Brief Report
Epidemiology of Rotavirus Infection in North-western Nigeria
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Summary
Rotaviruses (RV) are associated with ~33 000 deaths in children <5 years of age annually in Nigeria. However, limited data exist on RV infection in north-western Nigeria. During July 2002 to July 2004, 1063 (869 diarrhoeic and 194 control) stool samples were collected from children <5 years of age presenting with diarrhoea in north-western Nigeria. The stools were analysed for RV antigen and further characterized by antigenic and genomic methods. RV was detected in 18% of children with diarrhoea and 7.2% of the age-matched case controls. The highest RV burden was detected in children <6-months-old. Long electropherotypes and VP6 subgroup I + II specificity predominated.

Introduction
Recent estimates [1] attribute 527 000 deaths in children <5 years to rotavirus (RV) annually. Improvements in sanitation and the availability of clean water have not decreased the rate of RV diarrhoea and the development and implementation of an effective vaccine into the routine EPI schedule is considered the first strategy of prevention [2]. Epidemiological studies of RV infection reveal the greatest degree of diversity of RV strains in West Africa [3]. There is little or no information on RV-associated diseases in north-western Nigeria where there is the likelihood of strain diversity. While the introduction of the two new currently available oral, live attenuated RV vaccines may only occur in 4–6 years in these African settings, data on the epidemiology of RVs will be required to preamble vaccine implementation.

Materials and Methods
Faecal samples were obtained from 869 children <5-years-old who were presented or admitted at clinics or hospitals for diarrhoeal illness in north-western Nigeria. In addition, 194 control (non-diarrhoeic) samples were collected. Stool samples were stored frozen at –20°C and transported to the MRC/MEDUNSA Diarhoeal Pathogens Research Unit, University of Limpopo, Medunsa Campus, Pretoria, South Africa for further analysis. Upon delivery, a 10% faecal suspension was prepared using balanced salt solution and the suspension stored at 4°C.

RV detection
RV antigens were detected utilizing a commercially available Rotavirus IDEIA™ Kit (DakoCytomation, UK) according to the manufacturers' instructions.

Polyacrylamide gel electrophoresis (PAGE)
All RV-positive specimens were analysed by PAGE as previously described [4]. Briefly, RNA was extracted utilizing phenol–chloroform deproteinization and ethanol precipitation, electrophoresed overnight and visualized by silver staining according to the method described by Herring et al. [5].

Subgroup specificity (VP6)
All RV-positive specimens were analysed utilizing an ‘in-house’ VP6 ELISA as described by Steele and Alexander [6]. Group-specific [7] and subgroup-specific monoclonal antibodies [8] were a kind donation from H. B. Greenberg, Stanford University, USA.

Statistical analysis
Analysis of RV infection in children according to age and sex was done using statistical programme for social sciences (SPSS) version 11.0. Differences with
Results

RV antigen was detected in 18% (156/869) of the diarrhoeic samples and in 7.2% (14/194) of the control samples. Infection occurred throughout the study period with slightly higher peaks in the drier months (Fig. 1). Highest prevalence of RV infection was in children <6-months-old (p < 0.01) (Fig. 2). Viral shedding was slightly higher in males (16.4%: 100/608) than females (15.4%: 70/455) (p > 0.05). Electropherotypes could be obtained from 54% (92/170) of specimens analysed. Long RNA migration patterns predominated (80.4%: 74/92) and 10 distinct long (L) electropherotypes were noted (Fig. 3). In addition, six distinct short electropherotypes (n=18) and a small proportion of mixed patterns (2.2%) were detected. Subgroup specificity (SG) could be assigned to 146/170 specimens, with 22 specimens not reacting to any of the antibodies used and a further two specimens having insufficient stool for testing. Subgroup II specificity was found in 41/170 specimens, SGI in 37/170 specimens and SGnon-I/non-II in 16/170 specimens. Surprisingly, SGI+II specificity was detected in 52/170 specimens, by far the predominant subgroup in specimens from north-western Nigeria. Three strains exhibited the unusual combination of VP6 SGI specificity with a long electropherotype.

Discussion

Nigeria has recently been ranked second among six countries with the greatest number of RV disease-associated deaths per year in children <5-years-old [9]. The peak of RV infection in children <6-months-old implies greatest burden in the youngest and most vulnerable unlike developed countries where infections are more common in children 9- to 15-months-old [2]. Detection of RVs throughout the study period is not unexpected and similar seasonality trends have previously been reported in Africa [10].

Extensive genomic diversity was observed in this study as indicated by the 16 RNA electrophoretic variants identified. Mixed patterns noted for the first time in Nigeria may represent possible means of emergence of genetic reassortant RV strains. These strains might have originated from animals; because the study area is predominantly inhabited by nomadic pastoral farmers who live in close association with their animals and share common sources of drinking water.

Interestingly, 31% of samples were of SGI+II specificity indicating the possibility of mixed infections. This has been previously reported in very low level [11]. The unusual combination of a VP6 SGI long electropherotype noted have been previously described [6, 12] and may be a consequence of re-assortment process.

This study showed RVs to be important cause of diarrhoea in children 0–5 years in north-western Nigeria. Therefore, there is the need for additional studies in this region to provide data required to
expedite the introduction of RV vaccines to Nigerian children, who would clearly benefit from these interventions.

References