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Molecular Detection of Rotavirus Infection Associated with Diarrhoea among Children Aged 0 – 5 Years Attending Jahun General Hospital, Jigawa State

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Abstract

Group A Rotavirus causes infectious, severe and dehydrating diarrhoea which has great impact on childhood morbidity and mortality worldwide. However, there are limited data on this subject matter in the study area. This study was designed to investigate the disease prevalence and risk factors of Rotavirus infection among children 0 -5 years attending General Hospital Jahun, Jigawa State. A total of 227 stool samples were collected from children aged 0 - 60 months (mean age, 14 ± 0.2 months) and screened for Rotavirus Group A by Real – Time Polymerase Chain Reaction (RT-PCR) methods. Their sociodemographic information and clinical presentations were also recorded. Out of 227 children, 11(4.8%) were positive for Rotavirus Group A. The highest prevalence of Rotavirus diarrhoea in children was recorded among children aged 11-20 months- 3(1.3%), and the lowest was among those in age group 41-50 months, - 1(0.4%). The Rotavirus diarrhoea infection was observed to be slightly higher in females 7(3.1%) compared to males, 4(1.7%)with no statistical association based on gender of the children and Rotavirus diarrhoea infection in this study (P-value= 0.0640). However, there was a statistically significant association between the sources of drinking water and Rotavirus diarrhoea infection (P-value = 0.003). Also, there was strong association between duration of time of diarrhoea and Rotavirus diarrhoea infection (P - value = 0.001). In addition, there was an association between change in colour of stool and Rotavirus diarrhoea infection (P - value = 0.029). Although the prevalence of Rotavirus infection in children ≤ 5

years in this study is low, however, there is need for public enlightenment programme on mode of transmission, risk factors and preventive measures of Rotavirus diarrhoea infection in order to checkmate the spread of the disease and associated morbidity and mortality.

Keywords: Molecular Detection, Rotavirus, Diarrhoea, Children, Jahun General Hospital, Jigawa State.

Introduction

Diarrhoeal disease is the second leading cause of death in children under five years old, and is responsible for killing around 525 000 children every year. Diarrhoea can last several days, and can leave the body without the water and salts that are necessary for survival. In the past, for most people, severe dehydration and fluid loss were the main causes of diarrhoea deaths. Now, other causes such as septic bacterial infections are likely to account for an increasing proportion of all diarrhoea-associated deaths. Children who are malnourished or have impaired immunity as well as people living with HIV are most at risk of life-threatening diarrhoea, (Priyanka *et al*, 2015; WHO, 2017).

Rotavirus is the most important etiological agent of acquired diarrhea in infants and young children and is a vaccine preventable disease. It is considered to be most common pathogen responsible for causing severe diarrhea and dehydration in children less than 5 years of age (Muhammad *et al.* 2014). However, Rotaviruses are classified into serogroups A through G but only groups A - C have been shown to infect



humans with the severe disease mainly caused by members of group A. Rotavirus is highly transmissible and virtually all children will have experienced at least one rotavirus infection by the age of 5 years (Pennap and Umoh, 2010).

Each year, Rotavirus cause an estimated of 2 million hospitalizations and 450,000 deaths among children globally, with majority of death occurring in developing countries of Asia and Africa (Wang et al. 2014; Liu et al. 2015). In Nigeria a number of studies have reported higher infection rate in children less than two years of age, (Aminu et al, 2008; Junaid et al, 2011; Anochie et al., 2013). Rotavirus infection is associated with 33,000 deaths in children less than 5 years of age annually in Nigeria (Parashar et al., 2003). Some hospital-based studies (Audu et al, 2002; Pennap et al, 2002) have shown Rotavirus to be associated with 3.6-24.2% of childhood diarrhoea in Nigeria. Few studies conducted in some part of northwest region have associated Rotavirus infection with prevalence of 5.3% in Katsina and 16.3% in Zaria (Gambo, 2015).

Study Design and Study Area

The study was descriptive cross-sectional and hospital- based. It was conducted at Jahun General Hospital (JGH) situated in Jahun which is a Local Government Area of Jigawa State, Nigeria. The target populations of the study were children aged 0 - 5 years who presented with diarrhoea at the General Hospital, Jahun. Ethical approval was obtained from Ethical Committee of Jigawa State Ministry of Health and the approval letter was presented to the hospital before the study was conducted.

