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Molecular surveillance of rotavirus strains circulating in Yaoundé, Cameroon, September 2007–December 2012

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Abstract

Rotavirus is the most common cause of severe diarrheal disease in children under 5 years of age worldwide. The World Health Organization (WHO) estimated that 453,000 rotavirus-attributable deaths occur annually. Through the WHO, the Rotavirus Sentinel Surveillance Program was established in Cameroon in September 2007 with the Mother and Child Center (MCC) in Yaoundé playing the role of sentinel site and national laboratory for this program. The objectives of this surveillance were to assess the rotavirus disease burden and collect baseline information on rotavirus strains circulating in Cameroon. Diarrheal stool samples were collected in a pediatric hospital from children under 5, using the WHO case definition for rotavirus diarrhea. Antigen detection of rotavirus was performed by using an enzyme immunoassay (EIA). The genotypic characterization was performed using multiplexed semi-nested reverse transcription-polymerase chain reaction (RT-PCR) assays. Between September 2007 and December 2012, 2444 stool samples were received at the MCC laboratory for rotavirus antigen detection, of which 999 (41%) were EIA positive. Among EIA positive samples 898 were genotyped. Genotype prevalence varied each year. Genotype G9P[8] was the dominant type during 2007 (32%) and 2008 (24%), genotype G3P[6] predominated in 2010 (36%) and 2011 (25%), and G1P[8] was predominant in 2012 (44%). The findings showed that the rotavirus disease burden is high and there is a broad range of rotavirus strains circulating in Yaoundé. These data will help measure the impact of vaccination in the future.

Keywords

Molecular epidemiology; Rotavirus; Surveillance; Cameroon

1. Introduction

Rotavirus is the most common cause of severe gastroenteritis in infants and young children in the world. About 90% of all rotavirus-associated fatalities occur in low income countries in Africa and Asia. Despite the availability of rotavirus vaccines and World Health Organization's (WHO) recommendation that these vaccines be included in national immunization programs worldwide (WHO, 2009), rotavirus-attributable deaths remained unacceptably high. In 2008, WHO estimated that 5 countries in Africa (Nigeria, DRC, Ethiopia, Uganda, Angola) accounted for 121,353 (26.8%) of the estimated 453,000 deaths attributable to rotavirus worldwide (WHO, 2012a).

Rotavirus belongs to the *Reoviridae* family. The virus genome consists of 11 segments of double-stranded RNA and viruses have been classified traditionally based upon genetic and antigenic differences in the VP7 (G-type) and VP4 (P-type) genes (Gentsch et al., 1996). Group A rotavirus is one of the most common viral pathogens associated with gastroenteritis in humans and there are currently at least 27 rotavirus G genotypes and 37 P genotypes identified by nucleotide sequencing (Matthijnssens et al., 2011; Trojnar et al., 2013). Of these genotypes, 12 G types and 15 P types have been found in humans (Esona et al., 2009; Gentsch et al., 1996; Solberg et al., 2009).

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Cameroon is located in central Africa, bordering the Bight of Biafra, between Equatorial Guinea and Nigeria. The area of the country is 475,440 sq km and the 2014 population is estimated at 23,130,708 inhabitants, with a per capita income of $2,400 in 2013. Bordering countries are Central African Republic, Chad, Republic of the Congo, Equatorial Guinea, Gabon and Nigeria (CIA, 2014). In Cameroon, previous studies have reported the burden and genotype identification of rotavirus in different parts of the country. Molecular epidemiology data of rotavirus were first reported in 2003 in Western Cameroon (Esona et al., 2003). Further reports identified unusual rotavirus strains G5P[8] (Esona et al., 2004), G5P[7] (Esona et al., 2009), and G10P[8] (Esona et al., 2010a,b) in Cameroon. Recent studies in the North West and the Northern Regions of Cameroon revealed that the prevalence of rotavirus infection was 28.7% and 42.8%, respectively (Mbuh et al., 2012; Ndze et al., 2012).

