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Rotavirus serotypes/genotypes distribution and their implications in children with diarrhoea admitted to the Charles de Gaulle University Paediatric Hospital, Ouagadougou, Burkina Faso

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Abstract:

Background: Diarrhoeal diseases are a source of public health problems in low-income countries such as Burkina Faso, where the aetiology of rotavirus plays an important role. This led to the introduction of surveillance since the introduction of the rotavirus vaccine. The aim of this study is to take stock of rotavirus diarrhoea since the introduction of the vaccine.
Methodology: This was a retrospective cross-sectional study of children aged 0-56 months with diarrhoea admitted to the Charles de Gaulle Paediatric University Teaching Hospital, Ouagadougou between December 2013 and January 2021. Stool samples were collected for rapid diagnostic test for rotavirus, which was confirmed by ELISA for rotavirus group A VP6 antigen. ELISA positive samples were genotyped (for genotype G and P) using multiplex real-time RT-PCR.
Results: Of all the stool samples from 1223 children with suspected rotavirus diarrhoea, 401 were confirmed by ELISA, representing an average prevalence of 32.8%. The distribution of confirmed cases by year showed a considerable drop in positive cases between 2014 and 2015, with 61.7% and 17.6% prevalence rate respectively. This prevalence remained low in 2017 and increased in the other years without exceeding the 50.0% observed before the introduction of the vaccine. Genotyping identified P6(59.6%) and P8(40.4%) types for genotype P, and G12 (66.0%) for genotype G.
Conclusion: This study shows a considerable decrease in the prevalence of rotavirus diarrhoea which could be linked to the introduction of rotavirus vaccine in the Extended Vaccination Program (EVP) in Burkina Faso in 2013, although rotavirus diarrhoea cases are still considerably occurring.

Key words: Rotavirus, diarrhoea, vaccine, genotype, paediatrics, Burkina-Faso

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Distribution des sérotypes/génotypes du rotavirus et leurs implications chez les enfants atteints de diarrhée admis à l'hôpital pédiatrique universitaire Charles de Gaulle de Ouagadougou, Burkina Faso

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Résumé:

Contexte: Les maladies diarrhéiques constituent un problème de santé publique majeur dans les pays à faible revenu comme le Burkina Faso, où l'étiologie du rotavirus joue un rôle important. Ceci a conduit à la mise en place d'une surveillance depuis l'introduction du vaccin contre le rotavirus. L'objectif de cette étude est de dresser un bilan des cas de diarrhée à rotavirus depuis l'introduction de ce vaccin.
Méthodologie: Il s'agit d'une étude transversale rétrospective menée auprès d'enfants âgés de 0 à 56 mois, hospitalisés pour diarrhée au CHU Charles de Gaulle de Ouagadougou entre décembre 2013 et janvier 2021. Des échantillons de selles ont été prélevés pour un test de diagnostic rapide du rotavirus, confirmé par un test ELISA de l'antigène VP6 du rotavirus du groupe A. Les échantillons positifs à l'ELISA ont été génotypés (génotypes G et P) par RT-PCR multiplex en temps réel.
Résultats: Sur l'ensemble des échantillons de selles prélevés chez 1223 enfants présentant une suspicion de diarrhée à rotavirus, 401 ont été confirmés par ELISA, soit une prévalence moyenne de 32,8%. La répartition des cas confirmés par année a montré une baisse considérable du nombre de cas positifs entre 2014 et 2015, avec des taux de prévalence respectifs de 61,7% et 17,6%. Cette prévalence est restée faible en 2017 et a augmenté les années suivantes sans toutefois dépasser les 50,0% observés avant l'introduction du vaccin. Le génotypage a identifié les types P6 (59,6%) et P8 (40,4%) pour le génotype P, et G12 (66,0%) pour le génotype G.
Conclusion: Cette étude met en évidence une diminution considérable de la prévalence de la diarrhée à rotavirus, qui pourrait être liée à l'introduction du vaccin contre le rotavirus dans le Programme élargi de vaccination (PEV) au Burkina Faso en 2013, même si des cas de diarrhée à rotavirus persistent.

