Asymptomatic 

Rotavirus Infections among Children in Maiduguri, Borno State, Northeast, Nigeria

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Abstract

Background: Rotavirus remains one of the main causative agents of gastroenteritis in young children. This happens, especially in countries (e.g., Nigeria) that have not yet introduced the vaccine into the national immunization program. A significant prevalence of Rotavirus infection both in children and adults without major symptoms has earlier been reported. This study aimed at defining the prevalence of asymptomatic Rotavirus infection from apparently healthy children in Maiduguri, Borno State, Northeastern Nigeria. Methods: A total of 269 stool samples were randomly collected from apparently healthy children <15 years of age from July 2017 to June 2018. All samples were screened using a commercially available enzyme-linked immunosorbent assay kit for the presence of Rotavirus antigen. The Rotavirus-positive samples were further subjected to polyacrylamide gel electrophoresis (PAGE) to determine their RNA electropherotypes. Results: A total of 59 stool samples (19.9%) were Rotavirus positive with peaks observed in the cold dry season, among male children, and 6–10 years of age group. A total of 50 randomly selected Rotavirus-positive samples were subjected to PAGE, and none of the samples showed either long or short profiles. Conclusion: This study shows that Rotavirus can be shed into environments without any signs and symptoms. In view of this, the Rotavirus vaccine should be considered a priority and be introduced in the existing national immunization program in Nigeria, particularly in Borno State.

Keywords: Asymptomatic, children, healthy, Maiduguri, Rotavirus

Résumé

Contexte: Le rotavirus reste l’un des principaux agents responsables de la gastro-entérite chez les jeunes enfants. Cela se produit, en particulier dans les pays (par exemple, le Nigéria) qui n’ont pas encore introduit le vaccin dans le programme national de vaccination. Une prévalence significative de l’infection à rotavirus chez les enfants et les adultes sans symptômes majeurs a été signalée précédemment. Cette étude visait à définir la prévalence de l’infection asymptomatique à rotavirus chez des enfants apparemment en bonne santé à Maiduguri, dans l’État de Borno, au nord-est du Nigéria. Méthodes: Un total de 269 échantillons de selles ont été prélevés au hasard sur des enfants apparemment en bonne santé âgés de moins de 15 ans de juillet 2017 à juin 2018. Tous les échantillons ont été criblés à l’aide d’un kit de dosage immunosorbant disponible dans le commerce pour la présence d’antigène rotavirus. Les échantillons positifs pour le rotavirus ont été ensuite soumis à une électrophorèse sur gel de polyacrylamine (PAGE) pour déterminer leurs électrophérotypes d’ARN. Résultats: Un total de 59 échantillons de selles (19,9%) étaient positifs pour le rotavirus avec des pics observés pendant la saison sèche froide, chez les enfants de sexe masculin et le groupe d’âge de 6 à 10 ans. Un total de 50 échantillons positifs au Rotavirus sélectionnés au hasard ont été soumis à PAGE, et aucun des échantillons n’a montré de profils longs ou courts. Conclusion: Cette étude montre que le rotavirus peut être répandu dans des environnements sans aucun signe ni symptôme. Compte tenu de cela, le vaccin contre le rotavirus devrait être considéré comme une priorité et être introduit dans le programme national de vaccination existant au Nigéria, en particulier dans l’État de Borno.

