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Rotavirus-Associated Camel Calf Diarrhoea in Sudan

[1]Ali Y. H., [2]A. I. Khalafalla, [1]M. E. Gaffar, I. Peenze and A. D. Steele
[1]Central Veterinary Research Laboratory, Virology Department- P.O. Box 8067- Khartoum-Sudan
[2]Department of Microbiology, Faculty of Veterinary Medicine, University of Khartoum, Shambat, 13314, Khartoum North, Sudan; [3]MRC Diarrhoeal Pathogens Research Unit, Medunsa, Pretoria, South Africa

Abstract : The role of rotavirus in camel calf diarrhoea is studied. Faecal samples were collected from 245 diarrheic, 75 recovered and 12 clinically healthy camel calves at 4 different areas of Sudan (North, East, Central and West). The samples were collected during autumn, summer and winter seasons over 3 years period 2000-2002. All samples were tested for rotavirus antigen; using ELISA Kits, 46 (13.9%) were positive for Group A rotavirus. Latex agglutination test was applied on 144 samples, 9 positive (6.3%) results were found. Immunochromatographic test (IC) were applied on 213 of the samples with 38 positives. The overall group A rotavirus positives detected in the study was 66 (46 by ELISA and 20 by IC). Polyacrylamide gel electrophoresis (PAGE) was applied on 53 ELISA and IC positive samples. The characteristic Group A rotavirus electropherotype was seen in 11 samples. Electron microscopy examination was applied on 22 ELISA positive samples, 6 samples showed the characteristic wheel like appearance of rotavirus. None of 302 samples tested for coronavirus antigen using ELISA was positive. Most of the positive samples were collected from diarrheic calves (35 of 46 ELISA positives) The results showed the presence of rotavirus in stool samples of diarrheic as well as recovered and healthy camel calves indicating the significant role of rotavirus in camel calf diarrhoea in Sudan. The main age group affected was 0-3 months and males were found to be slightly more affected. Higher prevalence of rotavirus infection was noticed during autumn season than summer and winter seasons.

Key words: rotavirus, Camel calf, Diarrhoea, Sudan

Introduction

Camels are important animals in Saharan Africa and are uniquely adapted to dry and arid environment. The estimated camel population in Sudan is about three million animals. However, high calf mortality appears to be one of the major constraints to higher productivity in camels. Many factors contribute to calf mortality, among which calf diarrhea (Schwartz, 1992).

According to Schwartz and Diolli (1992) morbidity and mortality rates due to neonatal camel calf diarrhoea can reach up to 30% and 100% respectively. In a separate report, camel calf diarrhoea was shown to affect almost 33% of the neonatal calves and resulting in 23% of camel calf mortality in northeast Sudan (Abbas *et al.* 1992). Agab and Abbas (1998) reported that in 15 camel herds followed up for one year in eastern Sudan, calf diarrhoea was reported in 91 out of 415 one-year-old calves. During the study diarrhoea was reported to cause 30.2% of deaths due to different diseases in calves. Bacteria associated with calf diarrhoea in camels are *Escherichia coli*, Salmonella *spp*, and *Clostridium perfringens* (McGrane and Higgins., 1985; Mohamed *et al.*, 1998; Berrada *et al.*, 1998 and Salih *et al.*, 1998).

Viruses as causal agents of camel calf diarrhoea are poorly understood. A Medline literature search revealed only a few reports. Rotavirus-specific antibodies were detected in camel sera (Mahin *et al.*, 1983) and in camel milk (Elsayed *et al.* 1992). In an earlier report from Sudan, Mohamed *et al.* (1998) reported the detection of rotavirus antigens in diarrheic camel calf samples by ELISA and latex agglutination.

Since its initial discovery by Bishop *et al.* (1973), rotavirus has been identified as a major cause of diarrhoea in both humans and animals (Flewett *et al.*, 1974). Many reports describing the role of rotavirus in causation of diarrhoea was reviewed, in infants (Vesikari *et al.*, 1983 and Geyer *et al.*, 1993) as well as different animal species (Eugster and Whitford., 1978, Abraham *et al.*, 1992 and Munoz *et al.*, 1995). This study is intended to elucidate the role of rotavirus in camel calf diarrhoea in Sudan.

