

## Detection and isolation of group A rotavirus from camel calves in Sudan

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### ABSTRACT

A total of 332 fecal samples were collected from 245 diarrheic as well as 75 recovered and 12 healthy camel calves in four different areas in Sudan [north (River Nile), east (Gedarif), central to south (Sennar, Blue Nile) and west (Kordofan) States]. Using ELISA, 46 samples (13.9%) were found positive for group A rotavirus. Out of 46 ELISA positive calves, we found 35 diarrheic up to 3 months of age, 7 recovered 4-6 months of age and 4 clinically healthy 8-12 months of age. Using EM, 6 out of 22 ELISA positive samples showed the well defined wheel-like structure characteristic of rotavirus. The results indicate the significant role of rotavirus in the epidemiology of camel calf diarrhea in Sudan. Attempts to isolate the camel group A rotavirus in MA104 cell culture were carried out on 21 ELISA-positive samples with successful isolation of the virus in 18. The isolated viruses were identified by ELISA and EM. Cytopathic effects (CPE) were manifested as rounding, elongation, triangulation, vacuolation and granulation of cells while the cell sheet remains intact. The CPE appeared on days 3-5 on the first to the second passages after treatment of the inocula and the cell culture media with trypsin. To our knowledge this is the first report on the isolation of camel rotavirus in cell culture.

**Key words:** camel, group A rotavirus, isolation, Sudan

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### Introduction

Camels in Sudan are important animals with a population of about three million. High calf mortality is considered one of the major constraints to higher productivity in camels. Many factors contribute to calf mortality, among which calf diarrhea is important

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(SCHWARTZ, 1992). Thirty percent morbidity due to the disease was reported and mortality can reach 100% in untreated calves (SCHWARTZ and DIOLI, 1992).

Camel calf diarrhea affects about 33% of neonates (up to 3 months of age) causing 23% mortality in north-east Sudan (ABBAS et al., 1992). The detection of rotavirus antigens in diarrheic camel calf samples by ELISA and latex agglutination in eastern Sudan was reported previously (MOHAMED et al., 1998).

After much research work it was possible to isolate rotavirus in tissue cultures. Bovine rotavirus was successfully isolated after the addition of 10 µg/mL trypsin in the inocula and 5 µg/mL trypsin in media (BABIUK et al., 1977). A report describing that 1000 fold more bovine rotavirus is obtained when 5-10 µg/mL trypsin is added to the medium and allowed to remain throughout the growth cycle in primary calf kidneys and different pig, monkey and human cell lines was reviewed (ALMEIDA and HALL, 1978). The isolation of rotavirus from different species in MA104 cell was previously reported; bovine rotavirus (CASTRUCCI et al., 1983), equine rotavirus, (HOSHINO et al., 1983) canine rotavirus, (HOSHINO et al., 1982), lapine rotavirus, (CASTRUCCI et al., 1985) and ovine rotavirus (MAKABE et al., 1985).

The purpose of the present work is to determine the distribution of rotavirus in north, west, central in addition to eastern Sudan and to isolate the causative agent in cell culture in order to open the way to improve diagnosis and control of this economically important disease.

### **Materials and methods**

*Collection and preparation of fecal samples.* A total of 332 camel fecal samples from 245 diarrheic, 75 recovered and 12 healthy camel calves were collected, they were 28 from 20 pastoralists in River Nile State (North), 91 from 70 pastoralists in Gedarif State (East), 25 from 20 pastoralists in Sennar and Blue Nile States (Central to South) and 188 from 100 pastoralists in Kordofan States (West). Fecal samples were collected in sterilized plastic bags, stored at 4 °C and transported to the laboratory on ice. A ten percent suspension of fecal sample in phosphate buffered saline (PBS) was homogenized, centrifuged at 1000 x g for 15-20 minutes and the supernatant collected. Part of the supernatant was directly used for detection of rotavirus antigen by ELISA and the rest was layered on three times its volume of 30% sucrose solution and centrifuged at 100,000 X g for one hour in a Beckman L7-65 centrifuge using SW 41 Ti rotor. The virus pellet was re-suspended in 1 mL PBS and kept at -20 °C until used for examination by electron microscopy (EM) and cell culture inoculation. Prior to inoculation the virus suspension was incubated for 30-60 minutes with 10 µg/mL trypsin and 1000 I.U. penicillin, 1000 µg streptomycin, 2000 µg gentamycin and 250 µg nystatin per mL.