Mots-clés: Asymptomatique, enfants, en bonne santé, Maiduguri, Rotavirus

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INTRODUCTION

Rotavirus infection is a major cause of infant morbidity and mortality with 90% of Rotavirus-associated deaths occurring in low-income locales.\(^{[1]}\) Repeated infections occur from birth to old age, but natural immunity renders the majority of infections asymptomatic after the 1st year of life.\(^{[2]}\) The first Rotavirus infection tends to be the most severe because the body builds up immunity/resistance to the Rotavirus virus later in life.\(^{[3]}\) Moreover, Rotavirus infections can occur throughout life, and asymptomatic infections which occur in adults may maintain the transmission of infection in the community but due to immunity acquired in childhood, most adults are not susceptible to Rotavirus.\(^{[4]}\) In both children and adults, a significant prevalence (ranging from 3% to 31%, patient on the setting, and the age of the study population) of Rotavirus infection without infectious intestinal disease (asymptomatic) has been reported.\(^{[5]}\) Rotavirus infection is classified as asymptomatic because of the absence of diarrhea or vomiting, but infected persons may still display nonspecific symptoms such as fever, headache, nausea, and fatigue.\(^{[6]}\) The transmission of the asymptomatic Rotavirus infection is through the same route as Rotavirus infectious intestinal disease, which is mainly person-to-person contact. For instance, living in a household with a baby that still uses diapers is a risk factor in older adults likewise, attending day care is a risk factor for asymptomatic Rotavirus infection in children under the age of 5 years.\(^{[7]}\) Earlier studies have confirmed that during asymptomatic Rotavirus infections, smaller quantities of Rotavirus are shed than during symptomatic Rotavirus infections.\(^{[8,9]}\) This study was conducted to determine the prevalence of asymptomatic Rotavirus infections from apparently healthy children in Maiduguri, Borno State, Northeastern Nigeria, hence, indicating the importance of introduction of the Rotavirus vaccine in the national immunization program.

METHODS

Study area

This study focused basically on the prevalence of Rotavirus in Maiduguri, the capital city of Borno State, Northeast Nigeria. Borno State has about 69, 436 km\(^2\) and lies within the latitude 11°N and longitude 13.5°E. It is one of the largest States in the federation in terms of landmass.\(^{[10]}\)

Study population

Apparently healthy children (<15 years of age) in some of the selected settlements were the target population. Consent of the patients was sought orally and obtained before inclusion in the study. The ethical clearance for the study was obtained from the Ministry of Health, Borno State, Nigeria. Apparently healthy children (0–15 years) were inclusive in the study, whereas children >15 years were not considered for the study.

Sample collection

A total of 296 stool samples were collected from apparently healthy children. The samples were obtained with the help of consented parents. Sociodemographic information such as age, sex, and location was obtained from consented parents before the sample collection. The samples were collected in sterile, screw-capped universal containers and labeled appropriately. The samples obtained were transported on ice through vaccine carriers to the WHO National Polio Laboratory, University of Maiduguri Teaching Hospital (UMTH), Maiduguri, Borno State, where each sample collected were aliquoted into two and stored at −20°C before processed. Aliquot No. 1 was used for the research of the VP6 antigen, the major protein of the intermediate capsid by enzyme-linked immunosorbent assay (ELISA) technique in the WHO National Polio Laboratory, UMTH, and the aliquot No. 2 was transported on ice to the Noguchi Memorial Institute for Medical Research, West African Rotavirus Regional Reference Laboratory, Department of Electron Microscopy and Histopathology, University of Ghana, Accra, Ghana, for the determination of the gel electrophoretic profiles. The samples were also stored at −20°C until tested.

Detection of Rotavirus antigen by enzyme-linked immunosorbent assay

The frozen stool samples were allowed to thaw at room temperature. The sample preparation was done according to the manufacturer’s instructions. The samples were prepared in 1:5 dilutions by adding 1 g (approximately the size of a pea) to 4 ml of diluted wash buffer. The dilution was allowed to mix properly using the vortex mixer, and the supernatant was aliquoted into well-labeled 2-ml cryovials and used for the test immediately. The stool samples were tested for the presence of Rotavirus antigen using the sandwich ELISA technique\(^{[11]}\) (AccuDiag™ Rotavirus (Fecal) ELISA kit produced by Diagnostic Automation/Cortez Diagnostics, Inc. 21250 Califa Street, Suite 102 and 116, Woodland Hills, California 91367, USA., REF 8306-3).\(^{[12]}\) The assay sensitivity is 100%, and specificity is 97.1%. The test was conducted following the manufacturer’s instructions, and the microtiter plates were read at a wavelength of 450 nm, using the ELISA reader (E-max-reader, precision microtiter plate reader, MDS–Analytical technique, USA).