Materials and Methods

Faecal Samples: Faecal samples were collected from 245 diarrheic, 75 recovered & 12 healthy camel calves at North (River Nile state), Central (Sennar and Blue Nile states), East (Gedarif state) and West (Kordofan state) of Sudan. 114 faecal samples were collected during Autumn (July-October), 206 during Summer (March- June) and 12 during winter season (November-February) over three years period (2000-2002). The faecal samples were diluted 10% in phosphate buffered saline (PBS), centrifuged at 1000 g for 10 minutes and the supernatant was taken for analysis. The supernates of some samples were layered on top of 30% sucrose, centrifuged at 100,000 g for 90 minutes in Beckman L7-65 centrifuge with SW 41 Ti rotor and the pellets were collected for examination by PAGE and EM.

Enzyme-Linked Immunosorbent Assay (ELISA): All faecal samples (332) were examined by an enzyme immunoassay (Rotavirus IDEIA™, DAKO Diagnostics, United Kingdom). This test was performed as specified by the manufacturer instructions.

Latex Agglutination Test: 144 faecal samples were examined by a latex agglutination assay (Slidex-Rota-Kit 2, Bio Merieux (sa), France). The kits were used according to the manufacturer's instructions.

Immunochromatographic Test (IC): A total of 213 faecal samples were examined by an immunochromatographic test (Rota strip test, manufactured by Bio X Diagnostics- Belgium). The IC was performed according to the manufacturer's instruction.

Polyacrylamide Gel Electrophoresis (PAGE): Fifty three rotavirus positive specimens identified by ELISA or IC assay were subjected to analysis of the double stranded (ds) RNA viral genome, according to published methods (Steele and Alexander, 1987) with some modifications which includes the use of ultracentrifuged sample pellets for RNA extraction and the repetition of phenol/chloroform step

using 250 μl in the second round. In brief, the clarified 10% faecal suspension and ultracentrifuged sample pellet was mixed with 1/10 volume of 1M sodium acetate containing 1% SDS, and incubated for 15 min at 37°C. The viral dsRNA was extracted by 1 volume of 1:1 phenol-chloroform mixture at 56°C for 15 min, followed by centrifugation at 10,000g for 3 min. The RNA was precipitated from the aqueous phase with 1/10 volume of 3 M sodium acetate and 3 volume of cold ethanol at -20°C overnight and centrifugation at 10,000g for 10 min. The air-dried pellets were suspended in 40 μl of sample buffer (0.12 M tris hydrochloride, 0.1% SDS, 15% glycerol, 0.001% bromophenol blue).

PAGE was carried out in 10% polyacrylamide slab gels, with a 3% stacking gel, using the discontinous buffer system described by Laemeli (1970), but without SDS. Finally, 30 μl of sample was loaded and electrophoresis was performed at 100 V for 16-18 hrs.

The dsRNA segments were visualized by silver staining using the method described by Steele and Alexander (1987). The gels were fixed in 40% ethanol containing 10% acetic acid for 30 min and then in 10% ethanol with 0.5% acetic acid for a further 30 min. The gels were soaked in silver nitrate solution for 30 min before being washed in distilled water. The gels were developed in a solution of 0.75M NaOH containing 0.3% formaldehyde before the reaction was stopped with 5% acetic acid and dried.

Electron Microscopy (EM): A total of 22 ELISA positive samples were examined by EM after staining the ultracentifuged pellets by 3% phosphotungistic acid as described by Bishop *et al.,* (1973).

Detection of Coronavirus Antigen Using ELISA: A total of 302 faecal samples were tested for the detection of coronavirus antigen using ELISA Kits (Bio X diagnostics-Belgium) according to the instructions of the manufacturer.

Results

Detection of Group A Rotavirus Antigen: Using ELISA, 46 (13.9%) of the samples were positive. These rotavirus-positive specimens were from 35 calves with diarrhoea, 4 clinically healthy animals and 7 calves that had recovered from diarrhoea. The details are presented in Table 1.

Latex agglutination: Out of 144 samples tested using Slidex Rota-Kit2 and bioMerieux Kits, 9 were positive for rotavirus. All positive samples were collected from camel calves with diarrhoea (Table 2).

Immunochromatographic test (IC): Out of 213 samples tested by IC (Rota strip test) 38 were positive, the details are shown in Table 3.

Page: PAGE was applied to 53 of ELIS and IC rotavirus-positive samples, 11 samples (7 diarrheic, 3 recovered and 1 healthy) showed the characteristic RNA electrophoretype typical of Group A rotaviruses (Fig. 1).

Electron Microscopy (EM): A total of 22 ELISA positive camel calf faecal samples were examined by EM, 6 diarrheic samples showed the characteristic wheel like appearance of rotavirus (Fig. 2).