Methodology

Between September, 2017 to January, 2018 a total of 227 faecal specimens were collected from infants and young children aged 0-5 years attending the hospital with diarrhoea before starting therapy. All the 227 faecal specimens collected were analyzed for Rotaviruses, Simple random sampling was used to obtain the sample size and a closed ended structured questionnaire was used to obtain the socio-demographic and risk factors for infection. RNA Extraction was performed using commercially available

extraction kit by QIAGEN (QIAamp Viral RNA Mini Kits) according to the manufacturer's instruction. Rotavirus group A was detected using commercially available Rotavirus real time PCR kit by Life River. The test procedure was carried out according to the manufacturer's instructions and PCR compilation was carried out Using Real Time PCR instrument (RT-PCR ABI prism 7300 USA). Data generated were analyzed using SPSS Software Version 26.0 (2016, IBM Corp., USA). The prevalence of Rotavirus was expressed in simple proportion and percentages for the study groups. Chisquare was used to determine the relationship between Rotavirus infection and sociodemographic and risk factors. A P-value ≤ 0.05 was considered significant.

Results

Of the 227 children tested, the overall prevalence of Rotavirus infection was 4.8% (11 of 227) (Table 1), based on gender, the outcome of the present study reveals a Rotavirus infection prevalence of 1.7% (n = 4) among males and 3.1% (n=7) among their female counterparts (Table 2). However, when categorization of children tested based on age was considered, results showed that children in age groups 11-20 months and 21-30 months had the highest prevalence rate of 1.3% each (Table 2). Based on breastfeeding, the outcome of the present study reveals a Rotavirus infection prevalence of 3.5% (n = 8) among breastfeeding children and 1.3%(n = 3) among those that were not breastfed. Children in age group 21-30 months had the highest prevalence rate of 1.3% (3) among those children who stopped breastfeeding (Table 3). Furthermore, when the participants were categorized based on those that took other food outside breast milk and those that did not was considered, the results showed that those children who took other foods outside breast milk had the highest prevalence rate of 3.9% (9) while 0.9% (n=2) prevalence was observed among those that are taking breast milk only (Table 3). When vomiting as a clinical feature was considered, the result shows that the highest prevalence of 3.5% (n = 8) was recorded among those who were not vomiting and lowest of 1.3% (n=3) among those who were vomiting (Table: 4). Based on treatment, the highest prevalence of



Rotavirus infection was 2.6% (n = 6) among those children who were receiving treatment and 2.2% (n = 5) among those that were not receiving any treatment (Table: 5). However, when grouped children tested based on types of treatment were considered, the results revealed that the highest prevalence rate of 2.6% (n = 6) was among those receiving traditional treatment, followed by those with no treatment 2.2% (n=5) (Table: 5), those undergoing conventional has 0% prevalence.

 Table 1: Prevalence of Rotavirus Infection among Children Tested at Jahun General Hospital,

 Jigawa State

| Test/Assay | Rotavirus | Percentage |
|-------------------------|-----------|------------|
| No. of Positive Samples | 11 | 4.8 |
| No. of Negative Sample | 216 | 95.2 |
| Total | 227 | 100 |

Table 2: Distribution of Rotavirus Infection by Gender an Age

| Characteristics | Total No. (%) | No. (%) | No. (%) | P - value |
|--------------------|---------------|-------------|-----------|-----------|
| | Tested | Negative | Positive | |
| Gender | | | | |
| Male | 98 (43.2) | 94 (41.4) | 4 (1.7) | |
| Female | 129 (56.8) | 122 (53.7) | 7 (3.1) | |
| Total (%) | 227 (100) | 216 (95.2) | 11 (4.8) | 0.640 |
| Age Group (Months) | | | | |
| ≤10 | 75 (33.0) | 73 (32.1%) | 2 (0.9%) | |
| 11 - 20 | 40 (17.6%) | 37 (16.3%) | 3 (1.3%) | |
| 21 – 30 | 35 (15.4%) | 32 (14.1%) | 3 (1.3%) | |
| 31 - 40 | 18 (7.9%) | 17 (7.5%) | 1 (0.4%) | |
| 41 - 50 | 27 (11.9%) | 26 (11.5%) | 1 (0.4%) | |
| 51 - 60 | 32 (14.1%) | 31 (13.7%) | 1 (0.4%) | |
| Total | 227 (100%) | 216 (95.2%) | 11 (4.8%) | 0.740 |