Sentinel site surveillance for rotavirus was established in Cameroon in 2007 as part of the African Rotavirus Network with the support of the World Health Organization (WHO). The Mother and Child Center (MCC) hospital in Yaoundé, was chosen by the Cameroonian Ministry of Health to play the role of the pediatric hospital and national laboratory for rotavirus surveillance. In 2010, rotavirus gastroenteritis surveillance in Cameroon was strengthened and enhanced to include genotype surveillance for rotavirus strains at the national laboratory level through the Surveillance Epidemiologique en Afrique Centrale (SURVAC) Project (The_Laboratory_Working_Group_for_SURVAC, 2011).

The goal of this surveillance was to document the burden of rotavirus diarrhea and identify rotavirus genotypes circulating in Cameroon. This study reports the results of surveillance of rotavirus in one sentinel hospital in Cameroon, from September 2007 to December 2012. These data provide baseline information on the burden of rotavirus and rotavirus strains circulating in Cameroon before the upcoming introduction of rotavirus vaccination in the national childhood immunization program. These data will be used to assess the impact of the vaccination program and to monitor the changes in circulating genotypes.

2. Materials and methods

2.1. Case definition, patients and sample collection

The surveillance was conducted at one sentinel hospital, The Mother and Child Center, Foundation Chantal Biya, a pediatric hospital located in Yaoundé, Cameroon. From September 2007 to December 2012, stool specimens were collected within 48 h of admission from children less than 5 years old with a diagnosis of gastroenteritis. The WHO case definition of gastroenteritis was 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).

2.2. Laboratory testing for rotavirus

Collected samples were analyzed for rotavirus group A antigen in the laboratory of the sentinel site hospital and stored at −20 °C for subsequent genotyping analyzes. Before 2011, the genotyping assays were performed by multiplexed polymerase chain reaction (PCR)

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at the Regional Reference Laboratory in South Africa (Medical Research Council (MRC)/ Diarrhoeal Pathogens Research Unit, University of Limpopo, Medunsa). Since 2011, the genotyping assays have been performed by the sentinel site laboratory, with the support of the SURVAC project, using a multiplexed PCR technique. Samples subjected to genotyping were subsequently sent to the Centers for Disease Control and Prevention (CDC), Atlanta, USA for quality control.

2.2.1. Enzyme immunoassay (EIA)—A 10% suspension of each specimen was prepared as described in the ProSpecT™ Rotavirus Microplate assay procedure and analyzed using a Sandwich format enzyme immunoassay for detection of rotavirus in faecal specimens [ProSpecT™ Rotavirus Microplate Assay, Oxoid, Ltd., Basingstoke, Hampshire, UK] according to manufacturer's instructions.

2.2.2. Nucleic acid extraction—Viral RNA from randomly selected rotavirus-positive samples, collected during rotavirus seasons 2007–2008 and 2010–2012, was extracted from 140 μl of 10% stool suspension using the QIA-amp viral RNA Mini kit (Qiagen, Inc., Valencia, CA USA) according to manufacturer's instructions and stored at −80 °C. Due to some logistical issues, most of 2009 samples were lost and could not be genotyped.

2.2.3. Genotyping by multiplex RT-PCR—The RNA extracts were subjected to multiplexed semi-nested reverse transcription-polymerase chain reaction (RT-PCR). Two full-length genes, VP7 (896 bp) and VP4 (876 bp) were reverse-transcribed and amplified with primers 9Con1-L/VP7-R and Con3/Con2, respectively (Das et al., 1994; Gentsch et al., 1992). Reverse transcription of double-stranded RNA (dsRNA) was carried out with the OneStep RT-PCR Kit (Qiagen, Inc., Valencia, CA USA). After 5 min denaturation at 97 °C, the RNA was mixed with kit reagents and incubated at 42 °C for 30 min to obtain complementary DNA (cDNA), immediately followed by the PCR reaction. These first round RT-PCR products were then used in a semi-nested PCR to identify G and P types (Das et al., 1994; Gentsch et al., 1992). All PCR products were identified by electrophoresis in 2% agarose gels containing 10% Gel Red (Biotium, Hayward, CA USA) and visualized under UV illumination. To exclude false-positive mixed-infections, genotype-specific primers were used to generate amplicons and each amplicon was sequenced. Further analyses of theses samples by next generation sequencing are also underway.

