

Circulating Serotypes of Group A Rotavirus Identified in the Faeces of Children Aged 0-56 Months with Gastroenteritis

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Gastroenteritis is a public health problem in developing countries such as Burkina Faso. The introduction of the RotaShield rotavirus vaccine in the early 1980s had the side-effect of causing acute intestinal invaginations, which led to the introduction of surveillance. The aim of this study is to review the serotypes of rotavirus identified. This retrospective study consisted in collecting data on group A rotavirus serotypes identified from stool samples using a rapid diagnostic test, confirmed by an ELISA test and then serotyped by real-time multiplex RT-PCR. A total of 840 suspect stools were collected between December 2013 and December 2017. Confirmed cases represented an average of 37.7%. Genotyping identified the P6(59.6%) and P8(40.4%) types of the P genotype in the VP4 genotype and G12 which predominates with 66% in the VP7 genotype followed by G3 23.4%. The genotypes most involved in gastroenteritis are serotypes P6 and P8 of the P genotype and serotypes G1, G2, G3, G9 and G12 of the G genotype in this study showed that.

Keywords: Rotavirus; diarrhoea; serotype; genotype; paediatrics; Burkina Faso.

1. INTRODUCTION

Gastroenteritis is a public health problem in developing countries, where in most cases laboratory diagnosis focuses on bacterial aetiology. However, the aetiology of gastroenteritis in infants is dominated by rotavirus, which is not routinely investigated. Rotavirus diarrhoea causes around 185,390 deaths in children under the age of 5 worldwide, with more than 80% of mortality attributed to sub-Saharan Africa, including Burkina Faso, which is one of the 8 most affected countries [1]. The introduction of the RotaShield® rotavirus vaccine in the early 1980s had the side-effect of causing acute intestinal intussusception (AII) [2]. This led Burkina Faso, like other countries around the world, to set up an epidemiological surveillance system in 2010. Initially, this surveillance involved a single sentinel site, but it was subsequently extended to 04 sites made up of 04 health centres since the introduction of the Rotateq® vaccine in 2013 [3]. The aim of this study is to provide an update on the rota-virus serotypes identified at the Charles de Gaulle Paediatric Teaching Hospital in Ouagadougou since surveillance was set up in 2013, which is one of the sentinel surveillance sites.

2. MATERIALS AND METHODS

We conducted a retrospective study of rotavirus diarrhoea in children aged 0-56 months between December 2013 and January 2017. These children were seen at the Charles de Gaulle Paediatric Teaching Hospital (CHUP-CDG) in

Ouagadougou for biological diagnosis of diarrhoea. Stool samples in sterile jars were accompanied by a notification form containing socio-demographic data, medical history, vaccination status, etc. The stool samples were examined at the CHUP-CDG in Ouagadougou. The stools were examined in the laboratory by a rapid diagnostic test whose results were confirmed by an ELISA test using the ProSpecT™ Rotavirus Microplate Assay Kit (www.thermofisher.com/order/catalog/product/R240396). In this ELISA test, the capsular antigen VP6 of group A rotavirus was sought. The validity of the test was conditioned by the positivity of the positive control (optical density (O.D.) was >0.500) and the negativity of the negative control (O.D. was < 0.150).

The results of the samples were interpreted on the basis of the same OD threshold as the positive and negative controls. Once the group had been determined by this first test, the rotavirus A positive samples were genotyped by multiplex real-time RT-PCR after extraction with the Qiagen® kit. The targets were the VP7 and VP4 protein genes, which determine the G (glycoprotein) and P (protease-sensitive) genotypes respectively. We used 8 primers for G genotypes and 7 primers for P genotypes. The primers for the G genotypes were aBT1 (G1), aCT2 (G2), G3 (G3), aDT4 (G4), aAT8 (G8), G9 (G9), G10 (G10), the VP7 gene primer and the primers for the P genotypes were; 2T1 (P[4]), 3T1 (P[6]), 1T1D (P[8]), 4T1 (P[9]), 5T1 (P[10]), P[11] (P[11]) and the VP4 gene primer (Table 1) [4–8]. These primers were used at a