Polyacrylamide gel electrophoresis

A total of 50 of the Rotavirus-positive stool samples by ELISA were analyzed by polyacrylamide gel electrophoresis (PAGE). Electrophoresis was conducted at 100 volts for 18 hours at room temperature. The gels were stained using the silver nitrate method, and the PAGE profiles were documented using a digital camera.\(^{[13]}\)

RESULTS

Of the 296 stool samples examined by ELISA, Rotavirus antigen was detected in 59 of the samples, with a prevalence of 19.9%. From the study, 41 (29.9%) were positive from stool samples collected during the cold dry season, whereas 12 (14.8%) were positive from stool samples collected during the hot dry season, and 6 (7.7%) were positive from stool
Discriminant analysis (DA) was used to identify the factors associated with Rotavirus infection among apparently healthy children, taking into account the location (MMC, Konduga, Jere) and the age (0-5, 6-10, 11-15 years) of the study participants. The analysis revealed that the location of residence (MMC vs. Konduga vs. Jere) was a significant predictor of Rotavirus infection (P = 0.0003). The age group also showed a significant difference (P = 0.0003), with children aged 0-5 years having a higher prevalence of Rotavirus infection than those aged 6-10 years. No significant difference was found between the sexes (P = 0.2293). The difference in detection rates may be due to different conditions of the studies, for example, the season of sample collection and the sampling methods. It is also lower than 38% of prevalence reported in Zambia.

A significant difference was detected between the cold dry season (29.9%) and the hot dry season (14.8%), while the rainy season had the least rate of infection with 17.9% [17]. The difference in detection rates may be due to different conditions of the studies, for example, the season of sample collection and the sampling methods. It is also lower than 38% of prevalence reported in Zambia.

From this study, the prevalence of Rotavirus among apparently healthy children is 19.9%, which is higher than 11% reported in 2010[13] from the study “Asymptomatic Rotavirus infections in England: Prevalence, Characteristics, and Risk Factors?“ where some healthy persons were recruited at random from the general population of England during the Study of Infectious Intestinal Disease in England (1993–1996). It also higher than 7.6% from nondiarrheic children[17] and no detection of Rotavirus infection among asymptomatic carriage as reported in Gaza.[18] The report of the present study is higher than that of the Rotavirus prevalence of 7.2% among nondiarrheic children in a hospital setting in Northern Nigeria,[19] and in 2014, the prevalence of 7.7% which is lower than what it is obtainable in this present study was reported.[20] However, the prevalence among apparently healthy children in this present study is lower than the prevalence of 44.4% from asymptomatic neonates in India.[21] The difference in detection rates may be due to different conditions of the studies, for example, the season of sample collection and the sampling methods. It is also lower than 38% of prevalence reported in Zambia[22] before the introduction of Rotavirus vaccine in the country. Seasonal prevalence of Rotavirus among the apparently healthy children in the present study revealed that Rotavirus infection is more during the cold dry season (29.9%) than in hot dry (14.8%) and

**Table 1: Seasonal Variation and Rotavirus infection among apparently healthy children Maiduguri, Borno State**

| Season   | No Tested | No Positive (%) |
|----------|-----------|-----------------|
| Cold Dry | 137       | 41 (29.9)       |
| Hot Dry  | 81        | 12 (14.8)       |
| Rainy    | 78        | 6 (7.7)         |
| Total    | 296       | 59 (19.9)       |

χ²=17.23, df=2, P=0.0002 (S)

**Table 2: Sex distribution of apparently healthy children with Rotavirus Infection in Maiduguri, Borno State**

| Sex    | No Tested | No Positive (%) |
|--------|-----------|-----------------|
| Male   | 153       | 43 (28.1)       |
| Female | 143       | 16 (11.2)       |
| Total  | 296       | 59 (19.9)       |