3. Results

3.1. Seasonality of rotavirus infection

From September 2007 to December 2012, a total of 2444 specimens were collected from children under 5 years of age with diarrhea at the MCC hospital. The number of rotavirus cases in 2008 and 2009 was low compared to 2010–2012. Approximately 41% (999) were positive for rotavirus antigen by EIA testing. The yearly rotavirus detection rate varied from 21% in 2009 to 45% in 2012 (Table 1). Analysis of the monthly detection rate of rotavirus showed that the proportion of rotavirus varied consistently by season and was greatest during the dry season, from October to February (Fig. 1).

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3.2. Age distribution

The age distribution of children with rotavirus diarrhea as diagnosed by EIA is shown in Table 2. Rotavirus was detected in children of all age groups, but the highest detection rates were observed in children aged 3–5 months (50%), followed by 6–8 months (49%), 9–11 months (41%), and 12–17 months (36%). A cumulative age group distribution showed that 83% of rotavirus-infected children are between 3 and 17 months of age.

3.3. Prevalence of rotavirus G and P types amongst children in Yaoundé 2007–2012

3.3.1. Overall genotype distribution—Rotavirus genotyping was performed by multiplexed RT-PCR on 898 randomly selected samples among 971 (92%) specimens that were previously determined to be rotavirus-positive. G and P types could be assigned for 680 samples (76%), while 198 (22%) could only be assigned either genotype G or P (partially typed) and 20 (2%) could not be assigned genotype G and P (untypeable). The P[8] genotype was the most common being found in 356 (40%) specimens, followed by P[6] in 328 (37%) and P[4] in 97 (11%) samples (Table 3). The G types were identified, with 250 (28%) as G1, 178 (20%) as G3, 153 (17%) as G2, followed by G12 with 67 (7%), G9 with 40 (4%), G4 with 35 (4%), G8 with 29 (3%), and G6 with 12 (1%).

There was a large variety of rotavirus strains circulation in Cameroon with 33 G/P combinations fully identified during the study period. An overall predominance of G1P[8] was observed with an average frequency of 17%, followed next by genotype G3P[6] (14%) and G2P[4] (7%) (Table 3). Uncommon genotypes as G6P[6] (1%), G8P[4] (1%), G9P[6] (1%) were also found.

3.3.2. Temporal distribution of rotavirus strains in Yaoundé 2007–2012—The temporal distribution of rotavirus strains over the 5 year study period showed that the frequencies of the individual genotypes varied from year to year. Genotype G9P[8] was the dominant type during 2007 (32%) and 2008 (24%) seasons, immediately followed by genotype G8P[6] (14%) in 2007 and G1P[8] (16%) in 2008 (Fig. 2). Only 5 strains were identified in 2009; 3 G1P[6], 1 G9P[8] and 1 G12P[8] (data not shown in Fig. 2). Genotype G9P[8] was not found at all in 2010. Genotype G3P[6] (36%) was most common in 2010, followed G2P[4] (23%) and G2P[6] (17%). In 2011, G3P[6] (25%) and G2P[4] (14%) continued to be dominant. In 2012, the dominant strain changed to G1P[8] (44%) followed by G3P[6] (14%) and G1P[6] (9%).

Mixed infections were identified in 117 (13%) samples. The percentage of mixed infection varied per year: 22% (2007), 20% (2008), 19% (2010), 7% (2011) and 11% (2012). The most common strains involved in mixed infections were P[6,8] with either G1 or G9 and P[4,6] with G2 accounting for 11 strains (9%) each (Table 4). This was followed by G1,3 P[8] with 8 strains (6.7%) and G1 P[4,8] with 7 strains (6%).

