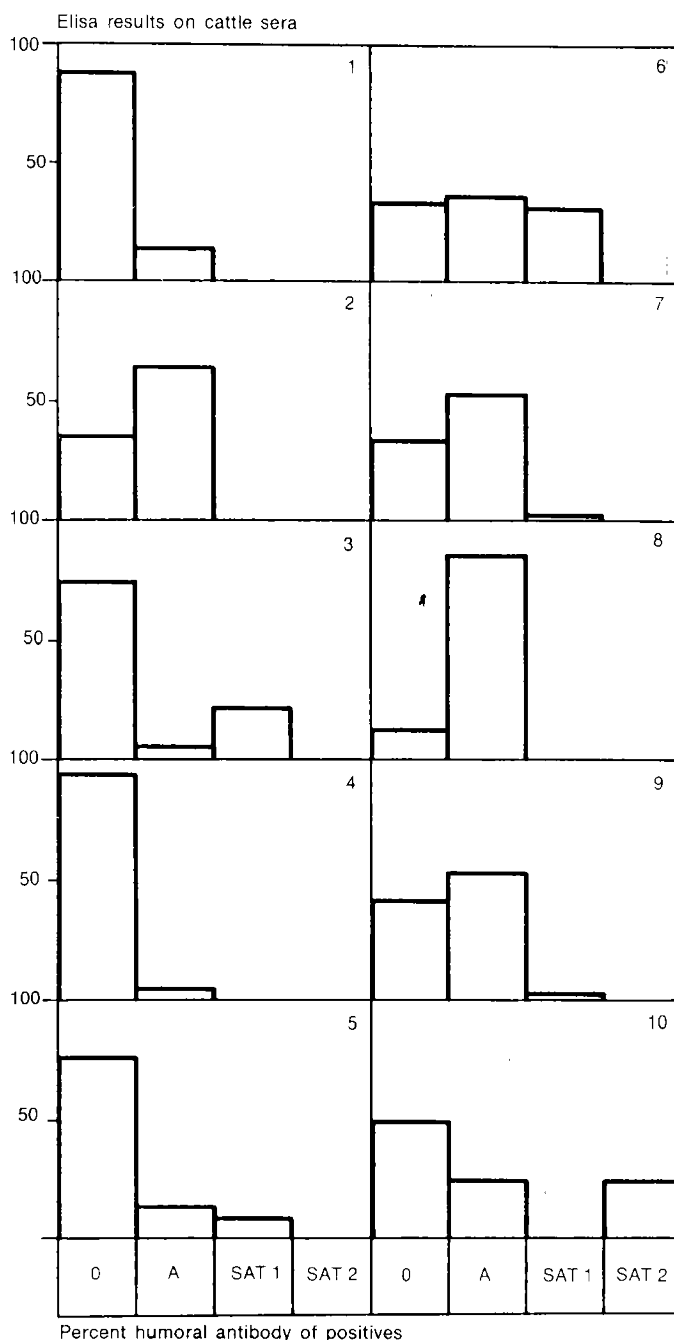


*Fig. 3 : 1 = Nishishiba, 2 = Atbara, 3 = Nyala, 4 = Omdurman, 5 = El Obeid, 6 = Shambat, 7 = Um Benein, 8 = Sennar, 9 = Jongoli, 10 = Jebel Moya.*

In sheep, antibodies to type O and A were predominant (Fig. 4) ; over 95 p. 100 of sheep in El Obeid were positive for type O and over 90 p. 100 in Atbara for type A. Antibodies to SAT 1 were detected only in