Table 1: Detection of group a rotavirus antigen in camel calf faeces by ELISA

States		No. of samples tested	No. of positive samples	No. of negative sampels	Percentage of positive positivies	Percentage of total
River Nile State	D	15	2	13		
	R	5	1	4		21.4%
	H	8	3	5		
Gedarif State	D	78	11	63		
	R	13	1	12		13.2%
	H	0	0	0		
Sennar and Blue Nile	D	23	4	19		
	R	2	0	2		16%
	H	0	0	0		
Kordofan State	D	129	18	110		
	R	55	5	50		12.8%
	H	4	1	3		
Sub Total	D	245	35	205		
	R	75	7	68		
	H	12	4	8		
Total		332	46	281		13.9%

D: Diarrheic R: Recovered H: Healthy

Table 2: Results of latex agglutination test (LA) for detection of group A rotavirus antigen in camel faecal samples

States	No. of tested samples	No. of positive samples	No. of negative	Percentage of positive
River Nile State	21	1	20	4.8
Gedarif State	76	3	70	4
Sennar and Blue Nile States	20	4	16	20
Kordofan States	27	1	26	3.7
Total	144	9	132	6.25

Table 3: Results of immunochromatographic test (IC) (Strip Rota test) for Detection of Rotavirus antigen in camel faecal samples

States		No. of tested samples	No. of positive samples	No. of negative samples	Percentage of positive sampels	Total Percentage of positivies
River Nile State	D	6	0	5	0	
	R	3	1	2	33.3	16.7
	H	3	1	0	33.3	
Gedarif State	D	14	5	7	35.7	
	R	13	0	13	0	18.5
	H	0	0	0	0	
Sennar and Blue	D	3	2	1	66.7	
Nile states	R	2	0	2	0	66.7
	H	0	0	0	0	
Kordofan State	D	119	25	85	21	
	R	46	4	40	8.7	17.2
	H	4	0	4	0	
Sub Total	D	142	32	98	22.5	
	R	64	5	57	7.8	17.8
	H	7	1	4	24.3	
Total		332	46	281		13.9%

D: Diarrheic R: Recovered H: Healthy

Table 4: Results of ELISA, LA, IC, PAGE and EM for detection of rotavirus antigeni n camel faecal samples

			Latex Agglutination				Immunochromatrographic Test				PAGE		EM			
Tests		No. tested	+	D	-	N	+	D	-	N	+	-	N	+	-	N
ELISa	+	46	8	1	17	20	17	12	9	8	11	34	1	6	16	24
	D	5	-	2	2	1	1	-	2	2	-	-	-	-	4	1
	-	281	1	-	113	167	20	4	148	109	-	34	247	-	16	265

+: Positive D: Doubtful -:Negative N: Not tested

Table 5: Age and sex distribution of camel calves examined fro group A rotavirus antigen using ELISA and IC

Percentage of positive	No. of positives	No. of tested samples	Sex	Average
24.6	30	122	M	0-3 month
14.4	17	118	F	
12.9	4	31	M	>3-6 months
14.3	3	21	F	
36.4	4	11	M	>6-9 months
0	0	7	F	
62.5	5	8	M	>9-18 months
22.2	2	9	F	
0	0	0	M	>18 months
40	2	5	F	
25	43	172	M	Subtotal
15	24	160	F	

M: Male F: Female

Table 6: Seasonal distribution of rotavirus infection in camel calves

Percentage of positive	No. of positives	No. of tested samples	Seasons
17.5	36	206	Summer season March-June
25.4	29	114	Autumn season July-October
16.7	2	12	Winter season November-February

Comparison Between the Different Techniques Used for Detection of Rotavirus Antigen in Camel Faecal Samples: A total of 332 samples tested by ELISA for detection of group A rotavirus were partially tested by latex agglutination (LA) test, immunochromatographic (IC) test, PAGE and EM. The results obtained were 46 positive, 5 doubtful and 281 negative by ELISA. Nine positive, 3 doubtful, 132 negative and 188 not tested by LA test. Thirty-eight positive, 16 doubtful, 159 negative and 119 not tested by IC test. Eleven positive, 34 negative and 279 not tested by PAGE, while, 6 positive, 26 negative and 310 were not tested by EM. The details of the results are presented in Table 4.

Age and Sex Distribution of the Affected Animals: Out of 332 animals sampled, 240 were at 0-3 month of age from which 47 (19.6%) were positive for group A rotavirus antigen. Out of 172 males examined, 43 (25%) were positive, while 24 of 160 (15%) females were positive; the details are shown in Table 5.