Table 1. The sequences of primers used

Nom	nt Position	Size	Sequence 5' → 3'	Reference
VP7-F	49-71	23	ATG TAT GGT ATT GAA TAT ACC AC	Iturriza-G´omara et al. (2001)
VP7-R	914-933	20	AAC TTG CCA CCA TTT TTT CC	Iturriza-G´omara et al. (2001)
aBT1-F	314-335	22	CAA GTA CTC AAA TCA ATG ATG G	Gouvea et al. [4]
aCT2-F	411-435	25	CAA TGA TAT TAA CAC ATT TTC TGT G	Gouvea et al. [4]
G3-F	250-268	19	ACG AAC TCA ACA CGA GAG G	Iturriza-G´omara et al. [5]
aDT4-F	480-498	19	CGT TTC TGG TGA GGA GTT G	Gouvea et al. [4]
aAT8-F	178-198	21	GTC ACA CCA TTT GTA AAT TCG	Gouvea et al. [4]
G9-F	757-776	20	CTT GAT GTG ACT AYA AAT AC	Iturriza-G´omara et al. [5]
G10-F	666-686	21	ATG TCA GAC TAC ARA TAC TGG	Iturriza-G´omara et al. [5]
2T-1-R	474-494	21	CTA TTG TTA GAG GTT AGA GTC	Gentsch et al. [6]
3T-1-R	259-278	20	TGT TGA TTA GTT GGA TTC AA	Gentsch et al. [6]
1T-1D-R	339-356	18	TCT ACT GGR TTR ACN TGC	Iturriza-G´omara et al. (2000)
4T-1-R	385-402	18	TGA GAC ATG CAA TTG GAC	Gentsch et al. [6]
5T-1-R	577-594	18	ATC ATA GTT AGT AGT CGG	Gentsch et al. [6]
P[11]-R	306-323	18	GTA AAC ATC CAG AAT GTG	Iturriza-G´omara et al. [5]

Nt : nucleotide

concentration of 20 µM each with a volume of 5 µL of 5X Buffer, 1 µL of dNTP mix (10 µM), 1 µL of enzyme, 5 µL of sample and RNase-free water in sufficient quantity to (q.s.p.) have 25 µL of final volume of reaction mixture. The thermal profile and amplification cycle was as follows: 1 cycle of 50°C for 30 minutes and 95°C for 15 minutes followed by 35 cycles each consisting of 94°C for 1 minute, 42°C for 1 minute, 72°C for 1 minute and finally 1 cycle of 72°C for 10 minutes.

3. RESULTS

3.1 General Results

Between 2013 and 2017, we collected 840 stool samples, distributed as follows: 45 in 2013, 305 in 2014, 124 in 2015, 117 in 2016 and 249 in 2017 (Table 2). The ELISA test yielded 325 positive samples out of the 840 samples analysed, representing 37.7%. The number of positive cases in male patients was 192 (59.08) compared with 133 (40.92) for female patients, which represents a sex ratio of 1.44. Children aged between 2 and 30 months were the most represented in the positive cases, with 85% (Table 2). Depending on the month of the year, the number of cases began to rise in December, peaked in January, then fell and reached its lowest level in May (Fig. 1). According to vaccination status, 35.66% of the 401 unvaccinated patients were positive, compared with 20.93% of the 645 vaccinated patients (Fig. 2).

3.2 Serotypes Identified by RT-PCR

We serotyped 47 strains by RT-PCR which enabled us to identify the G1, G2, G3, G9 and G12 genotypes in 4.3%, 2.1%, 23.4%, 4.3% and 66% respectively (Table 2). Genotypes P6 and P8 of serotype P were identified in 59.6% for P6 and 40.4% for P8 (Table). The combinations identified were G1P[8] (4.26%), G2P[6] (2.13%), G3P[6] (10.64%), G3P[8] (12.77%), G9P[8] (4.26%), G12P6 (46.81%) and G12P[8] (19.15%) (Table 2).

4. DISCUSSION

We found an average positivity rate of 37.7% for rotavirus infection in children. This rate remains high even though it is lower than the positivity rate observed between 2011 and 2012, which was 63.5% [9]. Positivity rates per year remained low until 2017 (18.5%) compared with the rates observed before 2013, the year when the vaccine was introduced. There was even a considerable drop in the number of cases between 2014 and 2015. This is probably linked to the introduction of the vaccine on 31 October 2013 in Burkina Faso, which is estimated to be 73.8% effective against rotavirus gastroenteritis, but could fall to around 60% in high-mortality regions [3,10–12]. This efficacy, combined with the vaccination coverage rate estimated at 91% in 2016 in Burkina Faso, could contribute to this drop in the positivity rate, especially as this study shows that the positivity rate in vaccinated children is lower than that in unvaccinated children [13].

