Infection, Genetics and Evolution xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Infection, Genetics and Evolution

journal homepage: www.elsevier.com/locate/meegid

Molecular surveillance of rotavirus infection in Bangui, Central African Republic, October 2011–September 2013

Virginie Banga-Mingo [a], Diane Waku-Kouomou [b,*], Jean Chrysostome Gody [c], Mathew D. Esona [b], Jean Fandema Yetimbi [a], Regis Mbary-Daba [d], Benjamin A. Dahl [e], Leon Dimanche [c], Thomas d'Aquin Koyazegbe [f], Vianney Tricou [a], Kathleen F. Cavallaro [e], Gilbert Guifara [d], Michael D. Bowen [b], Ionela Gouandjika-Vasilache [a]

[a] Institut Pasteur de Bangui, Bangui, Central African Republic
[b] Division of Viral Diseases, National Center for Immunization and Respiratory Diseases, United States Centers for Disease Control and Prevention, Atlanta, GA 30333, United States
[c] Complexe Pédiatrique de Bangui, Bangui, Central African Republic
[d] World Health Organization Country Office of Central African Republic, Bangui, Central African Republic
[e] Global Immunization Division, Center for Global Health, United States Centers for Disease Control and Prevention, Atlanta, GA 30333, United States
[f] Programme Elargi de vaccination, Bangui, Central African Republic

ARTICLE INFO

Article history:
Received 18 June 2014
Received in revised form 15 August 2014
Accepted 21 August 2014
Available online xxxx

Keywords:
Pediatrics
Rotavirus
Surveillance
Gastroenteritis
Central African Republic

ABSTRACT

Background: The World Health Organization (WHO) recommends the introduction of rotavirus vaccine in the immunization program of all countries. In the Central African Republic (CAR), sentinel surveillance for rotavirus gastroenteritis was established in 2011 by the Ministry of Health, with the support of the Surveillance en Afrique Centrale Project (SURVAC). The purpose of this study was to assess the burden of rotavirus gastroenteritis and to identify rotavirus strains circulating in CAR before the introduction of rotavirus vaccine planned for this year, 2014.
Methods: One sentinel site and one laboratory at the national level were designated by the CAR Ministry of Health to participate in this surveillance system. Stool samples were collected from children who met the WHO rotavirus gastroenteritis case definition (WHO, 2006). The samples were first screened for group A rotavirus antigen by enzyme immunoassay (EIA), and genotyping assays performed using a multiplex reverse transcriptase PCR (RT-PCR) technique.
Results: Between October 2011 and September 2013, 438 stool samples were collected and analyzed for detection of rotavirus antigen; 206 (47%) were positive. Among the 160 (78%) that could be genotyped, G2P[6] was the predominant strain (47%) followed by G1P[8] (25%) and G2P[4] (13%).
Conclusions: Almost half of stool samples obtained from children hospitalized with gastroenteritis were positive for rotavirus. These baseline rotavirus surveillance data will be useful to health authorities considering rotavirus vaccine introduction and for evaluating the efficacy of rotavirus vaccine once it is introduced into the routine immunization system.

Group A rotavirus is a major cause of severe gastroenteritis in young children, causing an estimated 453,000 deaths in 2008, mostly in Africa and South Asia (WHO, 2012). In 2009, WHO recommended the introduction of rotavirus vaccine in all countries (WHO, 2009).

Rotavirus belongs to the family of *Reoviridae*. The virus genome consists of 11 segments of double-stranded RNA which encodes 12 proteins. The protease sensitive outer capsid protein VP4 defines the P genotypes, and the surface glycoprotein VP7 defines the G genotypes. Rotavirus strain genotyping is commonly based on a dual nomenclature using both VP4 and VP7 genes (Hoshino et al., 1985). Studies in African countries have shown a diversity of rotavirus strains, including some less common human rotavirus types such as G12, G9, G8 and P[6] (Mwenda et al., 2010). The most prevalent rotavirus strains found in Africa are G1P[8], G2P[6], G8P[6], G3P[8] (Todd et al., 2010). There are two licensed rotavirus vaccines ; (1) Rotarix which is based on a monovalent human G1P[8] strain, and (2) RotaTeq, a pentavalent vaccine consist of 5 human-bovine reassortant strains, The genetic evolution or

