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Rotavirus infection in Humanand Domestic Animals in Sudan

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ABSTRACT: The role of rotavirus in causation of diarrhea in infants and young domestic animals in Sudan was studied during 2005-2006. A total of 198 fecal samples were collected from diarrheic as well as clinically healthy infants and domestic animals (85 infants, 50 camel calves, 40 cattle calves, 23 goat kids). All samples were screened for group A rotavirus antigen using ELISA. Positive results were noted in infants and in different animals. The highest percentage of positives was found in cattle samples (n=13, 32.5 %), then goat samples (n=5, 21.7 %); rotavirus positive infants (n=12; 14.1%) and camel calves with rotavirus (n=3; 6%) were also noted. Genomic characterization of the rotavirus samples was conducted by polyacrylamide gel electrophoresis (PAGE) and the characteristic group A rotavirus electropherotype was observed in human, bovine and camel samples.

Keywords: Rotavirus, Human, Domestic animals, Sudan,

INTRODUCTION

Rotaviruses are a well known enteric pathogen of the young of many mammalian species. In humans, rotavirus has been documented to cause about 111 million cases of gastroenteritis requiring home care annually, 25 million clinic visits, 2 million hospitalizations and a median of 440,000 deaths in children under 5 years of age world wide (Parashar et al., 2003). A study of diseases associated with diarrhea in Melit District in South Sudan in 1981-1982 showed that the major detected pathogens were amoebic dysentery and rotavirus (Sixl et al., 1987).

Similarly, rotavirus infection in the young of many species of domesticated livestock has been reported. For instance, in diarrheic camel calves in Sudan, Mohamed et al (1998) reported the detection of rotavirus in 8 out of 200 (4%) using latex agglutination test. Group A rotavirus has been reported previously by us in 13.9% of 332 camel calves with diarrhea in various locations across Sudan using an ELISA system, which is more sensitive (Ali et al., 2005).

The isolation of rotavirus from diarrheic bovine calves is also well known. An early report from Libya, described neonatal calf diarrhea (NCD) that was identified to cause 73% of neonatal bovine calf mortalities (Snodgrass, 1981). In Egypt, bovine rotavirus has also been described (Shalaby, 1981; Hassan, 1997); in the latter study rotavirus was detected in 10 out of 44 cattle calves and in 12 out

of 66 buffalo calves. In Ethiopia, 16.7% of 108 diarrheic bovine calves examined were rotavirus positive (Abraham et al., 1992), and in South Africa, rotavirus associated calf diarrhea was reported by Mendes et al. (1993), demonstrating the wide spread of bovine rotavirus across the African continent. In Sudan, in a study on neonatal calf diarrhea in Friesian dairy farm, rotavirus was detected in 77 out of 116 samples collected from diarrheic calves, (El Nour, 1994). The recovery and isolation of rotaviruses in young goat kids has also been documented in Africa. Berrios and colleagues (1988a) described the association of rotavirus infection with diarrhea in young goats, and Mendes *et al* (1994a) .reported the first isolation of 5 caprine rotaviruses in Saanen goats in South Africa.

Despite these widespread reports, there has been little research on the extent and characterization of rotavirus as a cause of diarrheal disease in the young of domesticated livestock in Sudan. This study was to conduct a preliminary evaluation of the extent of rotavirus infection in various livestock in Sudan with particular focus on animals of economic importance - camels, cattle and goats. In addition, despite widespread surveillance for rotavirus infection in human infants in many countries in Africa (Steele, 2003; WHO, 2008), there is virtually nothing known of rotavirus infection in Sudan, except for the early report by Sixl and colleagues (1987), so we conducted a preliminary investigation of rotavirus infection in infants.

MATERIALS and METHODS

Sample collection: A total of 198 stool samples were collected during 2005-2006 from various sources.

These were collected from 85 infants hospitalized with diarrhea in two health facilities in Khartoum State (n = 85). In addition, at field we collected fecal samples from camel calves (n = 50, 30 diarrheic and 20 clinically healthy), diarrheic cattle calves (n = 40), and diarrheic goat kids (n = 23). Cattle and goat samples were collected from animals submitted from Khartoum veterinary clinic to the Central Veterinary Laboratory for diagnosis. All fecal samples were kept at 40ºC until they were transported to the laboratory for analysis.

Group A rotavirus antigen detection: All fecal samples (n = 198) were examined by an enzyme immunoassay (Rotavirus IDEIA , DAKO Diagnostics, United Kingdom). The test was performed as specified by the manufacturer.

Polyacrylamide gel electrophoresis (PAGE): Twenty rotavirus ELISA positive samples,

6 from infants and 14 from different animal species (3 camel, 7 cattle and 4 goats), were subjected to analysis of the double stranded (ds) RNA viral genome according to published methods (Steele and Alexander, 1987). In brief, the dsRNA was extracted by phenol chloroform and precipitated by 3M sodium acetate and 3 volumes of cold ethanol at -20^{o}C overnight and centrifugation at 10,000g for 10 min. PAGE was carried out in 10% polyacrylamide slab gels, with a 3% stacking gel, using the discontinuous buffer system described by Laemeli (1970), but without sodium dodycil sulfate (SDS). Finally, 30 µl 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).