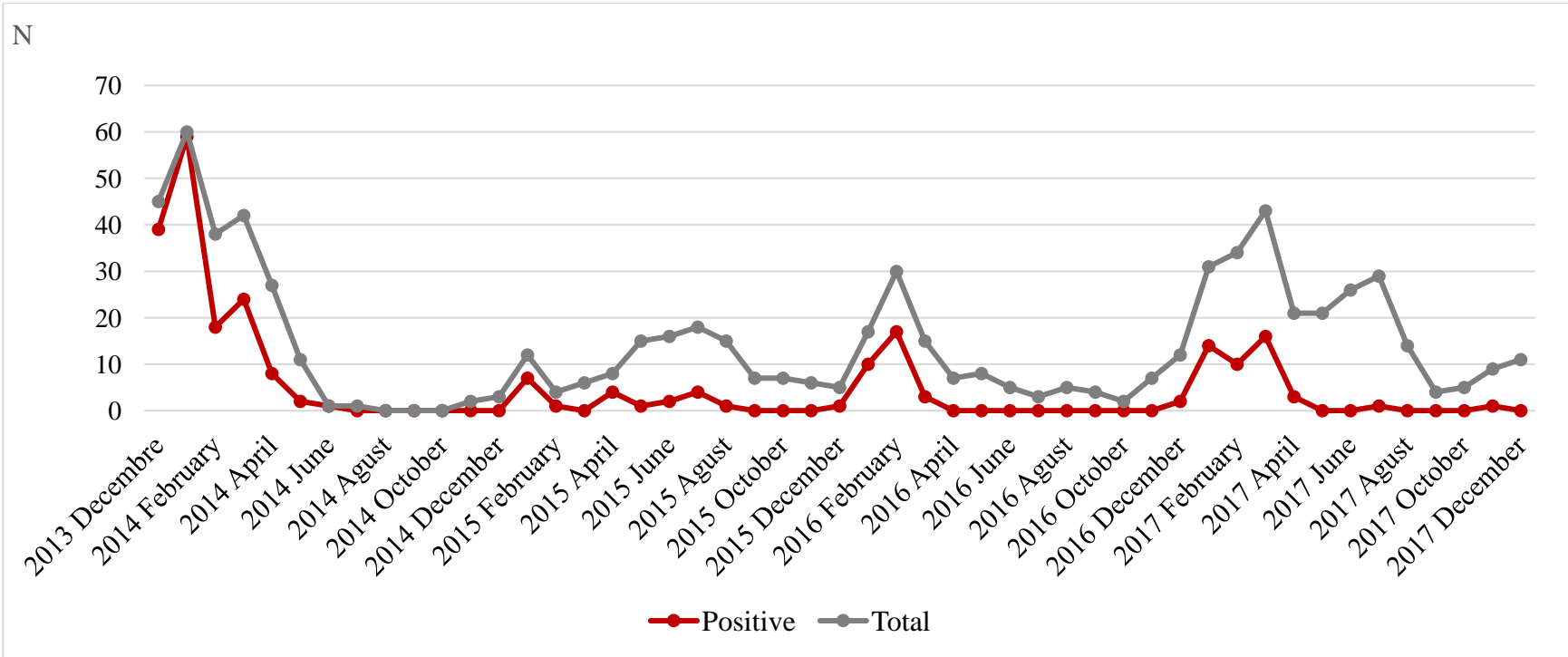


Fig. 1. Seasonal distribution of positive cases

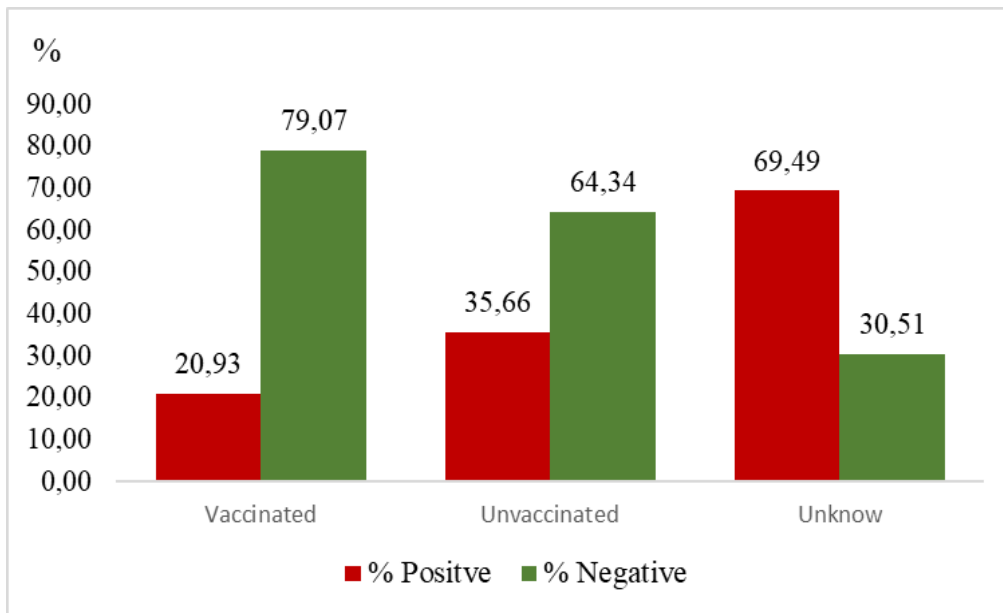


Fig. 2. Distribution of cases bay vaccination status

Table 2. Number of samples and positive cases by year, sex, year group and circulating genotypes between 2013 and 2017

Samples collected by year	2013	2014	2015	2016	2017	Total
Total by year	45	305	124	117	249	840
Positive samples n(%)	32(71.1)	223(63.7)	23(18.5)	33(28.2)	46(18.5)	325
Positive cases by sex n(%)						
Female	133(40.92)					
Male	192(59.08)					
Samples collected by age range in month	Negative n=515				Positive n=325	
	n	%			n	%
[0-1]	63	7,5			40	12,4
[2-30]	752	89,5			279	85,8
[31-56]	25	3,0			6	1,8
Genotypes n = 47	G(VP7) n(%)				P(VP4) n(%)	
	G1	2(4.3)			P6	28(59.6)
	G2	1(2.1)			P8	19(40.4)
	G3	11(23.4)				
	G9	2(4.3)				
	G12	31(66)				

In fact, 64.34% of samples from unvaccinated children were negative, while 79.07% of samples from vaccinated children were negative. Our results also show 20.93% infection despite vaccination, but it has been reported in the literature that there is a weak protective effect of the vaccine in low-income countries compared with industrialised countries [14], which could be linked to various co-morbidities such as malnutrition, variations in circulating genotypes within countries and individual variations in vaccine response [1,15]. Other factors such as the seasonal cycle of the year could also

influence the occurrence of infection. The number of cases increases between December and April, peaking between January and February. There is therefore an increase in the number of cases in December, which declines from April onwards, corresponding to the dry, cold season, which is favourable for rotavirus infection [16–18].

The diagnosis of diarrhoea on suspicion of rotavirus concerned the group A virus, where we found that in the P genotypes (VP4) P6 predominated with 59.6% of cases and P8 with