* Corresponding author. Address: Division of Viral Diseases, National Center for Immunization and Respiratory Diseases, United States Centers for Disease Control and Prevention, 1600 Clifton Road N.E., MS C-22, Atlanta, Georgia 30333, United States. Tel.: +1 404 639 2706; fax: +1 404 639 1516.
E-mail address: irf6@cdc.gov (D. Waku-Kouomou).

http://dx.doi.org/10.1016/j.meegid.2014.08.023
1567-1348/

changes in circulating virus population may represent a problem for vaccines efficacy. For this reason, it is important to collect genotype information before the introduction of the rotavirus vaccine. These data will help monitor the impact of the vaccine in the future.

CAR is a developing country located in Central African region with a population estimated at 5,166,510 in July 2013 (CIA, 2013). Very limited data on rotavirus were obtained in CAR during the 1980s (Georges-Courbot et al., 1988a,b). Since then, only few data on rotavirus epidemiology in CAR in 2008 have indicated that the most common genotypes found were G1P[8] followed by G1P[6] and G2P[4] (Gouandijka-Vasilache et al., 2014) . In July 2011, the Ministry of Health of CAR established sentinel surveillance for rotavirus gastroenteritis, with support of the SURVAC project (The_Laboratory_Working_Group_for_SURVAC, 2011), The goal of the SURVAC project was to strengthen disease surveillance and response in selected countries in Central Africa. Rotavirus surveillance was established in CAR using the generic protocol provided by the World Health Organization (WHO) (WHO, 2006).

This study aimed to describe the prevalence of rotavirus gastroenteritis among children less than 5 years of age and identify rotavirus strains circulating in CAR. The findings reported here describe the co-circulation of different rotavirus strains in CAR.

The surveillance was conducted at one sentinel hospital, the Complexe Pédiatrique de Bangui Hospital, located in Bangui, the capital of CAR. This hospital is the largest pediatric hospital in the country. From October 2011 to September 2013, stool specimens were collected as described in WHO manual (WHO, 2006), within 48 h of hospital admission, from all children less than 5 years old who met the WHO case definition of gastroenteritis: the occurrence of at least 3 looser than normal or watery stools in a 24 h period and/or two or more episodes of vomiting unexplained by other reasons (WHO, 2006).

Stool samples were first screened for group A Rotavirus antigen by EIA at the Complexe Pediatrique de Bangui Hospital laboratory. Aliquots were then stored at −20 °C before being transported to the Institut Pasteur de Bangui, where results were confirmed by EIA and genotyping assays were performed using a multiplex reverse transcription polymerase chain reaction (RT-PCR) technique as previously described (Pukuta et al., 2014). Samples subjected to genotyping were subsequently confirmed using the same method at the Centers for Disease Control and Prevention (CDC), Atlanta, USA, for quality control.

From October 2011 through September 2013, a total of 438 children <5 years old with diarrhea were enrolled at the Complex Pédiatrique sentinel site in Bangui, CAR. For each of these 438 children, a stool sample was tested for group A rotavirus antigen by EIA, and rotavirus was identified in 206 (47%), ranging from 37% in 2012 to 52% in 2013. This rotavirus prevalence is consistent with that reported in a 2008 Bangui study, where rotavirus was responsible for 40% of hospitalized diarrhea cases (Gouandjika et al., unpublished data) and with studies conducted in a neighboring country, Cameroon, where rotavirus was found in 42.8% of diarrhea cases (Ndze et al., 2012), as well as with an overall prevalence of rotavirus infection estimated at 40% in the African region (Mwenda et al., 2010). During the study period, rotavirus infection occurred year-round but was more common from November through March, with a high peak in January (2012) and March (2013), and from June to September with a smaller peak in August (2012) and June (2013) (Fig. 1). These biannual peaks correspond to the large and small dry seasons respectively in the country. This pattern is similar to that seen in Namibia in 1999 where rotavirus infection also showed biannual peaks (Page et al., 2010).