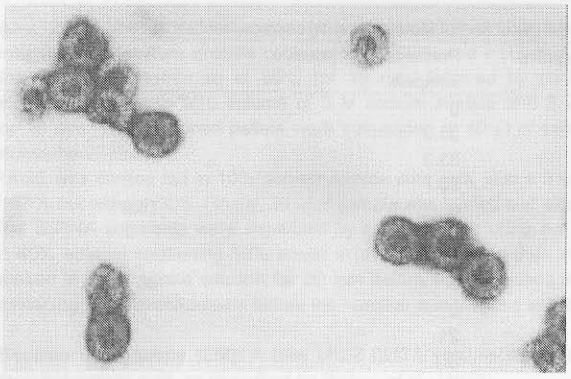

Fig. 1: Characteristics wheel like appearance of camel rotavirus detected by EM

Fig. 2: Group A rotavirus RNA profile detected by PAGE in two camel calf fecal samples (Kordofan and Gedarif)

Seasonal distribution of rotavirus associated camel calf diarrhoea: During the study period (2000-2002), a total of 206 faecal samples collected during Summer from diarrheic, recovered or clinically healthy camel calves tested for rotavirus antigen gave 36 (17.5%) positive results. Out of 114 samples collected during Autumn, 29 (25.4%) were found to be positive for rotavirus, while 2 of 12 calves sampled during winter were positive for group A rotavirus. The details are presented in Table 6.

Detection of Coronavirus Antigen: A total of 302 faecal samples were tested by ELISA for the detection of coronavirus antigen. All samples were found to be negative for coronavirus antigen.

Discussion

Detection of rotaviruses in diarrhoeal faeces can be conducted by different techniques including electron microscopy (Bishop *et al.,* 1973), latex agglutination (Hughes *et al.,* 1984), polyacrylamide gel electrophoresis (PAGE) of viral RNA (Kalica *et al.,* 1976), and enzyme-linked immunosorbent assay (ELISA) (Yolken *et al.,* 1978).

In this study, we describe the identification of rotaviruses in the diarrhoeal as well as non diarrhoeal faeces of camel calves in Sudan. The estimated camel population in Sudan is about 3 millions and the animals are a vital part of both social and economic life here. A continuous complaint of increased camel calf mortalities due to diarrhoeal disease raised the need to investigate the causative agents of diarrhoea. Some bacterial agents have been reported to cause camel calf diarrhoea, however, the role of rotavirus in camel calf diarrhoea is poorly understood or studied.

According to Mahin *et al.* (1983), the dromedary camel is susceptible to rotavirus infection. In a serological survey among 55 dromedary sera collected in Morocco, 27 possessed anti-rotavirus antibodies as detected by counter immuno-electrophoresis. Elsayed *et al.* (1992) described the detection of rotavirus antibodies in camel milk. Mohamed *et al.,* (1998) reported the detection of Group A rotavirus in 8 out of 200 diarrheic camel calf samples by latex agglutination assay, and 11 of 117 samples by ELISA and 4 out of 87 samples by electron microscopy in Sudan.

In this study faecal samples were collected from diarrheic, recovered and healthy camel calves from 4 different states of Sudan (Eastern, Western, Central and Northern states). Rotavirus was detected in 66 (20.2%) samples (46 using ELISA and other 20 using IC).

The detection of rotavirus antigen in faecal samples using ELISA is widely used in both human and animal faecal samples (Yolken *et al.,* 1977; Gerna *et al.,* 1987 and Iwona *et al.,* 1996). In this study, a total of 46 (13.9%) samples were found positive for Group A rotavirus using the IDEIA™ kit,. This level of detection of rotavirus is higher than that reported previously (Mohamed *et al.*, 1998). Furthermore, rotavirus was detected not only in diarrheic, but also in apparently healthy calves and in calves that had recovered from diarrhoea. This may indicate that the actual prevalence of rotavirus infection in camel herds is far higher than currently seen. The detection of rotavirus antigen in faecal samples collected from the four areas of study indicated the wide spread of rotavirus infection in camel calves in Sudan.

The use of latex agglutination test for the detection of rotavirus antigen in stool specimens was previously evaluated and shown to be useful (Hughes *et al.,* 1984; Kodituwakku and Harbour, 1990; Nussbaum *et al.,* 1999). In this study, the latex agglutination assay was less sensitive than the ELISA and only 9 samples (6.3%) were positive for rotavirus. Thus our results confirmed the less sensitivity of this test described previously (Mohamed *et al.,* 1998), nevertheless, this result is slightly higher than that recorded by him, he reported the detection of rotavirus in 4% (8 of 200) diarrheic camel calf samples in Eastern Sudan using latex agglutination test (Rota screen., Merica diagnostics, UK).