40.4% of cases. However, we were unable to identify the P4 genotype, which, along with P6 and P8, is the majority genotype circulating worldwide [19–21]. G12 was the predominant genotype identified in genotype G (VP7) with 66% followed by genotype G3 23.4%. This predominance of G12 corroborates the results of other studies in several sub-Saharan African countries where it is currently the most important phenotype since it was first identified in 2004 [22,23]. The genotypes identified in the minority were G1, G2, G3 and G9, despite the fact that, according to the literature, they are the most incriminating in humans [19].

Genotypes G8 and G6, which were reported in other studies in Burkina Faso and other African countries, were not identified in this study. The same applies to the G4 genotype, which has declined dramatically since 2003 [21,22,24]. According to the genotype combinations notified, G12P [8] accounts for almost half of all genotype combinations, followed by G12P [6]. These results corroborate the profile of combinations encountered in Africa, even though previous studies have shown the emergence of the G6P [6] genotype in Burkina Faso and other African countries, which we did not identify in this study [21,25].

5. CONCLUSION

This study demonstrated the involvement of a variety of group A rotavirus genotypes. The P genotypes implicated in this study are P6 and P8 and the G genotypes implicated are G1, G2, G3, G9 and G12. The G1, G2, G3 and G9 genotypes were in the minority despite being the most frequently incriminated in humans according to the literature. The genotypic combinations are dominated by G12P[6] followed by G12P[8]. We did not identify the G6P[6] genotype, although it has been identified in previous studies carried out in Burkina Faso.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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