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Hospital-based Surveillance for Rotavirus Gastroenteritis using Molecular Testing and Immunoassay during the 2011 Season in Botswana

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Abstract

We describe rotavirus testing results, demographics, seasonality and outcomes in children admitted with acute gastroenteritis to hospital during Botswana's 2011 rotavirus season.

Rotavirus season extended from mid-June until the end of October with the rotavirus-specific case-fatality being 2.8%. Using PCR as the reference, the immunochromatographic test had a sensitivity of 76.5% and specificity of 68.0%. Rotavirus vaccine may significantly reduce childhood morbidity and mortality in Botswana.

Keywords

Rotavirus; Botswana; diarrhea; PCR; immunoassay

Background

In Africa, rotavirus is the most common enteric pathogen causing hospitalization of children. It accounts for 40% of all gastroenteritis hospital admissions for under five-years-olds in the region (1).

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We describe the clinical characteristics and outcomes of children admitted to Botswana's two main referral hospitals during a single rotavirus season, before the introduction of rotavirus vaccine. We also evaluated the performance of a rapid immunochromatographic test (ITA) as compared to a real-time reverse transcriptase polymerase chain reaction (PCR) assay.

Methods

A prospective cohort study among children admitted with acute gastroenteritis to two referral hospitals--Princess Marina Hospital (PMH) in Gaborone, and Nyangabgwe Referral Hospital (NRH) in Francistown, has been ongoing since July 2010. Acute gastroenteritis was defined by two of the following: (1) change in stool consistency to liquid or semiformed; (2) increase in frequency of stool to ≥ 3 /day; (3) vomiting ≥ 2 episodes/day. Children were excluded if they had diarrhea ≥ 14 days or if they had diarrhea starting ≥ 24 hours after admission. Variables relating to demographics, clinical characteristics feeding practices, water sources, and outcomes, were collected on admission using a standardized data collection form. Participants who did not submit a stool sample were excluded from analysis. Children were enrolled from 8:00 to 17:00, Monday to Friday. The reason for missed enrollment was only recorded at Princess Marina Hospital.

Data analysis was done using Stata 12 (Stata Corp., College Station, TX). Associations between the primary outcome of interest (rotavirus infection) and potential explanatory variables were explored using the chi-square or Fisher's exact test (for categorical variables) or the Mann-Whitney test (for length of stay in hospital); similar analyses were done for the secondary outcomes of interest, including in-hospital mortality. Crude odds ratios were calculated using simple logistic regression. Multivariable analysis was not done as there were too few outcomes for a robust statistical model. Statistical significance was determined a priori as a two-tailed $P < 0.05$. This study was approved by Ethics Boards of all involved institutions.

Stool was collected after admission and tested using an ICT CombiStrip™ (Coris BioConcept, Belgium) according to manufacturer instructions. Stool was then immediately frozen at -80°C and shipped on dry ice in batches to McMaster University, Canada, for nucleic acid extraction and PCR detection. Fecal specimens were diluted 1 in 10 in either SL solution (Copan Italia, Brescia, Italy) or Lysis Buffer (bioMérieux, St. Laurent, Canada) and vortexed with glass beads for lysis. The pre-treated stools were then centrifuged at 14 000 rpm for 2 minutes. Subsequently, the pre-treated stools were divided in 200 μL aliquots and frozen at -80°C until ready for extraction. Extraction was performed using either the QIAasymphony® (Qiagen, Germantown, MD) or the easyMAG® (bioMérieux, St. Laurent, Canada) according to manufacturer instructions. Using reverse transcription-PCR with specific primers, Rotavirus A molecular detection was performed using an in-house viral multiplex reverse transcriptase PCR assay, performed on the Rotorgene Q® (Qiagen, Germantown MD), that also included primers and probes for adenovirus and norovirus GI/GII. The rotavirus A primers and probe (Forward- GGAKGTYCTGTACTCMTTGTC; Reverse- CCAGTTTGRAASTCATTTCC; Probe- FAM- GAATATAAT/ZEN/ GTACCTTCRACAATTTTGTCYCTAGCATC) were modified from those described by Logan *et al* (2). A case was considered to be rotavirus positive based on the results of PCR testing.

Rotavirus testing occurred at NRH between February and July 2011 and at PMH between May 2011 and January 2012. For the purposes of this study we defined the rotavirus season to have started with the first week (Sunday to Saturday) of two consecutive weeks where the rotavirus PCR positivity rate for tested stool specimens was $\geq 10\%$; the rotavirus season was

defined to have finished at the start of the last two consecutive weeks where rotavirus positivity was <10%. These definitions are similar to those used by previous investigators (3). Only testing during the rotavirus season was analyzed in this report.

Results

During the year-long study period (February 2nd, 2011 to January 31st, 2012), a total of 327 stool specimens were collected from individual patients. The first rotavirus -positive stool (by either PCR or ICT) was taken on June 13th, 2011, and the last rotavirus-positive stool was October 26th, 2011. The rotavirus season (as defined above) began June 12 and ended October 29, 2011. PCR testing was performed on all specimens.