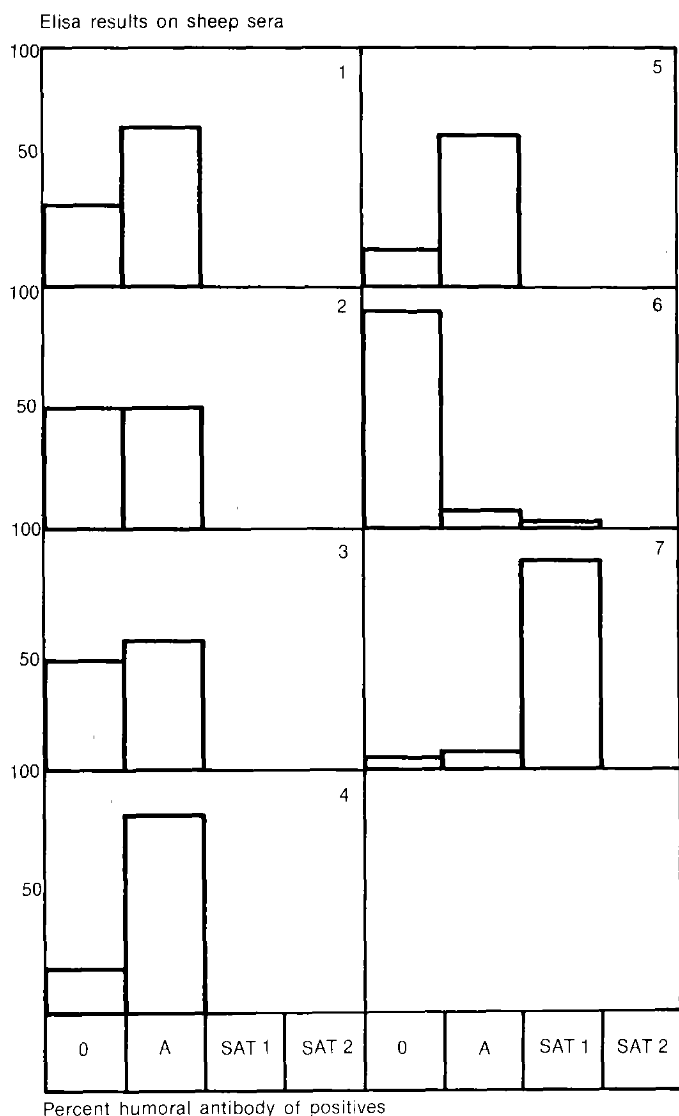


*Fig. 4 : 1 = Roseiris, 2 = Jongoli, 3 = Kassala, 4 = Atbara, 5 = Sennar, 6 = El Obeid, 7 = Omdurman.*

Omdurman (95 p. 100), Sennar (25 p. 100) and El Obeid (3 p. 100). No antibodies were detected against type SAT 2 or type C in sheep.

In goats, type O antibodies were detected in Jongoli (100 p. 100), Kassala (93 p. 100) and El Obeid (25 p. 100). Antibodies against SAT 1 were detected in Nyala (100 p. 100) and El Obeid (90 p.100). No SAT 2 or C antibodies were detected in goats.

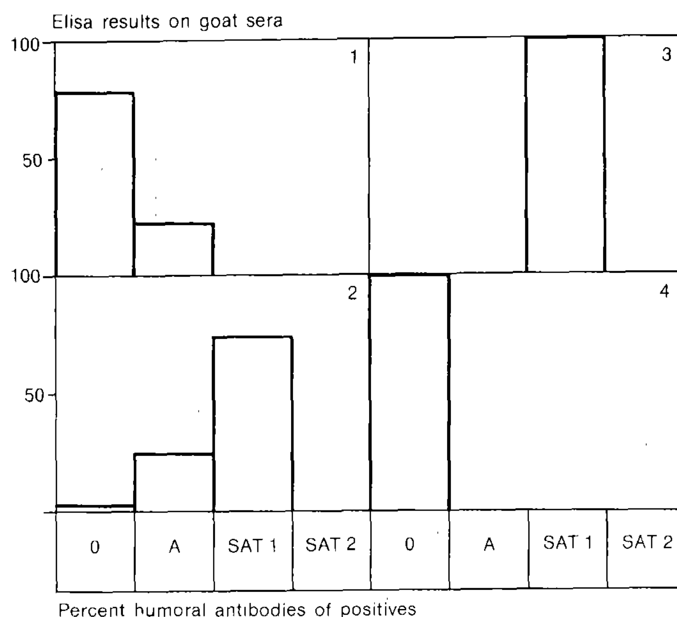


*Fig. 5 : 1 = Kassala, 2 = El Obeid, 3 = Nyala, 4 = Jongoli.*

The relationship between ELISA, SN test and VIA on cattle, sheep and goats sera (Fig. 6) indicated that ELISA detected more positives than both SN and VIA tests. The percentage of positives in cattle was high. A summary of the results is given in Table I.

| | No sera tested | Positive results | | |
|---|---|---|---|---|
| | | SNT | VIA | Elisa |
| Cattle | 100 | 73 | 70 | 82 |
| Sheep | 286 | 32 | 9 | 110 |
| Goats | 138 | 28 | 5 | 64 |

*Fig. 6 : Comparison of ELISA, DID-VIA and SNT on some selected sera.*

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*TABLE I Overall ELISA results on sera examined.*

| Species | No positive | p. 100 positives with antibodies against foot-and-mouth disease type O | | | | |
|---|---|---|---|---|---|---|
| | | O | A | SAT 1 | SAT 2 | C |
| Cattle | 599 | 75.6 | 18 | 6.4 | 0.2 | – |
| Sheep | 159 | 37.0 | 50 | 13.0 | – | – |
| Goats | 76 | 28.0 | 16 | 56 | – | – |
| Total | 834 | 47.0 | 28 | 24.9 | 0.1 | – |

– = *no antibodies detected.*

## DISCUSSION

Earlier studies have shown that ELISA could be used to detect FMD virus antibodies (1). The test described in this paper was found to be reliable, economical and easy to perform, especially when handling large numbers of sera.

