Brief Report
Prevalence of Rotavirus-induced Diarrhoea among Children under 5 Years in Ilorin, Nigeria

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Summary
Diarrhoea disease is an important cause of morbidity and mortality in Nigerian children and rotavirus has been identified as an important causative agent among children below 5 years. We determined the prevalence of rotavirus-induced diarrhoea among under-5s by electropherotyping. Stool samples were collected from eligible subjects who presented with acute diarrhoea. The samples were processed for viral studies by electropherotyping. Among the 299 subjects recruited, 55.9% were positive for rotavirus. Eighty percent of the recruited patients were below 24 months. There was a significant decrease (p-value = 0.00001) in the prevalence of rotavirus among diarrhoea patients above the age of 24 months when compared with those below this age group. Rotavirus was associated with higher morbidity and mortality. We conclude that rotavirus is the most important cause of diarrhoea among children <5 years in Ilorin, Nigeria and its prevalence is highest in the first 2 years of life. Adequate rehydration should be regarded as the mainstay of management.

Introduction
Diarrhoea disease is one of the major causes of morbidity and mortality in infants and young children, especially in the developing countries [1, 2]. The main cause of death from acute diarrhoea is dehydration, which results from loss of fluid and electrolytes in diarrhoea stools. The aetiology of diarrhoea in children can be viral, bacterial, parasitic or nutritional. Rotavirus is the most common cause of acute diarrhoea in children <2 years of age worldwide [3, 4]. Rotavirus has also been reported as a cause of endemic viral diarrhoea in children in Nigeria [5, 6].

Rotavirus can be detected by electron microscopy, using negatively stained stool specimen, antigen detection in stool by enzyme-linked immunosorbent assay (ELISA), electropherotyping technique and reverse transcriptase polymerase chain reaction (RT-PCR) [7, 8]. A previous study conducted in this environment found rotavirus to be the most prevalent viral agent in paediatric diarrhoea based on findings using ELISA detection technique. However, ELISA can only detect ‘group A’ rotavirus. Electropherotyping is a technique used to analyze rotavirus based on the arrangement and motility of the nucleic acid segments under the influence of an electric field [9, 10]. It can detect all types of rotavirus and also used in identification of type-specific strains [11, 12] hence its advantage in tracing nosocomial infection and in vaccine development.

We determined the prevalence of rotavirus-induced diarrhoea in children <5 years of age using electropherotyping.

Materials and Methods
This prospective study was carried out at the outpatient departments of the University of Ilorin Teaching Hospital, the children’s specialist hospital and two private hospitals within Ilorin metropolis.
Study was conducted between 1 December 2003 and 31 November 2004.

Children below 5 years who presented with watery stools with or without blood or fever at participating health facilities in Ilorin and those without diarrhoea for the preceding 2 weeks (as controls) were recruited into the study after informed consent was sought from parent or guardian.

At enrolment, the age, sex, nature and frequency of passage of stools and other relevant complaints were obtained from the caregiver. After history taking, complete clinical examinations were performed and those children that needed hospitalization due to severe dehydration were admitted.

Fresh stool samples were collected into clean specimen bottles. The specimens were transported to the Medical Microbiology Department of the University of Ilorin Teaching Hospital where it was stored at a temperature below 0°C until ready for analysis, which was done in three batches such that no sample was kept beyond 6 months before analysis. The study was approved by Ethical and research committee of the University of Ilorin Teaching Hospital, Nigeria.

Rotavirus dsRNA extraction from feces

Rotavirus RNA extraction was carried out according to the method of Herring et al. [10]. Briefly, 500 µl of 10–20% fecal extract was added to an Eppendorf tube containing 50 µl of 1 M sodium acetate (NaAc) at pH 5.0 with 1% sodium deodecyl sulphate (SDS) mixture (SDS added to NaAc just before use at 37°C to prevent SDS precipitating out) and incubated at 37°C in a water bath for 15 min. Phenol/chloroform mixture (500 µl) was added into the Eppendorf tube and vortexed for 1 min. (Care was taken to ensure that the lid was secured since phenol could cause skin burns!), then re-incubated at 56°C for 15 min. Vortexing was repeated for 1 min, then, centrifuged for 2–3 min at 12 000 r.p.m. The upper aqueous phase (containing the dsRNA) was removed into a new Eppendorf tube, taking care to avoid any interface material. Into this, 40 µl of 3 M NaAc and 1 ml of cold absolute ethanol were added. This was left at −20°C overnight to allow for precipitation of dsRNA of the rotavirus genome (Standard: −70°C for 20–30 min or at −20°C for at least 2 h.). Centrifugation of samples at 4°C was done for 10 min at 2000 r.p.m. and the supernatant was poured off and the pellet dried under vacuum. (The dried pellet contains dsRNA.) The pellet was re-suspended in 20 µl of TE buffer and stored at 4°C.

Electrophoretic typing

Ten percent agarose gel (1.5 mm thick) was prepared with the comb in place. This was introduced into the electrophoretic chamber at room temperature (22°C). The resuspended dsRNA pellet extract was well mixed with the aid of a micropipette. Five microlitres of the RNA-TE mixture was added to 1 µl of loading dye and loaded into the agarose gel. This was then run at 100 V for 1.5 h. Gels were stained with ethidium bromide and separated RNAs visualized on an UV trans-illuminator were photographed.

Analysis of data

Results were presented in tables and figures as applicable. Comparisons were made using standard statistical methods in which categorical data were compared by Chi-square.

Results

Five hundred and thirty-nine subjects were recruited over a period of 12 months. Two hundred and ninety-nine had acute diarrhoea while 240 were healthy children who served as controls. There were 298 males and 241 females giving a male to female ratio of 1.2:1. Age distribution of cases and their matched controls are shown in Table 1.