RESULTS

Rotavirus antigen detection: A total of 33 of the 198 (12 infants, 13 cattle,

5 goat and 3 camel) stool samples tested for group A rotavirus using ELISA were found positive (Table 1).

Polyacrylamide gel electrophoresis (PAGE): Of 20 rotavirus ELISA positive stool samples examined by PAGE, half (n=10) were positive for the classical group A rotavirus electropherotype with different profiles (Figure 1). These rotavirus samples were recovered from 5 infants, 1 camel, and 4 bovine samples.

DISCUSSION

Rotaviruses are one of the major causes of diarrhea in infants as well as a wide variety of animal species. Rotavirus is found to be the major cause of diarrhea in children in industrialized and developing countries, mostly occurs between 3 months and 2 years of age (Bresee, 1999), but is also an important pathogen in less developed countries. For instance, Nguyen et al. (2001) reported the detection of rotavirus antigen in 56% of stool specimens from children hospitalized with diarrheal illness in Vietnam, 41% in China, 56% in Myanmar and 29% in Hong Kong. In Africa, rotavirus has been reported to occur in a quarter to a third of cases of infants hospitalized with gastroenteritis (Steele, 2003).

Research on rotavirus in infants in Sudan is sparse; Sixl et al (1987) reported the role of rotavirus in infantile diarrhea in south Sudan, two decades ago. In a more recent study in Sudan, rotavirus antigen was detected in 26.1 % of 134 tested samples from young children admitted to hospital with diarrhoeal illness (Ali et al., unpublished data). In this study rotavirus antigen was detected in 14.1 % of 85 tested samples collected from two different health localities in Khartoum State. Compared to our previous unpublished results, and to other reports from developing countries, this may be considered to be low. This may be due to the collection of samples from only two health localities and some samples may have been collected from older children or from children in the convalescent phase of the disease where antigen is less commonly detectable. Nevertheless, the results indicate the role of rotavirus in causing diarrhea in infants in Sudan and it is recommended that the hospitalized based burden of diarrhoeal studies are urgently required in Sudan to assess this burden in a standardized way (WHO, 2008).

Mohamed et al. (1998) was the first to report 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. Ali et al (2005) reported 13.9 % prevalence rate of rotavirus in camel calf diarrhea in Sudan. In the present study rotavirus antigen was detected in 6 % of the 50 tested camel calves (30 diarrheic and 20 clinically healthy). This finding confirms the association of rotavirus with camel calf diarrhea.

In Canada, rotavirus was demonstrated in 124 out of 578 calves tested during an outbreak of diarrhea in calves (Durham et al., 1989). In Japan, 28 bovine group A rotavirus strains were isolated from 167 fecal samples collected from diarrheic calves (Fukai et al., 1998). In Mozambique, rotavirus associated diarrhea was detected in 20% of diarrheic calves (Baule et al., 1995).

In a dairy cattle herd in Khartoum, the calf mortality was 44.9% and declined to 12.3% during a 7 year period (El Nour, 1994). Further investigations carried out revealed that the main cause for calf mortality was rotavirus infection (El Nour, 1994). The results obtained in this study revealed detection of rotavirus antigen in 32.5 % of 40 tested calves, this was the highest incidence noticed in this work and is

higher than most of the previous reports, but emphasizes the need for further research to evaluate the significant role of rotavirus in causation of diarrhea in calves.

Scott et al. (1978) first described the detection of rotavirus in stools of scouring goat kids. Mendes *et al.* (1994) reported the first isolation and preliminary characterization of 5 caprine rotaviruses in Saanen goats in South Africa. To date, no other work was done on rotavirus diarrhea in goats in Sudan; however this study has shown the significant role of rotavirus in diarrhea in kids (21.7 %).

The use of PAGE in diagnosis as well as characterization of rotaviruses is well documented (Kalica., 1976, Smith and Tzipori, 1979, Herring, 1982, Steele and Alexander, 1987). PAGE was applied in 20 samples in this study, characteristic group A rotavirus long profile was seen in 10 samples, most of positives were human samples (for which this technique is well described and relatively easy to perform); no goat sample was positive and only one camel sample was positive, but it was previously reported (Ali et al., 2005) that there was a noticeable difficulty in the RNA extraction from camel samples as well as goat samples, which may mean that alternative methods need to be used.

This study investigating the presence of group A rotaviruses in human and domesticated animals in Sudan highlights the widespread of the infections, the ease with which they can be identified and indicates the urgent need for further research to understand the economic impact and the molecular epidemiology of rotavirus strains in various species in Sudan.

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Table 1 : Detection of group A rotavirus in fecal samples of infants and domestic animals in Sudan using ELISA.

Sample origin	Total tested	Positive	Negative	% positive
Infants	85	12	73	14.1
Camel	50	3	47	6
Cattle	40	13	27	32.5
Goat	23	5	18	21.7
Total	198	33	165	16.7

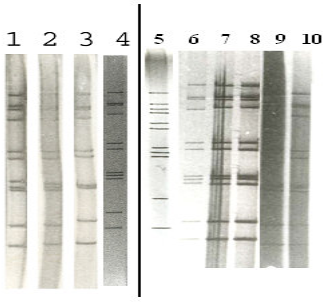

Figure 1: Characteristic group A rotavirus long profile detected by PAGE ,lane 1.2.3.4.10 human, lane 5 camel, lane 6,7,8,9 cattle samples