*Detection of rotavirus antigen by enzyme-linked immunosorbent assay (ELISA).* Sandwich ELISA kits for detection of group A rotavirus antigen (DAKO, Cambridgeshire, UK) were used. The test was performed according to the manufacturer's instructions. Briefly, 100 µL of each sample was put in a rotavirus specific rabbit polyclonal antibody labeled microtiter plate well, 100 µL of rotavirus specific rabbit polyclonal antibody conjugated to horse radish peroxidase was added to each well. After one hour incubation and washing 100 µL TMB substrate were added and results were read visually and photometrically at 450 nm.

*Electron microscopy (EM).* A total of 22 ELISA positive samples were examined by EM after staining by 3% phosphotungstic acid.

*Virus isolation.* A total of 21 group A rotavirus ELISA positive samples (5 from River Nile, 8 from Gedarif, 3 from Sennar and Blue Nile and 5 from Kordofan States) prepared as described above were used for inoculation. Foetal Rhesus monkey kidney cell line (Microbiological associate; MA 104) used for virus isolation was kindly supplied by the Virology Department, Faculty of Veterinary Medicine, Cairo University and also by the Virology Department, Onderstepoort Veterinary Laboratory, South Africa. Cells were grown in Glasgo minimum essential media (GMEM) with additives (tryptose phosphate broth, yeast extract, lact albumin hydrolysate) and antibiotics.

Each 25 cm<sup>3</sup> tissue culture flask of MA 104 cell line was inoculated with a volume of 0.2 mL of trypsin treated sample and incubated for 1-2 hours at 37 °C. The inoculum was then removed and the flask was washed three times, then GMEM media without serum, and containing 1 µg/mL trypsin was added. The flask was observed daily under an inverted microscope for the presence of cytopathic effect (CPE). Samples that did not show CPE after 15 days were subjected to 5 blind passages in new set of cells.

*Virus identification.* Samples showing 80% CPE were harvested and the harvests were tested for group A rotavirus using ELISA and EM as previously described.

## Results

*Detection of group A rotavirus antigen by ELISA.* A total of 332 fecal samples were tested for the detection of group A rotavirus antigen. Forty-six (13.9%) were found to be positive. Out of 46 positive samples, 24 (52.2%) were from Kordofan State, 12 (26.1%) from Gedarif, 6 (13%) from River Nile State and 4 (8.7%) from Sennar and Blue Nile States. The details of ELISA results are presented in Table 1.

*Electron microscopy (EM).* A total of 22 ELISA positives fecal samples were tested using EM. Six (5 from Gedarif, 1 from Sennar and Blue Nile states), out of 22 samples, 6 were found positive and showed the characteristic morphology of rotavirus of wheel-like appearance of rotavirus particles (Fig. 1).

Table 1. Detection of group A rotavirus antigen in camel calf fecal samples by ELISA

States	No. of samples tested		N° of positive calves	Age of positive calves (month)	Percentage of positive samples	Percentage of total positives
River Nile State	D	15	2	0-3	13.3%	21.4%
	R	5	1	9-18	20%	
	H	8	3	1 (0-3 ), 2 (4-6 )	37.5%	
Gedarif State	D	78	11	9 (0-3 ), 1 (4-6), 1 (6-9 )	14.1%	13.2%
	R	13	1	4-6	7.7%	
	H	0	0	0	0	
Sennar and Blue Nile	D	23	4	0-3	17.4%	16%
	R	2	0	0	0%	
	H	0	0	0	0	
Kordofan State	D	129	18	14 (0-3), 2 (4-6), 2 (9-18)	14%	12.8%
	R	55	5	4 (0-3 ), 1(4-6 )	9%	
	H	4	1	9-18	25%	
Total	332		46	35 (0-3 ), 7 (4-6 ), 1 (6-9 ), 3 (9-18 )		13.9%

D: Diarrheic, R: Recovered, H: Healthy

Table 2. Results of camel rotavirus isolation in MA104 cells

States	River Nile State	Gedarif State	Sennar and Blue Nile States	Kordofan State	Total
N° positive / N° of tested samples	5 / 5	6 / 8	3 / 3	4 / 5	18 / 21

