



## Detection of Human Rotavirus in Faeces from Diarrhoeic Calves in North-east Nigeria

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### ABSTRACT

Information on the epidemiology of rotavirus in any particular area is necessary for vaccine development against the disease caused by the virus. This study presents preliminary information on the prevalence of human rotavirus in diarrhoeic calves in North-east Nigeria. Faecal samples from 188 diarrhoeic calves in various farms in North-east Nigeria, obtained between November 1998 and February 1999, were analysed by ELISA for the presence of rotaviruses. A prevalence rate of 3.2% was recorded, with the virus being prevalent among calves aged 29–56 days ( $p < 0.05$ ). The implications of these findings are discussed with respect to the close association between the herdsmen and their animals and the sharing of a common source of drinking water in the predominantly livestock-producing communities of North-east Nigeria.

*Keywords:* calves, diarrhoea, epidemiology, prevalence, rotavirus, strain

*Abbreviations:* ELISA, enzyme-linked immunosorbent assay

### INTRODUCTION

Gastroenteritis caused by rotavirus continues to be a major cause of death of neonates and infants in humans and animals in developing countries and a significant cause of high morbidity in the industrialized nations of the world (Kapikian and Chanock, 1996). While it is true that many other viruses, such as those causing Newcastle disease and infectious bursal disease in chickens, measles in children, rinderpest and *peste des petits ruminants* in ruminants, also cause diarrhoea among other signs, the enteric viruses are those for which the only significant sign of infection is diarrhoea.

The rotaviruses are the most important of the enteric viruses (Ojeh, 1991; Kapikian and Chanock, 1996). Rotaviruses have also been reported in association with syndromes other than diarrhoea, such as exanthema subitum, otitis media, necrotizing enterocolitis and liver abscesses (Estes, 1996). However, there is no convincing evidence that they cause these syndromes.

In both human and veterinary medicine, rotaviruses pose a serious health problem, being reported to be responsible for 5–20 million infantile deaths per annum or 15–

34% of all deaths in certain developing countries, as well as high morbidity in developed countries (Kapikian and Chanock, 1996). On average, every child contracts diarrhoeal disease several times per year during the first five years of life, with the potential for severe dehydration, nutritional impairment and death (Bern and Glass, 1994).

Group A bovine rotaviruses are a major cause of enteric disease in calves of 1–3 weeks of age (Luchelli *et al.*, 1992). Affected calves may die as a result of severe dehydration or secondary bacterial infections (Theil, 1990). The presence of rotaviruses in domestic livestock is an epidemiological problem whose significance is evident from the detection in humans of serotypes and genotypes of the virus of animal origin (Nakagomi *et al.*, 1992). Animals may serve as reservoirs for human rotavirus infections.

Extensive epidemiological and molecular studies on the natural distribution in humans of rotavirus serotypes and genotypes have been conducted in southern Africa (Steel *et al.*, 1995, 1998) and in Nigeria (Adah *et al.*, 1997a,b). Also, hospital-based and community-based studies in Nigeria have shown that up to 33% of childhood diarrhoeas are associated with rotavirus infection (Adah *et al.*, 1997b; Omotade *et al.*, 1995). However, there have been no similar studies in animals in Nigeria. Such information would be useful from an epidemiological standpoint, especially as a recent report indicates that there may be cross-species transmission of rotaviruses in Nigeria (Adah, 1997a).

This study was undertaken to generate preliminary information on the prevalence of human rotaviruses among diarrhoeic calves in North-east Nigeria.

## MATERIALS AND METHODS

### *Faecal samples*

One hundred and eight faecal specimens from calves under one year of age that presented with diarrhoea in various farms in Borno (Maiduguri, Benisheik, Ngala and Ngamdu), Yobe (Potiskum and Damaturu) and Gombe (Gombe and Ashaka) states were collected between November 1998 and February 1999. The specimens were transported on ice to the laboratory and stored at  $-20^{\circ}\text{C}$  for up to 7 days before they were examined for the presence of rotavirus antigen.

### *Enzyme immunoassay for the detection of rotavirus*

All the specimens were examined for the presence of human rotavirus antigen using a commercial ELISA test kit (RIDASCREEN Rotavirus, r-Biopharm, Darmstadt, Germany) according to the instructions of the manufacturers as follows.

All the reagents were brought to room temperature ( $20-30^{\circ}\text{C}$ ) before use. Sample diluent (reagent 1 containing buffered saline), 1 ml, was added to a set of labelled tubes. Then 100  $\mu\text{l}$  or 100 mg of the stool sample was added and resuspended. A sufficient

number of wells for the samples and the controls were snapped off and inserted into the microtitre well holder. The positions of the samples were recorded. The diluted faecal sample or the positive control (reagent 2 containing inactivated Simian rotavirus, SA11) or the negative control (reagent 1), 100  $\mu$ l, was added to separate wells. Enzyme conjugate (reagent 3 containing horseradish peroxidase conjugated to an anti-rotavirus monoclonal antibody in a buffered protein solution), 100  $\mu$ l, was then added to each well. After gentle mixing, the plates were incubated at room temperature for  $60 \pm 5$  min. The liquid in the wells was discarded and all the wells were washed five times with 250  $\mu$ l deionized water each time. Chromogen solution (reagent 5 containing tetramethylbenzidine), 100  $\mu$ l, was then added to each well and incubated for 10 min at room temperature before the reaction was stopped with reagent 6 (1 mol/L sulphuric acid).