4. Discussion

The objective of this study was to assess disease burden and establish a baseline prevalence for rotavirus strains circulating in Cameroon, in anticipation of rotavirus vaccine introduction. Using a standardized protocol (WHO, 2006) in the sentinel site hospital in

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Yaoundé, Cameroon, rotavirus was found to be a common cause of severe diarrhea among children less than 5 years. Rotavirus was detected in 41% of total stool specimens by EIA, ranging from 22% in 2009 to 45% in 2012. This detection rate is similar to those estimated from WHO global surveillance at 38% (WHO, 2012b), in the African region at 40% (Mwenda et al., 2010, 2014; Tsolenyanu et al., 2014) and in the Northern Region of Cameroon at 42.8% (Ndze et al., 2012). However the prevalence of rotavirus infection in this study was higher than the 28.7% reported from another study in the North West region of Cameroon in 2003–2004 (Mbuh et al., 2012) and (21.9%) in Western region in 2003 (Esona et al., 2003). We attribute the low number of rotavirus cases in 2008 and 2009, compared to 2010–2012, to a lack of financial incentives for personnel involved in the surveillance and a shortage of investigation forms and stool collection supplies in 2008–09. In addition, since late 2009, the SURVAC project provided funding for rotavirus surveillance in Cameroon that likely contributed to the increase in the number of cases detected in 2010–2012.

This study reveals that rotavirus infection in Yaoundé is significant in children less than 2 years of age, predominantly in infants between 3 and 17 months of age. This is in agreement with the situation observed in others studies in Cameroon and in other sub-Saharan African countries (Mbuh et al., 2012; Mustafa et al., 2014; Ndze et al., 2012). Based on these observations, the 1st rotavirus vaccine dose should be administered to infants before 3 months of age to maximize its effects in Cameroon. In this study, the peak of rotavirus transmission was observed during the dry season which is the seasonal pattern reported for other sub-Saharan African countries (Cunliffe et al., 1998; Mwenda et al., 2010). Rotavirus is transmitted by the faecal-oral route, via contact with contaminated hands, surfaces, objects and possibly by the respiratory route. Levy and colleagues have reviewed the seasonality of rotavirus diseases in the tropics and speculate that the airborne component of rotavirus transmission might be responsible for the seasonal pattern of rotavirus diseases (Levy et al., 2009). In Cameroon, the dry season is windy and therefore, highest temperature during dry season leads to formation of dust that might contain viral particles from dried, contaminated fecal material and the wind might increase their aerial transport. Also, the congregation of children inside the house, due to high temperatures outside, might increase the transmission of rotavirus as it has been shown for other infectious diseases such as measles (Grassly and Fraser, 2006). There is some evidence that rotavirus is a water-borne pathogen however the spread of rotavirus in many countries in the winter suggests that water is a minor mechanism of rotavirus transmission. This said the role of climatic conditions in the transmission of rotavirus remains unclear and needs to be address for a better understanding of rotavirus epidemiology in Central Africa.

This 5-year study clearly showed that there is a high rotavirus genotype variation, with 33 G–P combinations identified over the time in Yaoundé, Cameroon. Genotype G1[P8] was the most detected genotype within the overall study period and is known to be the most common strain globally; however, unusual combinations like G9P[6], G2P[6], G3P[6], G8P[6], G6P[6] were detected and this reaffirmed the circulation of such strains in Africa, as previously reported in West Africa (Akran et al., 2010; Armah et al., 2010b; Nordgren et al., 2012). The genotype G12P[8] was also detected and has been circulating in Yaoundé since 2007. This genotype is rare in Africa and so far has been detected only in few African countries and most recently, in a study in Northern Cameroon in 2010/2011, by Ndze and

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colleagues, G12P[8] was the most predominant strain identified with a high prevalence (54.1%) (Ndze et al., 2013). G12P[8] was also found in the neighboring country, in Nigeria in 2010–2011 (Cunliffe et al., 2009; Oluwatoyin Japhet et al., 2012; Page et al., 2009). Of note, 13% of strains identified in this study included more than 1 P and/or G type. Mixed infections involving G1 and/or G2, G3, G9 were common (84%) as well as mixed infections involving P[6] and or P[8] (96%). Overcrowding in communities is one factor that favors mixed infections and provides an opportunity for in vivo reassortment, possibly explaining the large diversity of circulating genotypes in this study. However, it was noted that the percentage of mixed infections decreased over the years, from 22% in 2007 to 11% in 2012. This may be due to the use of sequencing techniques for confirmation of mixed infection during 2011–2012 seasons which came into use during the SURVAC-funded portion of the study.