The detection of VIA antibodies in animals in the absence of vaccination suggests that the animals have undergone infection with FMD virus (7). Since all the animals were aged a year or older, the possibility that the antibodies came through colostrum from infected dams is not feasible (9).

The overall results indicate that antibodies against type O are the most prevalent, followed by type A and type SAT 1. SAT 2 antibodies were found in samples from only one site. No antibodies against type C were detected. This order is the same as that obtained from virus isolation tests on samples received at AVRI, Pirbright from Sudan during 1952-1981 (WRL records, Pirbright). This shows that 55 p. 100 of the positively typed samples were of type O, 20 p. 100 of type A, 20 p. 100 of type SAT 1 and 5 p. 100 of type SAT 2 and that no other FMD virus serotype was isolated from the country.

Types O, A and SAT 1 would appear to be endemic in Sudan, but the SAT 2 outbreak in 1977 could have been introduced from outside the country. However, further studies on the carrier state and on wild animals should be carried out.

## CONCLUSION

It is difficult from the relatively small numbers of animals sampled to indicate the geographical distribution of the virus. In addition, the source of the animals at the slaughterhouses is uncertain. However, there does appear to be a difference in distribution and prevalence of antibodies between cattle and small ruminants in some areas. There may be independent circulation in these species depending on the husbandry of the area.

The presence of antibodies and the lack of clinical reports suggest that sheep and goats may undergo inapparent or subclinical infection with foot-and-mouth disease. Further studies are needed to see if they act as virus carriers. As animals from southern Sudan were not examined in this study, we believe that a similar survey on animals from that region is important for the understanding of the epidemiology of the disease in the whole country. However, it has been indicated (4) that FMD is endemic in southern Sudan. The role of the camels and wild animals in the epidemiology of the disease in the country also needs to be investigated.

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**ABU ELZEIN (E. M. E.), NEWMAN (B. J.), CROWTHER (J. R.), BARNETT (I. T. R.), McGRANE (J. J.).** Prevalence of antibodies to foot-and-mouth disease virus in various species of Sudanese livestock following natural infection. *Rev. Elev. Méd. vét. Pays trop.*, 1987, **40** (1) : 7-12.

**ABU ELZEIN (E. M. E.), NEWMAN (B. J.), CROWTHER (J. R.), BARNETT (I. T. R.), McGRANE (J. J.).** Frecuencia de los anticuerpos contra el virus de la fiebre aftosa en rumiantes domésticos sudaneses después de una infección natural. *Rev. Elev. Méd. vét. Pays trop.*, 1987, **40** (1) : 7-12.

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**The prevalence of antibodies to foot-and-mouth disease (FMD) virus was determined in 1 611 sera collected in 1979 and 1980 from cattle, sheep and goats in Sudan, using the enzyme-linked immunosorbent assay (ELISA) and serum neutralisation (SN) tests. The double immunodiffusion (DID) test was used to detect antibodies against the virus infection associated antigen (VIA). Antibodies against VIA antigen, indicating infection with FMD virus was detected in 53 p. 100 of the cattle, 2 p. 100 of the sheep and 4 p. 100 of the goats tested. Antibodies to type O were predominant and were tested in 47 p. 100 of all animals tested. Antibodies to types A and SAT 1 were detected in 28 p. 100 and 25 p. 100, respectively, of all animals examined. Type SAT 2 antibodies were detected only in cattle in one location. The highest incidence of type O antibodies was demonstrated in cattle, whereas that of type SAT 1 was detected in goats and that of type A in sheep.** *Key words* : Cattle - Sheep - Goat - Foot-and-mouth disease - Antibody - Immunological test - Sudan.

**En Sudán, se determinó la frecuencia de los anticuerpos contra el virus de la fiebre aftosa en 1 611 sueros, colectados en 1979 y 1980 a partir de bovinos, ovinos y cabras, por las técnicas ELISA, de doble inmunodifusión (DID) y de seroneutralización (SN). La prueba ELISA fue más sensible que los otras. Se evidenciaron los anticuerpos contra el antigeno asociado VIA, indicator de infección por el virus aftoso, en 53 p. 100 de los bovinos, 2 p. 100 de los ovinos y 4 p. 100 de las cabras examinados. Los anticuerpos contra el tipo O predominan en 47 p. 100 del total de los sueros examinados. Se observan los anticuerpos contra los tipos A y SAT 1 respectivamente en 28 p. 100 y 25 p. 100 de los animales examinados. No se señalan los contra el tipo SAT 2 más que en los bovinos y una sola vez. Se nota la frecuencia más elevada de anticuerpos del tipo O en los bovinos mientras que la del tipo SAT 1 se encuentra en las cabras y la del tipo A en los ovinos.** *Palabras claves* : Bovino - Ovino - Cabra - Fiebre aftosa - Anticuerpo - Técnicas inmunologicas - Sudán.

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