The absorbance of each well was read at 450 nm against an air blank in an ELISA Reader (MR700 Microplate Reader, Dynatech Laboratories, Guernsey, UK). Samples giving absorbance equal to or greater than 0.150 units were considered positive. Samples giving absorbance less than 0.150 units were considered negative.

#### *Statistical analysis*

The difference in the distribution of positive results was assessed using the  $\chi^2$  test.

## RESULTS

Of the 188 diarrhoeic faecal samples tested for the presence of rotavirus antigen, 6 were positive. This represents a prevalence rate of 3.2%.

A higher proportion of samples from calves aged 29–56 days tested positive to rotavirus antigen compared with samples from those up to 28 days old and those at least 57 days old (Table I) ( $p < 0.05$ ).

More samples from male calves tested positive to rotavirus antigen than those from female calves (Table II) but the difference was not statistically significant ( $p > 0.05$ ).

## DISCUSSION

Rotaviruses are the major infectious cause of diarrhoea in young calves in many countries (Snodgrass *et al.*, 1990). In Nigeria, most of the work on rotaviruses has focused on infections in human infants and children (Adah *et al.*, 1997a,b), although a few studies have been carried out in pigs and sheep (Ojeh, 1991). The present study is the first report of human rotavirus infection in livestock in North-east Nigeria, a predominantly livestock-producing region accounting for 40% of the total livestock production in the country (Alaku and Igene, 1986).

The prevalence of 3.2% is below the range recorded among human infants in northern Nigeria (Gomwalk *et al.*, 1990; Adah *et al.*, 1997b). The lower prevalence rate recorded in this study may have arisen from a low infection rate by human

TABLE I  
Age distribution of rotavirus infection among calves in North-east Nigeria

Age (days)	No. of samples tested	No positive (%)
0–28	124	1 (0.8)
29–56	31	4 (12.9)
57 and above	33	1 (3.0)
Total	188	6 (3.2)

TABLE II  
Sex distribution of rotavirus antigen among diarrhoeic calves in North-east Nigeria

Sex	No. of samples tested	No. positive (%)
Male	75	4 (5.3)
Female	113	2 (1.77)
Total	188	6 (3.2)

rotavirus in calves owing to the absence of or insufficient human rotavirus receptors on calf cells. In addition, the number of samples tested in this study is small compared to the other studies, which may have influenced the result. One study (Adah *et al.*, 1997a) demonstrated the presence of a virus of serotype G8 in a human infant that is closely related to the bovine serotype G8, perhaps indicating interspecies transmission of rotavirus in Nigeria. This rotavirus strain (HMG 89) was detected among the samples from the current study area. Similar studies in other parts of the world have reported evidence of interspecies transmission of rotavirus (Nakagomi *et al.*, 1992). In view of the close association between the herdsmen and their animals in the livestock-producing communities of North-east Nigeria, including sharing common sources of drinking water, coupled with evidence from the previous report (Adah *et al.*, 1997a), it seems possible that human rotaviruses constitute a meaningful risk of animal rotavirus infection and vice versa. There is, therefore, a need to undertake a detailed molecular epidemiological study of rotavirus infection among calves and other farm animals in this community. Such a study is planned.

The study showed that calves aged 29–56 days were most susceptible to rotavirus infection. In human infants in Nigeria, infection with rotavirus was found to be most common in the first 6 months of life, decreasing with age (Gomwalk *et al.*, 1990; Adah

*et al.*, 1997b). The lower prevalence of infection in the first four weeks of the life of the calves may be attributable to maternally derived antibodies (Snodgrass *et al.*, 1980). If so, calves appear to begin to pick up infection as the maternal antibodies wane and later their own immune response probably protects them against infection. This hypothesis requires further investigation.

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#### **Détection de rotavirus humains chez des veaux diarrhéiques au Nord-Est du Nigéria**

**Résumé** – Il est nécessaire d'obtenir des informations sur l'épidémiologie des rotavirus, quelle que soit la discipline, pour développer un vaccin contre les maladies induites par ceux-ci. Cette étude présente des informations préliminaires sur la prévalence des rotavirus humains chez des veaux diarrhéiques du Nord-Est du Nigéria. Des prélèvements fécaux furent obtenus chez 188 veaux entre 1998 et 1999. Les échantillons furent analysés par ELISA pour la présence de rotavirus. Une prévalence de 3,2% fut observée, le virus étant plus souvent rencontré chez les veaux âgés de 29 à 56 jours ( $p < 0,05$ ). Ces résultats sont discutés par rapport à la relation particulière entre le berger et les animaux, en particulier le partage de l'eau potable entre ces deux groupes dans cette région.

#### **Detección de rotavirus humano en heces diarreicas de terneros en el nordeste de Nigeria**

**Resumen** – Para el desarrollo de una vacuna contra la enfermedad provocada por el rotavirus, es necesario obtener información sobre su epidemiología. Este estudio presenta información preliminar sobre la prevalencia de rotavirus humanos en terneros afectados de diarrea en el nordeste de Nigeria. Con el propósito de detectar el rotavirus, se analizaron mediante ELISA 188 muestras fecales, obtenidas entre Noviembre de 1998 y Febrero de 1999, de terneros con diarrea procedentes de varias granjas del nordeste de Nigeria. Se observó una tasa de prevalencia del 3,2%, siendo superior en terneros de 29 a 56 días ( $p < 0,05$ ). Las implicaciones de estos descubrimientos son discutidas respecto a la estrecha relación entre los ganaderos y sus animales, incluyendo la utilización de la misma fuente de agua de bebida en las principales comunidades productoras de ganado del nordeste de Nigeria.