During the rotavirus season a total of 231 (34 at NRH and 197 at PMH) children were enrolled. At NRH there were 20 missed patients during the rotavirus season. At PMH there were 88 missed enrollments, 11 (12.5%) were missed as they had died prior to enrollment, 63 were missed as they were discharged, 7 subjects did not have a suitable guardian for consent, and 7 had guardians that declined. Among enrolled subjects, 212 (92%) had stool collected within 3 days of admission. The majority (75%) of the children enrolled during the rotavirus season were < 1 year of age, and the majority of these infants were not exclusively breastfed (see Table – supplemental digital content 1).

During the rotavirus season, 178 stool specimens (78%) were positive for rotavirus by PCR. Children < 1 year of age ($p=0.002$), those with severe acute malnutrition ($p=0.05$), and HIV positive children ($p=0.04$) were more likely to test negative for rotavirus, although the absolute numbers were very small for the latter two variables. The overall case fatality for enrolled cases during the rotavirus season was 3.5% (8/231), which was significantly lower than the 12.5 % (11/88) mortality seen among non-enrolled children ($p < 0.01$). The rotavirus specific case fatality was 2.8% (5/178) and did not differ significantly from the rotavirus negative case fatality of 5.7% (3/53). Of the variables listed in Table 1, only severe acute malnutrition was associated with death ($p=0.03$).

Comparative testing during the rotavirus season is summarized in Table 1. Most specimens (212/231, 92%) were tested by ICT; using the PCR assay as the reference standard, ICT had a sensitivity of 76.5% (95% CI 70 – 82%) and a specificity of 68.0% (95% CI 54 – 79%). McNemar test for paired proportions revealed a significant difference in rotavirus detection between the two tests ($p=0.004$).

Discussion

These data from Botswana's two largest referral hospitals describe the 2011 rotavirus season. Key findings were a relative high case fatality; the improved sensitivity of PCR as compared to ICT; and the winter seasonality (June–September in the southern hemisphere). The rotavirus season extended from mid June 2011 until late October 2011 in our study, which is similar to those reported in the southern African region (4). Our study limitations included enrolment in the northern region of Botswana at NRH (Francistown) from only February 2011 until July of 2011 and in the southern region (PMH) from May 2011 until January 2012. We cannot therefore be certain as to discrepancies in seasonality between these regions although the first rotavirus positive cases at NRH occurred 3 weeks before PMH (we did not capture the end of the season at NRH).

The rotavirus specific in-hospital case fatality we report, 2.8%, is relatively high for sub-Saharan Africa. Reported mortality in neighboring South Africa is estimated at 0.9–1.8% (9), 2% in Kenya (10), and the highest reported was 8% in Guinea Bissau (11) We likely under-estimated mortality rate in our study as mortality among missed cases was higher

(12.5%) when compared to the 3.5% mortality among the enrolled cases ($p < 0.01$). The majority of missed cases however were due to discharge from hospital (63/88) and these may represent more mild disease. Data is not available, but possible explanations for the relatively high case fatality observed include the relatively high number of HIV exposed children, low rate of breastfeeding, and malnutrition. Logistic regression only found severe malnutrition to be associated with death. The sample size and number of deaths may have been too low to detect a difference for other factors.

Interestingly, using the PCR assay as the reference standard, the ICT had a sensitivity of 76.5% and a specificity of 68.0%. Others have also reported reduced sensitivity (8) and specificity (9) of rotavirus immunoassays when compared with molecular testing. None of the ICT positive/PCR negative samples demonstrated inhibition of the MS2 phage RNA internal control and several ($n=5$) were positive for other viral pathogens (suggesting no significant PCR inhibition) and all tested negative with a separate rotavirus A RT-PCR assay which had different primer/probe targets (data not shown). Potential explanations for this finding include 1) instability and degradation of nucleic acid due to sample storage/transport, 2) false positive immunoassay, and 3) differential sensitivity of the PCR assay vs. immunoassay for specific rotavirus strains. The ICT we used had previously been evaluated using specimens from South Africa and its performance was found to be similar to enzyme linked immunoassay (10). The World Health Organization recommends against using molecular assays for rotavirus surveillance because “low viral loads, not associated with disease, can be detected in cases of gastroenteritis in which another co-infecting pathogen is likely to be causing the symptoms” (11). We plan to further analyze these discrepant samples using other comparator assays in order to hopefully clarify these discordant samples. Given the mixed performance of immunoassays, it may be important to include molecular assays as a component of ongoing surveillance, particularly after introduction of a vaccination program where there may be a shift in prevalent serotypes.

In conclusion, rotavirus continues to cause significant morbidity and mortality in children in Botswana. Implementation of a universal rotavirus vaccine program holds real potential to prevent childhood morbidity and mortality in Botswana.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

Detection of Rotavirus A in Stool Specimens from Children Admitted to Hospital during 2011 Rotavirus Season in Botswana by Method.

	RT-PCR Positive	RT-PCR Negative
ICT positive	124	16
ICT negative	38	34

Sensitivity of ICT using RT-PCR as reference = 76.5%, Specificity of ICT = 68.0%

ICT= Immunochromatographic assay

RT-PCR = reverse transcriptase polymerase chain reaction