| Characteristics | Total No. Tested | No. (%) Negative | No. (%) Positive | P-Value |
|--|------------------|------------------|---------------------|---------|
| Children Breast feeding? | | | | |
| Yes | 126 (55.5%) | 118 (52.0%) | 8 (3.5%) | |
| No | 101 (44.5%) | 98 (43.2%) | 3 (1.3%) | |
| Total (%) | 227 (100%) | 216 (95.2%) | 11 (4.8%) | 0.239 |
| Children not breast feeding | | | | |
| ≤10 | 21 (9.3%) | 20 (8.8%) | 1 (0.4%) | |
| 11-20 | 38 (16.7%) | 38 (16.7%) | 0 | |
| 21-30 | 47 (20.7%) | 44 (19.4%) | 3 (1.3%) | |
| No | 121 (53.3%) | 114 (50.2%) | 7 (3.1%) | |
| Total (%) | 227(100%) | 216 (95.2%) | 11 (4.8%) | 0.492 |
| Taking other foods outside breast feeding | | | | |
| Yes | 172 (75.8%) | 163 (71.8%) | 9 (3.9%) | |
| No | 55 (24.2%) | 53 (23.4%) | 2 (0.9%) | |
| Total (%) | 227 (100%) | 216 (95.2%) | 11 (4.8%) | 0.631 |

Table 3: Distribution of Rotavirus Infection by Breast feeding and taking other food outside Breast feeding

Table 4: Distribution of Rotavirus Infection by Source of Drinking Water and Type of Toilet

| Characteristics | Total No. (%) | No. (%) | No. (%) Positive | P-Value |
|-----------------|---------------|-------------|------------------|---------|
| | Tested | Negative | | |
| Source of | | | | |
| Drinking Water | | | | |
| Borehole | 121 (53.3%) | 118 (52.0%) | 3(1.3%) | |
| Others | 40 (25.6%) | 35 (15.5%) | 5(2.2%) | |
| Pond | 1 (0.4%) | 1 (0.4%) | 0 | |
| Stream | 7 (3.1%) | 5 (2.2%) | 2(0.9%) | |
| Тар | 58 (17.6%) | 57 (25.1%) | 1 (0.4%) | |
| Total (%) | 227 (100%) | 216 (95.2%) | 11 (4.8%) | 0.003 |
| Type of Toilet | | | | |
| In the field | 17 (7.5%) | 15 (6.6%) | 2 (0.9%) | |
| Pit latrine | 145 (63.9%) | 137 (60.4%) | 8 (3.5%) | |
| Water system | 65 (28.6%) | 64 (28.2%) | 1 (0.4%) | |
| Total (%) | 227 (227%) | 216 (95.2%) | 11 (4.8%) | 0.178 |

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| Characteristics | Total No. (%) Tested | No. (%) Negative | No. (%) Positive | P - Value |
|-----------------------------|-------------------------|------------------|------------------|-----------|
| Duration of Diarrhoea(days) | | | | |
| >4 | 40 (17.6%) | 39 (17.2%) | 1 (0.4%) | |
| 1 | 7 (3.1%) | 7 (3.1%) | 0 | |
| 2 | 43 (18.9%) | 42 (18.5%) | 1 (0.4%) | |
| 3 | 62 (27.3%) | 59 (26.0%) | 3 (1.3%) | |
| 4 | 74 (32.6%) | 69 (30.4%) | 5 (2.2%) | |
| 5 | 1 (0.4%) | 0 | 1 (0.4%) | |
| Total (%) | 227 (100%) | 216 (95.2%) | 11 (4.8%) | 0.001 |
| Liquid Stool loss per day | | | | |
| <3 | 57 (25.1%) | 56 (24.7%) | 1 (0.4%) | |
| >4 | 4 (1.8%) | 4 (1.8%) | 0 | |
| >5 | 7 (3.1%) | 6 (2.6%) | 1 (0.4%) | |
| 2 | 1 (0.4%) | 1 (0.4%) | 0 | |
| 3 | 76 (33.5%) | 72 (31.7%) | 4 (1.8%) | |
| 4 | 58 (25.6%) | 55 (24.2%) | 3 (1.3%) | |
| 5 | 24 (10.6%) | 22 (9.7%) | 2 (0.9%) | |
| Total (%) | 227 (100%) | 216 (95.2%) | 11 (4.8%) | 0.746 |
| Blood in Stool | | | | |
| Yes | 71 (31.3%) | 65 (28.7%) | 6 (2.6%) | |
| No | 156 (68. 7%) | 151 (66.5%) | 5 (2.2%) | |
| Total (%) | 227 (100%) | 216 (95.2%) | 11 (4.8%) | 0.088 |
| Change of Stool colour | | | | |
| Yes | 135 (59.5%) | 125 (55.1%) | 10 (4.4%) | |
| No | 92 (40.5%) | 91 (40.1%) | 1 (0.4%) | |
| Total (%) | 227 (100%) | 216 (95.2%) | 11 (4.8%) | 0.029 |