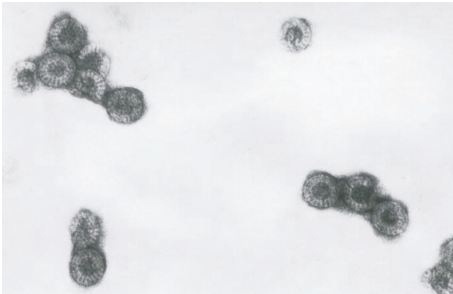


Fig. 1. Characteristic wheel like appearance of camel rotavirus detected by EM

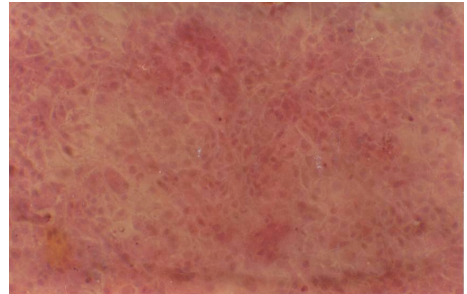


Fig. 2a. Normal non infected MA104 cells

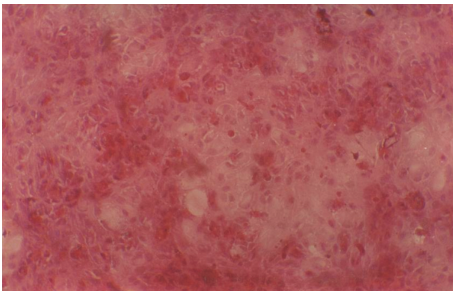


Fig. 2b. Cytopathic effect (CPE) of camel Group A rotavirus in MA104 cells

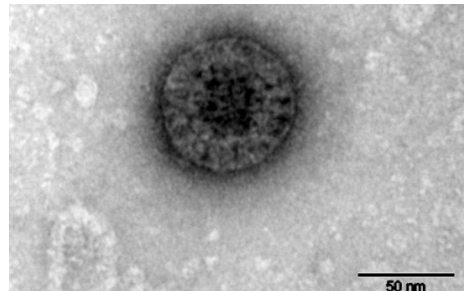


Fig. 3. Camel calf rotavirus appearance in tissue culture harvest detected by EM

*Virus isolation in cell culture.* The concentrated samples after ultracentrifugation were inoculated into MA104 cells for virus isolation. Out of 21 samples, 18 showed a progressive CPE and 3 were negative. The details of the results are shown in Table 2.

Ten group A ELISA positives showed round floating cells in the first passage on day 3 to 5 post inoculation (PI), which reached 80-90% on day 6 PI. In the first passage a progressive changes in the cells, which included very large amount of round floating cells, giant cells, elongated cells, refractive, ring shaped cells, multinucleated cells, triangular cells, vacuolated cells and granulated cells were observed. From passage 12-17, the CPE appeared on day 1 and reached 80% on day 3 PI. In all propagated isolates it was noticed that the cell sheet never detached completely and there was no difference in the CPE

detected by all tested samples. The normal MA104 cells and the CPE of camel rotavirus are shown in Figures 2a and 2b.

*Identification of virus isolates.* Rotavirus antigen was detected in tissue culture harvests of the 18 isolates by ELISA and EM (Fig. 3).

## Discussion

The detection of group A rotavirus in 11 out of 117 (9.4%) samples from 1-3 month age diarrheic camel calves in eastern Sudan using ELISA was reported (MOHAMED et al., 1998). The present study revealed the detection of group A rotavirus in different parts of the Sudan. Beside Gedarif state in which rotavirus was previously detected (MOHAMED et al., 1998), group A rotavirus was detected in Sennar, Blue Nile, River Nile and Kordofan States with more or less the same prevalence rate and most positive samples were from diarrheic calves indicating the wide spread of rotavirus infection in the country. Rotavirus antigen was detected in diarrheic, recovered as well as clinically healthy animals which indicate that samples collected from animals with different clinical status could be used for investigating rotavirus.

In this study EM was used to confirm ELISA results, the characteristic morphology of rotavirus was seen in 6 group A ELISA positive samples from Gedarif, Sennar and Blue Nile states.