The age distribution of diarrhea cases showed that the highest rotavirus detection rate was among children less than 5 months old (56%), followed by children 6–11 (49%), 12–23 (34%) and 24–59 (17%) months of age (data not shown). The cumulative rotavirus positivity rate showed that 180 of 206 (87.4%) positive specimens were from children in the 0–11 month age group, including 38% from the 0–5 months age group (Fig. 2). Only 5% of positive cases were observed in the 24–59 month age group. These findings are consistent with those from other Central African countries such as the Democratic Republic of the Congo, where in 2003–2005 the majority of the patients affected by rotavirus infection were less than 12 months old (Kabue et al., 2010). Similar results were observed in other African countries, including Ghana, Kenya, Ethiopia (Mwenda et al., 2010).

All rotavirus positive samples (206) and 22 rotavirus negative samples (228 total) were subjected to RT-PCR for rotavirus genotyping and genotypes could be assigned to 160 strains (i.e., 160 samples). Rotavirus strains bearing the G2 genotype were found in 105 of 160 (66%) samples and were the most common found during the study period, followed by G1, found in 45 (28%) samples, and 5 (3%) each of G9 and G12 genotypes (Table 1). The most common P types associated with rotavirus infections in Bangui were P[6] found in 83 (52%) specimens, followed by P[8] in 56 (35%), and P[4] in 21 (13%). The most common G and P combinations were G2P[6] (47%), followed by G1P[8] (25%) and G2P[4] (13%). Unusual combinations, such as G12P[6], were also detected (2%). These findings are consistent with 2008 data from Bangui, which also showed that G1P[8] was the most common rotavirus strain detected (Gouandijka-Vasilache et al., 2014) . Genotype G1P[8] is recognized as the most prevalent in Africa (Audu et al., 2002; Steele et al., 1998) and worldwide (Gentsch et al., 2005). Among the P types, genotype P[6], found in 52% of strains, was the most common. Our findings confirm the high prevalence of P[6], which previously was documented to be 18–75% in African countries (Gentsch et al., 2005); recent studies have reported the rapid expansion of P[6] prevalence in Africa (Seheri et al., 2014; Steele and Ivanoff, 2003). During the two years of this evaluation, G2 accounted for 66% of G types and were found in combination with P[6] (71%), P[4] (20%) and P[8] (9%). Previous studies of rotavirus infection in CAR in the 1980's reported G2 strains in only 15.4% (Georges-Courbot et al., 1988a). More recently, in 2008, G2 strains were reported to account for 10% of G types (Gouandijka-Vasilache et al., 2014) . The disparities among these studies are most likely due to the fact that neither of the two first studies was sentinel site based surveillance and the population targeted was probably different; however, they may also reflect natural temporal changes in circulating strains. It is noteworthy that G12 was detected for the first time in CAR in association with P[6] and P[8]. G12 strains have been reported previously to circulate in Cameroon and in Ethiopia (Mwenda et al., 2010), in South Africa (Page et al., 2009), in India (Das et al., 2003) and worldwide (Rahman et al., 2007) .

As the emergence of new genotypes and the changes in circulating genotypes represents potential problem for vaccines protection, knowledge of circulating rotavirus strains will help measure the efficacy of vaccine. Both rotavirus vaccines (Rotarix and Rotateq) have been shown to be efficacious against the common strains causing disease but protection against rare or novel strains is yet to be documented. Hence, rotavirus strain surveillance after vaccine introduction will be critical for a better understanding of vaccine impact in CAR and will help detect genotype replacement after vaccine introduction.

There are some limitations in this study related to the fact that this surveillance was inpatient hospital-based surveillance of children with diarrhea, in only one sentinel site in Bangui, and therefore, these data may not be representative of the entire country. Additional sentinel sites for rotavirus surveillance in other cities would likely capture a more complete picture of rotavirus burden in the country. In addition, enrollment at the sentinel site in Febru-

Please cite this article in press as: Banga-Mingo, V., et al. Molecular surveillance of rotavirus infection in Bangui, Central African Republic, October 2011–September 2013. Infect. Genet. Evol. (2014), http://dx.doi.org/10.1016/j.meegid.2014.08.023