Table 5: Distribution of Rotavirus infection in relation to Diarrhoea Stool



| Characteristics | Total No. (%) Tested | No. (%) Negative | No. (%) Positive | P-Valu e |
|-----------------|-------------------------|------------------|------------------|----------|
| Vomiting? | | | | - |
| Yes | 71 (31.3%) | 68 (30.0%) | 3 (1.3%) | |
| No | 156 (68.7%) | 148 (65.2%) | 8 (3.5%) | |
| Total (%) | 227 (100%) | 216 (95.2%) | 11 (4.8%) | 0.769 |

Table 6: Distribution of Rotavirus infection based on Vomiting

 Table 7: Distribution of Rotavirus infection by Treatment and Type of Treatment

| Characteristics | Total No. (%) Tested | No. (%) | No. (%) | P-Value |
|----------------------------|----------------------|-------------|-----------|---------|
| | | Negative | Positive | |
| Received any Treatment? | | | | |
| Yes | 113 (49.8%) | 107 (47.1%) | 6 (2.6%) | |
| No | 114 (50.2%) | 109 (48.1%) | 5 (2.2%) | |
| Total (%) | 227 (100%) | 216 (95.2%) | 11 (4.8%) | 0.746 |
| Type of Treatment | t | | | |
| Conventional | 33 (14.5%) | 33 (14.5%) | 0 | |
| Traditional | 88 (38.8%) | 82 (36.2%) | 6 (2.6%) | |
| No Treatment | 106 (46.7%) | 101 (44.5%) | 5 (2.2%) | |
| Total (%) | 227 (100%) | 216 (95.2%) | 11 (4.8%) | 0.297 |

Discussion

In this study, of the two hundred and twenty seven (227) children tested for Rotavirus infection, a prevalence of 4.8% (n=11) was recorded (Table: 1) .The prevalence of 4.8% of Rotavirus infection in this study is similar to the report of Kuta et al, (2014) where a prevalence of 4.5% was reported in their study in three North Central States and Federal Capital Territory Abuja, Nigeria. The prevalence rate is also comparable to the reports of Mukhtar et al. (2016) who reported a prevalence rate of 5.3% in Katsina State. However, the overall prevalence in this study is low as compared to the reports of Aminu et al., (2008) who reported prevalence rate of 18% in Northwestern States of Nigeria. Nevertheless, the prevalence of this study contradicts the reports of Wada-Kura (2011) who

reported a prevalence rate of 36.5% in Kano State and Tagbo et al. (2014) who also reported high prevalence rate of 56% in Enugu, South east, Nigeria respectively. The difference in the prevalence rates of Rotavirus infection observed in various studies could be due to differences in size of samples, age groups that were studied in each research, study areas of the research, target populations, duration of time in each study, and geographical variations of the study areas. In addition, it could be due to different level of hygiene practices, source of water used, and level of sanitation.

The outcome of the present study reveals a Rotavirus infection prevalence of 1.7% (n = 4) among males as compared to 3.1% (n=7) prevalence among their female counterparts



(Table: 2). This is similar to the finding of Ndze et al. (2012) who reported highest detection rates in females (45.3%) than in males (40.8%). However, the result contrasts that of Mukhtar et al (2016) who reported that Rotavirus infection was slightly higher in males (5.5%) than in female children in Katsina State. Aminu et al. (2008) also reported that Rotavirus infection was slightly higher in males than females.

