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MOLECULAR DETECTION OF NOROVIRUS GI AND GII GENOTYPES IN CHILDREN LESS THAN TWO YEARS OF AGE AND IMPACT ON CHILD GROWTH.

BY

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ABSTRACT

Background: Noroviruses (NoVs) are, after Rotaviruses, the second most common causative agents of acute gastroenteritis in young children. In South Africa, NoV was first reported in 1993 in gastroenteritis associated with the consumption of salad. NoV antibody prevalence was later reported in both urban and rural South African populations, and Norwalk and Hawaii strains were detected. However very few studies have been conducted to identify strains of NoV in the country and their impact on child growth is not well understood. This study aims to identify common NoV genogroups and the strains that are more prevalent in diarrhoeal and non-diarrhoeal stool samples of children less than 2 years of age in rural areas in Vhembe district, South Africa, using reverse transcriptase Real Time PCR. Moreover, an investigation of the impact of different genotypes on child group was determined.

Methodology: In the present study, 185 children were recruited of whom 88 were males and 97 females. Of all the children 141 had experienced diarrhoea at least once while 44 never had diarrhoea. Samples were treated with Sodium Chloride (NaCl), and RNA was purified from them using the QiaAmp viral RNA purification kit and stored at -20°C. Following RNA purification, samples were subjected to One step reverse transcriptase real-time PCR to detect NoV genotypes. Positive samples were further run in reverse transcriptase PCR using specific primers that amplify genogroup-specific sequences of the N-terminal and shell (N/S) region of the NoV VP1 gene, and the cDNA synthesized was run in a conventional PCR. Successfully amplified conventional PCR products for NoV GI and NoV GII were sequenced and the sequences aligned and compared with the existing sequences in the GeneBank, in order to determine the genetic relationship and variability of strains of NoV in Vhembe district, South Africa.

Results: In the study, 708 samples were tested, of which 256 (36.2%) were diarrhoeal samples and 453 (63.8%) were non-diarrhoeal samples. Norovirus GII was the most common genogroup detected in the present study. Among the 256 diarrhoeal samples, 34 (24.1%) were GI, and 93 (66.0%) were GII. Five (13.2%) of the children who were born stunted presented with NoV GI, whilst 22 (57.9%) of them presented with NoV GII at 12 months. The number of infection increased with the children's age. About 12 (61.7%) of the children who were stunted presented with NoV GI and 29 (61.7%) of the children who were stunted presented with NoV GII. Norovirus GII infections showed to be the highest in June (48.1%), October (48.0%) and November (50.6%) whilst infection with Norovirus GI was the highest in October (20.0%). Analysis of sequences showed that the NoV strains differed in their sequences up to 40%. Comparison of study strains with reference has shown the difference in the sequences of the strains, indicating a high mutation rate among Norovirus strains.

Discussion: The present study shows that infection with GII is the most common variant among children and contracted easily during the winter months of the year. Reverse-transcriptase RT-PCR can be recommended as a rapid and sensitive method that can be used to detect NoV, in order to give a quick response to eliminate and prevent infection. Though sequences detected are of similar strains, their nucleotide alignment varies due to the high mutation rate of NoV. Mutations in NoV can cause a problem with vaccine development or an alteration to confer vaccination in order to prevent infection, and spread of NoV.

Conclusion: The present study shows how NoV affects the children's health and has an effect on the child's nutritional state and growth. It also shows that Norovirus strains differ from one country to another, this also include seasonal variation of Norovirus genogroups, which calls for a lot of attention on vaccine development. Norovirus has shown to be more active during winter season and has NoV GII as a predominant strain of which other studies also agree with these results.

Keywords: Norovirus, GI genotype, GII genotype, reverse-transcriptase RT-PCR, Vhembe, South Africa