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SHORT COMMUNICATION

Distribution of rotavirus genotypes in the postvaccine introduction era in Ashaiman, Greater Accra Region, Ghana, 2014-2016

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Abstract

Group A Rotaviruses (RVAs) are the most important etiological agents of acute gastroenteritis (AGE) in children less than 5 years of age. Mortality resulting from RVA gastroenteritis is higher in developing countries than in developed ones, causing a huge public health burden in global regions like Africa and South-East Asia. This study reports RVA genotypes detected in Ashaiman, Greater Accra Region, Ghana, in the postvaccine introduction era for the period 2014-2016. Stool samples were collected from children less than 5 years of age who visited Ashaiman Polyclinic with AGE from November 2014 to May 2015 and from December 2015 to June 2016. The samples were tested by enzyme immunoassay (EIA), and one-step multiplex reverse transcription polymerase chain reaction was performed on the EIA positive samples for gel-based binomial genotyping. Of the 369 stool samples collected from children with AGE, 145 (39%) tested positive by EIA. Five VP7 (G1, G3, G9, G10, and G12) and three VP4 (P[4], P[6] and P[8]) genotypes were detected. Eight G/P combinations were identified of which, G3P[6], G12P[8], G1P[8], and G9P[4] were the most prevalent and responsible for 93 (68%) of the AGE cases, and seven mixed-types were detected which represented 8% of the RVA cases. High prevalence, diversity, and mixed-types of RVAs were detected from Ashaiman with the emergence of unusual genotypes.

KEYWORDS

Ashaiman, gastroenteritis, genotypes, rotavirus, RT-PCR, VP7 and VP4

1 | INTRODUCTION

Group A rotavirus (RVA) infection is the leading cause of acute gastroenteritis in children under the age of 5 years and a global pediatric health burden, especially in Africa and South-East Asia.[1] RVA are double-stranded RNA viruses belonging to the genus *Rotavirus* and the family *Reoviridae*,[2] and are classified into eight groups (A–H) based on antigenicity of the VP6 gene.[3] The rotavirus

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genome is made up of 11-gene segments encased within three icosahedral protein shells.[4] Global epidemiologic surveys have identified G1P[8], G2P[4], G3P[8], G4P[8], G9P[8], and G12[P8] as the most common circulating strains associated with diarrhea in humans,[3,4] but the prevalence of the common strains has been shown to fluctuate in different geographical regions.[5] However, recent studies in developing countries have shown a greater diversity of RVA genotypes with several novel combinations.[6,7] The most effective measure of reducing the burden due to RVAs is by the use of vaccines.[8] Two licensed RVA vaccines (Rotarix and RotaTeq) have been recommended by the World Health Organization (WHO) for use globally.

In Ghana, the monovalent RVA vaccine Rotarix was introduced into the national Expanded Programme on Immunization (EPI) in May 2012 to manage diarrhea due to RVA in children under 5 years of age. A recent study on the RVA strain distribution in the pre- and post-RVA vaccine eras suggest that even though the G1P[8] genotype seem to has been replaced by G12P[8], the replacement could be merely transient and not necessarily due to vaccine immune pressure,[9] and therefore suggesting the need for continued RVA surveillance to monitor the circulating genotypes after the introduction of the Rotarix vaccine.

Ashaiman is a one-town municipality with a total population of approximately 200 000 people and is located in the Greater Accra Region of Ghana. The Ashaiman Municipality is considered an unhygienic setting due to the fact that as many as 63.8% of houses lack a domestic toilet facility[10] and this high dependence on public toilets promotes indiscriminate open defecation and the disposal of human excreta into public drains and into refuse dumps. Thus, the municipality is faced with gross waste management problems.[10] In addition, animal rearing is one of the primary occupations of the people in the municipality and domestic animals are allowed to stray into household settings in search of food. The high frequency of human-animal interactions in combination with a poor sanitary environment may promote RVA zoonotic and mixed infections and increase RVA strain diversity at the study area. The purpose of this study was to investigate RVA strains in children in Ashaiman and look for evidence of zoonotic infection and high strain diversity.

2 | MATERIALS AND METHODS

Stool samples and demographic data were collected from children under the age of 5 years who reported with severe nonbloody diarrhea to the Emergency Department of the Ashaiman Polyclinic, Ashaiman from November 2014 to May 2015 and from December 2015 to June 2016. The samples were collected from the children after the study was explained to their parents/guardians and informed consent to participate in the study has been obtained. Institutional Review Board (IRB) approval for the study was obtained from the Noguchi Memorial Institute for Medical Research (IRB# 067/14-15).