The isolation of bovine rotavirus in monkey kidney cells was previously reported (BABIUK et al., 1977). The propagation of bovine rotavirus in MDBK cells was also reported (McNULTY et al., 1977). 1000 fold more bovine rotavirus is obtained in calf kidney; continuous monkey kidney (BSC-1, LLC-MK2), semi-continuous human fibroblast (MRC-5) and continuous pig kidney (IBRS-2) when 5-10 µg/mL trypsin is added in the media (ALMEIDA and HALL, 1978). Many authors described the increased titers of rotavirus in different cultures in the presence of trypsin. This was proved in bovine rotavirus (SCOTT et al., 1979; THEODORIDIS et al., 1979), chicken and turkey rotaviruses (McNULTY et al 1979), canine rotavirus (HOSHINO et al., 1982), equine rotavirus (HOSHINO et al., 1983), rabbit rotavirus (CASTRUCCI et al., 1985), and lamb rotavirus (MAKABE et al., 1985). The CPE observed in MA104, CV-1 and BSC-1 cells inoculated with simian rotavirus was described (ESTES et al., 1979). Distinct CPE of human, porcine, equine and bovine rotaviruses in MA104 cells were previously described (KUTSUZAWA et al., 1982; ALBERT et al., 1987).

In the present study the isolation of camel group A rotavirus in cell culture is described. Eighteen out of 21 group A rotavirus ELISA positive samples were successfully propagated in MA104 cells. In all propagated rotavirus isolates, the CPE observed were almost similar but varied in the time of appearance (ranging between days 3 to 5 PI). The CPE observed did not differ from that described in the previous reports for rotavirus

isolated from different species of animals. However, the only difference could be that the cell sheet was not observed to detach in the case of camel rotavirus.

This is the first report for the propagation of camel group A rotavirus in cell culture in Sudan. This success was made possible by the selection of highly ELISA positive samples (absorbance at 450 nm higher than 2.0) and by the method we used to concentrate the samples by ultracentrifugation. The successful growth of camel rotavirus in cell culture can aid in further research work to prepare specific reagents for testing camel rotavirus, either serologically or on the molecular biology level as well as developing a relevant vaccine to control the disease.

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**ALI, Y. H., A. I. KHALAFALLA, M. E. GAFFAR, I. PEENZE, A. D. STEELE: Dokaz i izdvajanje skupine A rotavirusa iz devine teladi u Sudanu. Vet. arhiv 78, 477-485, 2008.**

**SAŽETAK**

Prikupljena su 332 uzorka fecesa od 245 devine teladi s proljevom, 75 teladi oporavljene od proljeva i 12 od zdrave devine teladi sa četiri različita područja u Sudanu: sjevernoga (područje rijeke Nila), istočnoga (područje Gedarif), središnjega i južnoga (područje Sennar, Plavi Nil) te zapadnoga područja (Kordofan). Pretragom imunoenzimnim testom 46 uzoraka (13,9%) bilo je pozitivno za skupinu A rotavirusa. Od tih 46 pozitivnih životinja imunoenzimnim testom, 35 s proljevom bilo je u dobi do tri mjeseca, sedam s preboljelim proljevom bilo je u dobi od četiri do šest mjeseci, a četiri životinje u dobi od osam do 12 mjeseci bile su klinički zdrave. Elektronskom mikroskopijom dokazane su čestice karakteristične za rotavirus u šest od 22 uzorka pozitivna pretragom imunoenzimnim testom. Rezultati ukazuju na znatnu ulogu rotavirusa u epizootologiji proljeva u devine mladunčadi u Sudanu. Skupina A rotavirusa izdvojena je na MA104 staničnoj kulturi iz 18 od 21 uzorka pozitivnoga pretragom imunoenzimnim testom. Izdvojeni virusi bili su identificirani imunoenzimnim testom i elektronskom mikroskopijom. Citopatski učinak očitovao se zaokruživanjem stanica, njihovim izduživanjem, triangulacijom, vakuolacijom i granulacijom, dok je stanični sloj ostao netaknut. Citopatski učinak javljao se za tri do pet dana u prvoj i drugoj pasaži nakon obradbe inokula i staničnog medija tripsinom. Ovo je prvo izvješće o izdvajanju rotavirusa deva u staničnoj kulturi.

**Cljučne riječi:** deva, skupina A rotavirusa, izdvajanje, Sudan

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