The use of the immunochromatographic test (IC) for detection of group A rotavirus was evaluated by Klingenberg, and Esfandiari (1996). IC was compared with ELISA for group A rotavirus antigen in 161 bovine, porcine and equine faecal samples. Eighty nine percent sensitivity and 99% specificity of the IC test was found. In the present study IC test detected rotavirus antigen in diarrheic, recovered and healthy camel calf samples. The results showed a comparable sensitivity of IC and ELISA; although the results of both tests revealed some differences. Out of 38 ELISA positive samples, 17 were positive, 12 doubtful and 9 were IC negative while out of 38 IC positive samples, 17 were positive, 1 doubtful and 20 were ELISA negative. Nevertheless, the statistical analysis of these results revealed 77.5% level of agreement, which is considered moderate between the two tests.

PAGE is one of the commonly used techniques for identification of rotavirus, because the technique is cheap, easy to perform and can recognize different viral RNA electropherotypes (Kalica *et al.,* 1976; Rodger *et al.,* 1979). The use of this technique, describing its reliability and sensitivity has been previously reported by other authors (Kalica *et al.,* 1976, Herring *et al.,* 1982; Steele *et al.,* 1987). In the present study, the characteristic Group A rotavirus electropherotype was detected in only 11 of the 53 ELISA and IC-reactive

samples tested. This is likely to be due to the observed difficulty in RNA extraction from camel faeces and the increased sensitivity of the ELISA techniques in determining the presence of rotavirus antigens in faeces (Gerna *et al.*, 1987; Khattar and Pandey, 1990).

Electron microscopy (EM) was the first technique used for detection and nomination of rotaviruses (Flewett *et al.*, 1974). In this study 6 out of 22 faecal samples examined by EM were rotavirus positive. The higher percentage of positives detected by EM in this study than that reported by Mohamed *et al.*, (1998) could be due to the examination of only ELISA positive samples, however ELISA was found to be more sensitive than EM for detecting rotavirus in this study.

According to this study, and in support to the finding of Mohamed *et al.*, (1998), the ELISA test was confirmed as being more sensitive than the latex agglutination and PAGE for the detection of rotavirus. We also confirm the role of group A rotavirus as an important causative agent for camel calf diarrhoea, as this study had covered almost all areas where camels are raised in Sudan.

The commercial kits used in this study can only detect the presence of group A rotavirus antigen. Thus the results of PAGE, which can detect the presence of Group B or C rotaviruses, were not encouraging and need to be studied further. It was noticed that using the standard RNA extraction method, positive results were obtained when four RNA extractions of each sample applied and the pellets were loaded on one gel . Clear bands were seen only when using ultracentrifuged sample pellets for RNA extraction, with repeating the phenol/chloroform step. It is possible that camel calf faeces contain factors that make the extraction and electrophoresis of the viral dsRNA difficult.

The detection of group A rotavirus antigen in 20.2% of tested samples beside the failure of detecting coronavirus antigen in all tested samples indicated the major role of rotavirus in causation of camel calf diarrhoea in Sudan. This is the first report for detection of group A rotavirus in camel calf faeces in areas other eastern Sudan.

The main age group found to be affected by rotavirus associated diarrhea in camels was 0-3 months. This is in agreement with the previous reports describing the high prevalence of rotavirus infection during the first three months of age in bovine (Deleeuw *et al.*, 1980, Abraham *et al.*, 1992 and El Nour 1994). Males were found to be more affected than females in different age groups in this study, which was previously noticed in bovine calves in Sudan (EL Nour, 1994).

It was noticed that higher prevalence of rotavirus infection was detected during autumn season. In Sudan the calving season of camels is mainly during autumn season (July-October) with some calving during winter season (November-February) Higgins (1985). The results obtained were in agreement with the previous reports that the incidence of calf diarrhoea increases during the calving season due to the increase in susceptible individuals with the persistence of the causative agent in the environment (Babuik *et al.*, 1985).

In Sudan, camels are mainly owned by nomads and it is not easy to find them and to collect samples at the appropriate time. The camels share water sources with their owners and pasture with different animal species. Therefore the relationship between rotavirus isolates from humans, camels and other animal species should be investigated further. Further studies to detect Group B,C and other rotavirus Groups, as well as genotyping of detected rotavirus isolates are to be considered. A trial for vaccination against rotavirus infection in camel calves is highly recommended.

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