Fisher exact test, P=0.0003 (S)

**Table 3: Age distribution of apparently healthy children with Rotavirus Infection in Maiduguri, Borno State**

| Age    | No Tested | No (%) Positive |
|--------|-----------|-----------------|
| 0-5    | 223       | 40 (17.9)       |
| 6-10   | 33        | 10 (30.3)       |
| 11-15  | 40        | 9 (22.2)        |
| Total  | 296       | 59 (19.9)       |

χ²=2.945, df=2, P=0.2293 (NS)

**Table 4: Distribution of rotavirus infection among apparently healthy children according to location in Maiduguri, Borno State**

| Location | No tested | No positive (%) |
|----------|-----------|-----------------|
| MMC      | 129       | 28 (21.7)       |
| Konduga  | 72        | 14 (19.4)       |
| Jere     | 95        | 17 (17.9)       |
| Total    | 296       | 59 (19.9)       |

χ²=0.6028, df=2, P=0.7398 (NS). MMC=Maiduguri municipal council, NS=Not significant

**Figure 1:** Electrophoretic patterns of human stool samples analyzed by polyacrylamide gel electrophoresis

Among 50 Rotavirus ELISA-positive stool samples examined by PAGE, none of the positive samples yielded typical Rotavirus electrophoretic migration profiles [Figure 1].
rainy season (7.7%). The finding from this study tallies with a report from Tunisia[23,24] with the peak detection of Rotavirus during the months of the cool dry season. The survival of infective Rotavirus is favored in cooler conditions with low relative humidity and a relative drop in humidity and rainfall combined with drying of aerial transport of dried contaminated fecal materials.[25] There is a higher detection of Rotavirus among males (28.1%) than in females (11.2%) in this study. This is similar to report from Nigeria[26] in 2016 with a higher prevalence among males than females. The high prevalence in a male could be due to male children preferring playing with their mates outside than females who are mostly indoor.[26] The report observed in this study revealed the highest prevalence of 30.3% in ages 6–10 years. This is similar to results in Iraq,[7] where the prevalence of Rotavirus was highest in children under the age of 18 years among asymptomatic Rotavirus infection. The high detection of Rotavirus infection among children between the ages of 6–10 could be because they had acquired the virus while in younger age and are now carriers shedding the virus without showing any symptoms of the disease and since virtually all humans experience at least one Rotavirus infection by 3 years of age and circulating Rotavirus antibodies stay detectable indefinitely.[27] Studies in Nigeria[28] have revealed similar observations, and they explained that older children acquire protective immunity during repeated exposures to the virus, and therefore, subsequent infections are mild or asymptomatic. None detection of Rotavirus by PAGE in this study proofed that the patients were shedding Rotavirus antigen lower than the detection limits of the test assay, which was able to be detected by ELISA.

Conclusion

Higher prevalence of Rotavirus infection was observed in apparently healthy children. The seasonal prevalence of Rotavirus among the patients in the present study revealed that Rotavirus infection is more during the cold dry season than in hot dry and rainy season. The highest prevalence of Rotavirus infection was noted in ages 6–10 years and males than in females in this study among the population. There was no detection of either long or short electropherotypes from the ELISA-positive samples. None detection of Rotavirus by PAGE in this study may be the RNA might have been degenerated during storage due to constant power failure, which might have led to the disintegration of the viral particles, hence, insufficient antigen in the specimen. In addition, the patients might be shedding Rotavirus antigen lower than the detection limits of the test assay.

This study reveals that children who are considered apparently healthy can still shed Rotavirus into the environments, susceptible individuals contracting the disease, and the Rotavirus circulation continues in the environment. There should be introduction of Rotavirus vaccine to the national immunization program in Maiduguri, Borno State and Nigeria at large.

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Conflicts of interest

There are no conflicts of interest.

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