A total of 369 diarrhea stool samples were collected and tested for RVA by enzyme immunoassay (EIA). Total RNA was extracted from 145 EIA positive samples using phenol/chloroform/RNAID extraction method as previously described.[11] The RNA was subjected to VP7 (G) and VP4 (P) genotyping using either two-step reverse transcription polymerase chain reaction (RT-PCR)[11] or multiplexed one-step RT-PCR[12] assays.

3 | RESULTS

Of the 369 stool samples collected from children under 5 years with AGE, 145 were found RVA antigen positive by EIA, giving a prevalence of 39%. Out of the 145 EIA positive samples, 136 (94%) produced amplicons during 1st round or 1st and 2nd round PCR reactions for genotyping (partially or fully genotyped for either VP4 or VP7 or both). The age group of 7 to 12 months had the highest number of rotavirus-positive samples of 101 (27.4%) cases.

Five G genotypes (G1, G3, G9, G10, and G12) along with three P genotypes (P[4], P[6], and P[8]) were detected. The most prevalent G genotypes G3, G12, G1, and G9 accounted for 39%, 15%, 14%, and 10%, respectively (Figure 1A), and the three P genotypes P[6], P[8], and P[4] accounted for 51%, 28%, and 9%, respectively (Figure 1B). Mixed G and P genotypes were detected with a prevalence of 6% and 9%, respectively, and G non-typables (GNTs) were 15% for G and 3% for P (Figure 1A and 1B).

Figure 2 shows the distribution of all the strains; G3P[6], G12P[8], G1P[8], and G9P[4] were the most prevalent and accounted for 93 (68%) of the AGE cases in children less than 5 years of age in Ashaiman over the period of the study. Of the different genotype combinations detected, eight were single-types (G1P[8], G1P[6], G3P [6], G3P[8], G9P[4], G9P[8], G10P[8], and G12P[8]), seven were mixed-types (G1P[6][8], G1G12P[8], G3G12P[6][8], G12P[6][8], G3G12P[8], G1G3G12P[6], and G1G3G12P[6][8]), and three were partially characterized (GNTP[6], GNTP[6][8] and G3P[NT]) (Figure 2).

4 | DISCUSSION

This study is important in providing information on RVA genotypes circulating in southern Ghana during the postvaccine introduction era. In Ghana, postvaccine introduction era studies have reported a reduction in pediatric diarrheal diseases hospitalization following the introduction of the Rotarix vaccine by approximately 45%.[13] Studies have reported the seasonality of RVA infection to peak during the cool dry months (September to February) globally, as well as in Ghana[14] and these are the periods during which the samples were collected for the current study.

RVA prevaccine introduction era surveillance studies have reported G1 as the globally most common VP7 genotype.[6] In the postvaccine introduction era, however, several studies have reported the emergence of other predominant VP7 genotypes which have substituted the global dominance of the G1 genotype. The dominance of G3 genotype has been shown in a previous report from Ghana[15] and is similar to the observation made in this study. The mixed

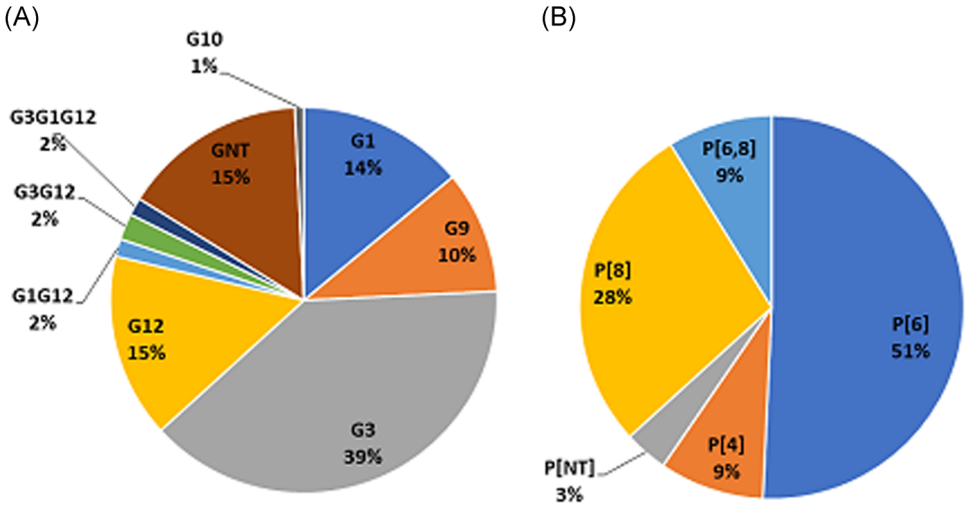

FIGURE 1 Frequency (%) distribution of G and P genotypes of rotavirus strains detected from Ashaiman, 2014-2016. A, G genotypes. B, P genotypes