The level of mixed infection observed in this study is higher compared to other reports from Cameroon (Esona et al., 2003; Mbuh et al., 2012) and from other African countries (Akran et al., 2010; Cunliffe et al., 2010); however, it was consistent with the data from the Democratic Republic of the Congo (Kabue et al., 2010). The co-circulation of different rotavirus genotypes in one city is commonly observed in epidemiology studies in Africa. However, the high diversity of rotavirus strains found in this study is of epidemiological importance and could challenge the efficacy of the upcoming rotavirus vaccine. The are two licensed rotavirus vaccines available; The human monovalent rotavirus vaccine (Rotarix; GlaxoSmithKline Biologicals) derived from a wild-type human rotavirus strain G1P[8] and the pentavalent human-bovine reassortant vaccine (RotaTeq; Merck) that carries human rotavirus genes encoding for VP7 G1, G2, G3, and G4 proteins and human rotavirus VP4 gene encoding the P[8] protein. Both vaccines have shown a good protection against common rotavirus strains (Armah et al., 2010a; Madhi et al., 2010; Steele et al., 2012), however their efficacy against unusual strains such as G9P[6], G6P[6], G8P[6], found in this study and new strains that may emerge in the future are yet to be determined. It is therefore important to continue the surveillance of rotavirus circulating strains as this will be vital for the evaluation of rotavirus vaccine impact in Cameroon. This study has limitations as it was restricted only to one town, Yaoundé and therefore may not be representative of the whole country. It is now indispensable to expand the surveillance of rotavirus infection to other regions in order to get a full picture of circulating rotavirus strains in Cameroon. Genotyping were performed at different laboratory institutes and therefore, genotyping results may not be completely consistent between these laboratories. These findings reinforce the importance of monitoring rotavirus strains before and after rotavirus vaccine introduction in the childhood immunization program in Cameroon.

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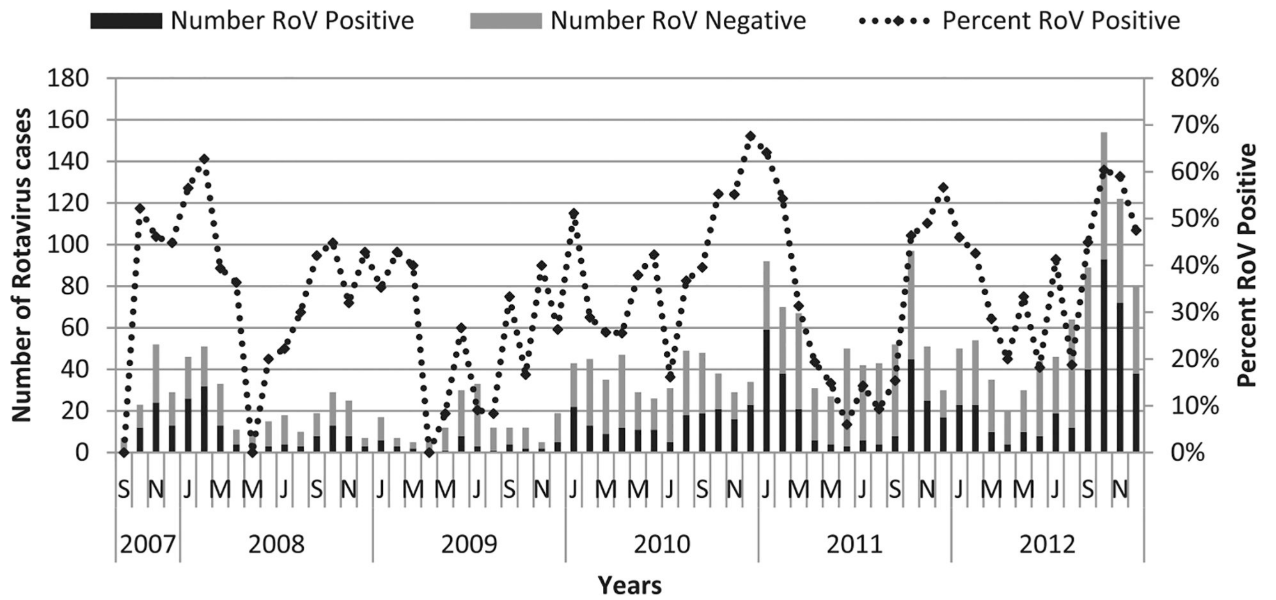

Fig. 1.