The results of the present study based on age group showed that children between 11-20 and 21-30 months had the highest prevalence rate of 1.3% each (Table 2). This age-group distribution is comparable to previous reports by Junaid et al. (2011) and Kajbaf et al. (2013) where they reported higher prevalence in children 7-12 months old, while the lowest prevalence of was found in children in the age groups of 31-40, 41-50 and 51-60 months.

Rotavirus infection has prevalence of 3.5% (n = 8) among breastfeeding children as compared to 1.3% (n=3) prevalence among that were not breastfeeding (Table: 3). This study supported the finding of Eric et al. (2011) which revealed that breastfeeding was not associated with Rotavirus diarrhea. In contrast, the prevalence was least among infants who were breast fed and highest among infants on artificial formula. These findings were consistent with the finding of most other published reports especially in 0-6 month age group (Rawaa et al, 2013; Sushmita et al, 2016). After analysis of the study population, it was observed that children in age group 21-30 had the highest prevalence of 1.3% (n = 3) among those children who stopped breastfeeding (Table: 3). In contrary, Sushmita et al, 2016 demonstrated that Rotavirus-diarrhea was mostly common among the in 0-6 months age group. However, in previous reports, the prevalence of Rotavirus infection was in children between 6 - 11 months of age group (Isidore et al, 2010).

In this present study, children tested based on those that took other food outside breast milk and those that were exclusively breastfed were considered, the results showed that those children who took other foods outside breast milk had the highest prevalence of 3.9% (n = 9) as compared to 0.9% (n=2) among those that were exclusively breastfed. Quigley et al. (2007) further estimated that optimal breastfeeding practices could prevent substantial hospital admissions due to diarrhoea and lower respiratory tract infection. However, Kramer and Kakuma (2012) indicated that infants who are exclusively breastfed for the first six months are less likely to die of gastrointestinal infection than infants who switched from exclusive to partial breastfeeding at three to four months. Nevertheless, Aleksandra et al. (2015) reported that exclusive breastfeeding was found to be effective in the prevention of Rotavirus infection.

When the results of sources of drinking water as risk factors used in houses of tested children were analyzed, the results showed that children in houses with other sources of drinking water apart from pipe-borne water (such as wells) had the highest prevalence of 2.2% (n = 5). The Chisquare indicates there is strong association between Rotavirus and source of drinking water (P<0.05) (Table:4). This is in agreement with Isidore et al. (2010) and Gambo (2015) who reported that Rotavirus infection was significantly associated with source of drinking water. In a study conducted in Albania, Rotavirus infection occurrence in drinking water was monitored in association with outbreaks resulting from contamination of groundwater or surface waters (Da silva et al, 2018). In contrary, Maryam (2006) and Aminu et al. (2014) reported that there was no association between Rotavirus infection and the type of water supply in area where a child lived.

In the present study, children tested and analyzed based on type of toilet used as a risk factor, the results revealed the children that used pit latrines had the highest prevalence of 3.5% (n = 8) (Table: 4). Statistical analysis of type of toilet as a risk factor of infection shows no significant association between the Rotavirus and type of toilet used in houses (P>0.05) (Table: 4). This is similar to the finding of Surajudeen et al. (2011) that; distance of toilet from water sources, were observed to be associated with the incidence of Rotavirus infection. In contrary, Aminu et al., (2014) reported that Rotavirus infection was significantly associated with types of toilet used in houses of their studied population. This implies that toilets may be a factor depending on the distance from the source of water.



Similarly, when duration of diarrhoea was considered, the result shows that children who had 4 days diarrhoea had the highest prevalence of 2.2% (n=5) (Table 5). Statistical analysis revealed that there is strong association between Rotavirus infection and duration of diarrhoea of the children tested (P-value = 0.001; P<0.05) (Table 5). This study supported the finding of Pennap et al, 2010 who revealed that no statistically significant association between the start of the diarrheal episode and the detection of rotavirus in the stool.

Nevertheless, the present study revealed that there was no significant association between the liquid stool loss per day and Rotavirus infection, (P>0.05). Although, those who discharged liquid stool 3 times per day had the highest prevalence of 1.8% (n = 4) (Table: 5). This is in contrary to the finding of Surajudeen et al. (2011) which revealed that Rotavirus infection is associated with diarrhoea.