Eighty-two percent of the 299 patients with diarrhoea were 24 months old and below. Among the control group, 58.8% of the children were aged 24 months or below.

<table>
<thead>
<tr>
<th>Age range of subjects recruited into the study</th>
<th>Patients (%)</th>
<th>Control (%)</th>
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</thead>
<tbody>
<tr>
<td>&lt;1–12</td>
<td>166 (55.5)</td>
<td>59 (24.6)</td>
</tr>
<tr>
<td>13–24</td>
<td>78 (26.1)</td>
<td>82 (34.2)</td>
</tr>
<tr>
<td>25–36</td>
<td>24 (8.0)</td>
<td>55 (22.9)</td>
</tr>
<tr>
<td>37–48</td>
<td>23 (7.7)</td>
<td>26 (10.8)</td>
</tr>
<tr>
<td>49–60</td>
<td>8 (2.7)</td>
<td>18 (7.5)</td>
</tr>
<tr>
<td>Total</td>
<td>299 (100)</td>
<td>240 (100)</td>
</tr>
</tbody>
</table>

Fig. 1. Sample of electropherotypes.
Stool analysis using electropherotyping
Figure 1 shows a sample of electropherotyping results. The electrophoretic pattern of diarrhoea stools showed rotavirus positivity in the wells marked 1, 2, 3 and 4. The double-stranded RNA segments were separated by electrophoresis on agarose gel. One hundred and sixty-seven (55%) of the 290 subjects were positive for rotavirus infection while none of the 240 controls were positive (Table 2). The negative findings in all the 240 controls further confirm the importance of rotavirus as an important causative agent in childhood diarrhea.

Age and rotavirus diarrhoea
Figure 2 shows that among the 166 case subjects aged between 0 and 12 months, 109 (65.3%) were positive for rotavirus; 42 (53.8%) of the 78 children in the 13–24-month age group were positive for rotavirus while 29% of the children above 24 months were positive for rotavirus infection. The younger the age higher the risk of rotavirus-induced diarrhoea (df 2; \( \chi^2 = 22.58, p = 0.0001 \)). Also, there is a significant increase in the risk of acquiring rotavirus-induced diarrhoea among patients aged 24 months and below when compared with those age above 24 months (RR 2.13; 95% CI: 1.39–3.25).

Morbidity from rotavirus infection
Rotavirus-induced diarrhoea is associated with more severe morbidity. As can be seen from admission pattern (Table 3), 44% of children with rotavirus-induced diarrhoea were hospitalized compared to 28% of children with non-rotavirus-induced diarrhoea \((p = 0.005)\).

Discussion
In this study, the prevalence of rotavirus-induced diarrhoea was 55.9%. This rate is higher than that reported earlier in Ilorin [6] (16.2%), Ibadan (13%) [10] and Lagos (37.5%) [11]. The reason for lower prevalence in these other studies may be accounted for by the limitations of ELISA (which were used) which can only detect group A rotaviruses in contrast to electropherotyping which can detect all groups of human rotaviruses. Our finding was however similar to that of Franco et al. [13] who, using electropherotyping method, reported the prevalence of rotavirus-induced diarrhoea to be between 55% and 71%. Electropherotyping has a high specificity and can also detect non-group A rotaviruses [13]. This is the first time in reporting rotavirus prevalence using electropherotyping for all samples in this environment, thereby allowing for the detection of both group A and non-group A rotaviruses. The sensitivity of electropherotyping is further enhanced by phenol–chloroform extraction, which was used in this study. Electropherotyping has the great advantage in that there are no false-positives and the identification of specific electropherotypes is of special utility in identifying strain-identical infections and this may be invaluable in tracing nosocomial transmission of rotavirus infection [14] and development of effective rotavirus vaccine. Electropherotyping is labor intensive and therefore not recommended for routine clinical laboratory tests. However it is invaluable in monitoring epidemiology.

<table>
<thead>
<tr>
<th>Results</th>
<th>Subjects</th>
<th>Controls</th>
</tr>
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<tbody>
<tr>
<td>Negative</td>
<td>132</td>
<td>240</td>
</tr>
<tr>
<td>Positive</td>
<td>167</td>
<td>0</td>
</tr>
<tr>
<td>Total no.</td>
<td>299</td>
<td>240</td>
</tr>
<tr>
<td>Positivity (%)</td>
<td>55.9</td>
<td>0</td>
</tr>
</tbody>
</table>

\( \chi^2 = 194.22, p\text{-value} = 0.00000. \)

<table>
<thead>
<tr>
<th>Mode of care</th>
<th>Rotavirus positive</th>
<th>Rotavirus negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>In-patient care</td>
<td>74</td>
<td>38</td>
<td>112</td>
</tr>
<tr>
<td>Out-patient care</td>
<td>93</td>
<td>94</td>
<td>187</td>
</tr>
<tr>
<td>Total</td>
<td>167</td>
<td>132</td>
<td>299</td>
</tr>
</tbody>
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\( p = 0.0057. \)
In this study, the prevalence of rotavirus in the first 24 months of life was significantly higher than those between 24 and 60 months, showing that the ages commonly associated with diarrhoea are also commonly associated with rotavirus infection. Furthermore, rotavirus-induced diarrhoea is associated with high morbidity from dehydration requiring more admission.

We conclude that rotavirus is the most important cause of diarrhoea among children <5 years in Ilorin, Nigeria and the prevalence of diarrhoea induced by rotavirus is highest in the first 2 years of life. We recommend adequate rehydration rather than the use of antibiotics in the care of diarrhoea patients especially those below 2 years of age in this environment.

References