Seasonality of rotavirus diarrhea in Yaoundé, Cameroon, from September 2007–December 2012. Number of rotavirus-positives (shaded dark bars), rotavirus-negatives (shaded grey bars) and percentage of rotavirus–positives (dotted line) is indicated.

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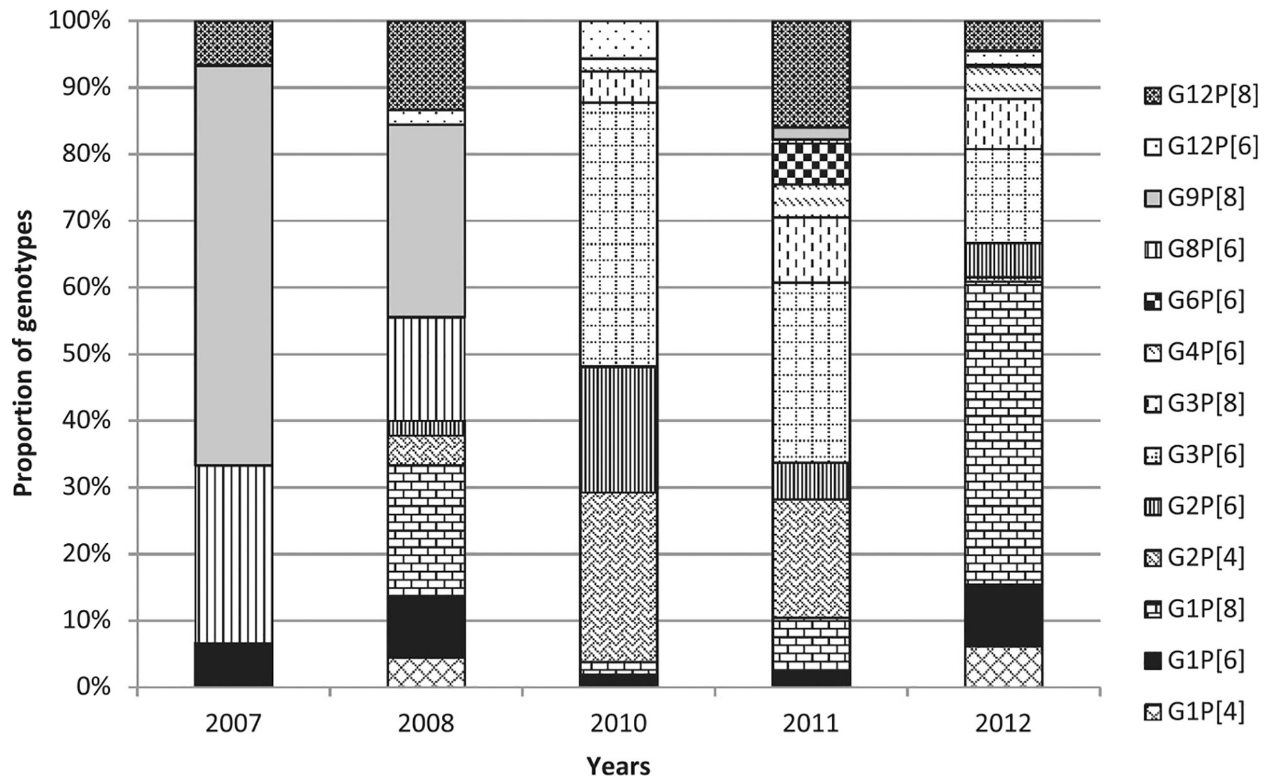

Fig. 2.