G-types were most likely due to RVA with different G-types coinfecting the same host. The outcome of coinfections may lead to reassortment and the generation of new genotype constellations.[3] As many as 15% of the samples could not be genotyped for VP7 by PCR (GNT) and this relatively high level of GNTs corresponds with those reported in previous studies from southern Ghana.[14]

In keeping with the findings from this study, other studies from Ghana have also reported genotypes P[4], P[6], and P[8], with the P[6] genotype being the most prevalent.[7,15] Strains with the VP7/VP4 genotypes: G3P[6], G12P[8], G1P[8], and G9P[4] were the most prevalent and accounted for 68% of the characterized strains, which differs somewhat from genotype prevalence reported previously—G2P[6], G3P[4] and G9P[8] as the most prevalent strains in northern Ghana,[16] and G1P[8], G3P[6], and G2P[6] as the most prevalent strains in southern Ghana.[14] The global emergence of the G12 strains in the postvaccine introduction era[17] probably explains the high incidence of the G12P[8] strain found in this study. The unusual G9P[4] strain was shown to be emerging in the postvaccine introduction era in Ghana[9] after it was first detected about 15 years ago.[18] G9 and G10 have been shown to be more commonly associated with RVA infections in animals,[19-21] and suggest that the unusual G9P[4] and G10P[8] strains detected in this study may be due to zoonotic transmissions. The unusual strains will be investigated further by nucleotide sequencing to ascertain the possibility of zoonotic transmission.

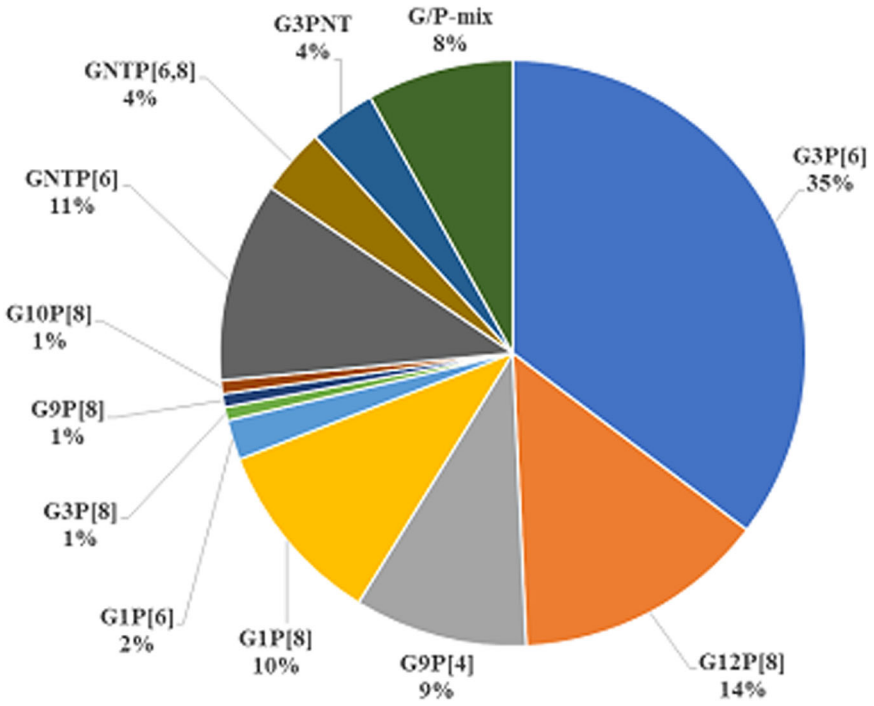

FIGURE 2 Frequency (%) distribution of G/P combinations among 136 RV genotypes detected from Ashaiman, 2014-2016

Indiscriminate disposal of human excreta due to a high number of houses lacking toilet facility and animal-human interactions as a result of domestic livestock breeding,[10] may have promoted RVA zoonotic transmission and mixed infections, and increased RVA strain diversity in the Ashaiman Municipality

5 | CONCLUSION

High prevalence, diversity, and mixed infections of RVAs were detected in Ashaiman along with unusual genotypes, which may be due to zoonotic transmissions facilitated by the unhygienic environment. To better understand the dynamics of RVA transmission in Ashaiman, a follow-up study looking at RVAs in animals as well as the environment is warranted.

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CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

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