The Chi-square analysis of presence of blood indicates no significant association between Rotavirus infection and presence of blood in stool of tested children (P>0.05) (Table: 5). The present result supported the finding of Pennap et al. (2010) who observed that blood tinged diarrhoea was rare in rotavirus infection.

However, change in colour of stool among children tested in the study population had the highest prevalence of 2.6% (n = 6) as compared to 2.2% (n=5) among those with no change in colour in stool (Table: 5). The chi-square analysis of change in stool colour indicates significant association between Rotavirus and change in stool colour (P<0.05) (Table: 5). Earlier studies indicated that stools in Rotavirus diarrhoea were non- bloody and generally lack faecal leukocytes but mucus may be found in about 20% of cases (Alkali et al, 2015).

When vomiting as the clinical feature of Rotavirus infection were considered, statistical analysis shows no significant association (P>0.05) between Rotavirus infection and vomiting (Table: 6). This was in agreement with that of Thrissawan et al (2006) who reported that no significant differences were found between clinical manifestations of children with

Rotavirus infection and non-Rotavirus infection as both groups displayed nausea or vomiting (p=0.08). In contrary reports, vomiting was found to have a strong association (P<0.01) with Rotavirus infection (Maryam, 2006). However, Alkali et al. (2015) and Surajudeen et al. (2011) revealed significant association between vomiting and rotavirus infection.

In the study population, the highest prevalence of Rotavirus infection was 2.6% (n = 6) among those children who were receiving treatment and 2.2% (n = 5) among those that were not receiving any treatment (Table: 7). The chi-square analysis shows that there was no significant association between Rotavirus infection and treatment received (P – Value = 0.746; P>0.05). Similarly, statistical analysis of type of treatment received shows no significant association (P>0.05) between the Rotavirus and type of treatment received. This study agrees with the previous finding which suggested that drugs formulated to stop diarrhoea, did not affect viral infection since they do not have antiviral properties.

The indiscriminate use of drugs for the treatment of diarrhoea in children by mothers observed in this study had earlier been reported in Nigeria (Aminu et al., 2005; Eugenia et al, 2018). For instance, drugs are such as antibiotics, antiprotozoans, anti-diarrhoeal and herbal remedies are commonly used (without prescription by certified healthcare givers) mainly in the developing countries (including Nigeria) for the treatment of diarrhoea. Indeed, there is no specific treatment for Rotavirus infection at the moment. Antibiotics and antivirals appear not to have any useful impact at Rotavirus infection. Usually, the infection resolves within three to seven days (Mayo, 2018).

Conclusion

Findings from this study showed that Rotavirus causes significant diarrhea in children 0 - 5 years of age attending Jahun General Hospital, Jigawa State, Nigeria. A low prevalence of 4.8% Rotavirus was identified among the studied children whereby children in age groups 11 - 20 months and 21 - 30 months had the highest prevalence rate of 1.3% each. The duration of time for Rotavirus diarrhoea and change in stool colour were significantly associated with



Rotavirus infection in this study. However, source of drinking water particularly well used in homes was a possible risk factor of Rotavirus infection.

Recommendations

In view of the findings obtained from this study, the following recommendations are made: The study provides useful data base for assessing the potential benefits of introducing a Rotavirus vaccine in the study area, especially for those aged less than 3 years and may be considered for National Immunization Program.

- Governments at all levels should provide portable and safe drinking water and for domestic use. Possible ways of delivering treated water by pipe lines and ensuring regular checks for possible leakages and improper connection of pipes from sources so as to avoid contamination of the water. Also, survey of the area should be done before constructions of Boreholes, to ensure the hygiene and safety of the water.
- 2. Mothers should be encouraged to adopt exclusive breastfeeding from 0-6 months to avoid early of children exposure to Rotavirus infection from sources like water and other food items.
- 3. Rotavirus infection with diarrhoea cases often present with change in colour of stool, therefore this study recommends the inclusion of Rotavirus screening as part of the routine laboratory tests for diarrhoea cases in our hospitals.
- 4. Further studies should be carried out to determine the genetic strains of Rotavirus circulating in the study area to aid specific vaccine production.

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