Yearly occurrence of rotavirus genotypes identified in children hospitalized with acute gastroenteritis in Yaoundé, Cameroon between 2007 and 2012. Only genotypes with at least 10 samples during the study period are represented here.

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Table 1

Yearly distribution of rotavirus cases in Yaoundé Cameroon, September 2007–December 2012.

Years	Number of diarrhea samples collected	Number of rotavirus-positive samples	Percentage of rotavirus positive
2007	112	50	45%
2008	276	118	43%
2009	165	35	21%
2010	448	177	40%
2011	657	263	40%
2012	786	356	45%
Total	2444	999	41%

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Table 2

Age group distribution of rotavirus cases in Yaoundé, Cameroon, from September 2007 to December 2012.

Age group (months)	Number of rotavirus positives cases	Number of rotavirus negatives cases	Total	Percentage of rotavirus positive
0 – 2	94	165	259	36%
3 – 5	230	232	462	50%
6 – 8	285	293	578	49%
9 – 11	158	277	385	41%
12 – 17	154	271	425	36%
18 – 23	34	98	132	26%
24 – 35	31	91	122	25%
36 – 47	11	37	48	23%
48 – 59	2	31	33	6%
Total	999	1495	2444	41%

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Table 3

Distribution of rotavirus G and P types in Yaoundé, Cameroon from September 2007 to December 2012.

P-type (%)	G-type (%)										
	G1	G2	G3	G4	G6	G8	G9	G12	GMix	GNT	Total
P[4]	20 (2)	60 (7)	1 (0)	–	–	7 (1)	1 (0)	4 (0)	2 (0)	2 (0)	97 (11)
P[6]	41 (5)	46 (5)	128 (14)	25 (3)	10 (1)	12 (1)	8 (1)	13 (1)	31 (3)	14 (2)	328 (37)
P[8]	156 (17)	19 (2)	43 (5)	9 (1)	2 (0)	3 (0)	26 (3)	47 (5)	19 (2)	32 (4)	356 (40)
PMix	20 (2)	21 (2)	2 (0)	–	–	7 (1)	2 (0)	1 (0)	4 (0)	3 (0)	60 (6)
PNT	13 (1)	7 (1)	4 (0)	1 (0)	–	–	2 (0)	1 (0)	5 (1)	20 (2)	54 (6)
P[9]	–	–	–	–	–	–	–	–	–	2 (0)	2 (0)
P[14]	–	–	–	–	–	–	1 (0)	–	–	–	1 (0)
Total	250 (28)	153 (17)	178 (20)	35 (4)	12 (1)	29 (3)	40 (4)	67 (7)	61 (7)	73 (8)	898 (100)

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Table 4

P–G type combination in 117 mixed infections from gastroenteritis cases in Yaoundé, Cameroon, 2007–2012.

P-type	G-type	Number of infections	P-type	G-type	Number of infections
P[4]	G1,3	1	P[6,8]	G1	11
P[4]	G1,8	1	P[6,8]	G2	3
P[4,6]	G1	1	P[6,8]	G3	2
P[4,6]	G2	10	P[6,8]	G8	4
P[4,6,8]	G2	1	P[6,8]	G9	11
P[4,8]	G1	7	P[6,8]	G1,4	1
P[4,8]	G2	5	P[6,8]	G8,12,3	1
P[4,8]	G8	1	P[6,8]	GNT	3
P[6]	G1,8	2	P[6,9]	G1	1
P[6]	G2,4	1	P[8]	G1,2	2
P[6]	G2,9	1	P[8]	G1,8	2
P[6]	G3,1	5	P[8]	G1,9	2
P[6]	G3,2	6	P[8]	G1,3	8
P[6]	G3,1,2	1	P[8]	G1,4	1
P[6]	G3,12	2	P[8]	G2,4	1
P[6]	G4,1	1	P[8]	G2,3	4
P[6]	G8,3	4	P[8]	G3,4	1
P[6]	G8,1,3	1	P[8]	G3,4,2	1
P[6]	G9,1	1	PNT	G3,1	1
P[6,4]	G3,2	1	PNT	G3,2	1
P[6,4]	G9	1	PNT	G9,1	2