Mots clés: Rotavirus, diarrhée, vaccin, génotype, pédiatrie, Burkina Faso

Introduction:

Infant mortality has been a public health challenge for many years, and one of the most frequently cited causes is infection. In 2010, of the 6 to 7 million children who died before their fifth birthday, about two-thirds died of infectious causes, most of which are preventable (1). Among infectious diseases, diarrhoeal illnesses, in addition to pneumonia, remain top of the list of causes of death in children under 5 years of age (2,3). In low-income countries, the situation is worse. The sub-Saharan Africa countries such as Burkina Faso, Mali, Niger and Nigeria are among the 10 countries most affected by diarrhoeal diseases worldwide (3).

In Burkina Faso, the number of deaths due to diarrhoeal diseases in children under 5 years of age remains high, with a large number attributable to rotavirus (4,5). Rotavirus diarrhoea causes around 185,390 deaths in children under 5 years of age worldwide, with more than 80% of deaths attributed to sub-Saharan Africa, including Burkina Faso, which is one of the 8 countries most affected (6). Rotavirus diarrhoea has been the subject of epidemiological surveillance in Burkina Faso since 2010 at a single sentinel site, which has been extended to 4 sites comprising 4 health centres since the introduction of the Rotateq® vaccine in 2013, with a laboratory chosen as the national reference for case confirmation (7).

This study aims at reviewing cases of rotavirus diarrhoea at the Charles de Gaulle Paediatric University Teaching Hospital in Ouagadougou since surveillance was set up in 2013, which is one of the sentinel surveillance sites.

Materials and method:

Study setting and design:

This was a retrospective study of children aged 0-56 months between December 2013 and January 2021 admitted for diarrhoea illnesses to the Charles de Gaulle Paediatric University Teaching Hospital in Ouagadougou, Burkina Faso.

Study participants and data collection:

A total of 1223 children were studied over 8 years (December 2014 and January 2021). After a clinical examination and when infectious diarrhoea was suspected in a child, stool sample was taken and accompanied by a notification form. This notification form included socio-demographic data, medical history, vaccination status, and other relevant clinical information. The stool sample was collected from each patient into a sterile jar and transported to the laboratory for analysis.

Laboratory analysis:

***Rotavirus antigen detection test on stool samples*:**

Once in the laboratory, the faeces were first examined by a rotavirus rapid diagnostic test and the results were confirmed by an ELISA test. ELISA testing was performed using the ProSpecT™ Rotavirus Microplate Assay Kit (www.thermofisher.com/order/catalog/product/R240396) which detects group A rotavirus antigen, i. e. VP6 capsular antigen. The positive control was considered valid when the optical density (O.D.) was >0.500, the nega-

tive control when the O.D. was < 0.150. The results were interpreted on the basis of the same O.D. threshold as the positive and negative controls.

Rotavirus genotyping by multiplex real-time PCR assay:

The rotavirus A positive samples were genotyped by. amplifying the VP7 and VP4 protein genes, which determine the G (glycoprotein) and P (protease-sensitive) genotypes respectively, using multiplex real-time RT-PCR. This amplification was carried out after DNA extraction using the Qiagen® kit. A total of 8 primers were used for the G genotypes and 7 for the P genotypes. For genotypes G, the primers were aBT1 (G1), aCT2 (G2), G3 (G3), aDT4 (G4), aAT8 (G8), G9 (G9), G10 (G10), and the VP7 primer, and for P genotypes the primers for the genes were 2T1 (P[4]), 3T1 (P[6]), 1T1D (P[8]), 4T1 (P[9]), 5T1 (P[10]), P[11] (P[11]) and the VP4 gene primers (8–12).

The concentration of primers for genotyping the G and P genotypes was 20µM for each primer with a volume of 5µL of 5X Buffer, 1µL of dNTP mix (10µM), 1µL of enzyme, 5µL of sample and RNAse-free water in sufficient quantity to have 25µL of final volume of reaction mixture. The thermal profile and amplification cycles were 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.

Statistical analysis:

Data was entered on a computer using Microsoft Excel 2021, analyzed by EPI INFO version 3.5.4 software and the Chi-square test was used to compare qualitative variables.

Results:

Prevalence of rotavirus diarrhoea in the children:

Of the stool samples analysed for 1223 children, 726 were from male patients and 497 from female patients, representing a sex ratio (M/F) of 1.46 (Table 1). There were 401 ELISA positive samples, representing rotavirus positivity rate of 32.8% (Table 1). There were 245 positive samples in male patients (33.7%, n=245/726) and 156 in female patients (31.4%, n=156/497), representing a male/female ratio of 1.57 (OR=1.113, 95% CI=0.8722-1.421, p=0.4232) (Table 1).

The age group analysis shows that neonates (0-1 month of age) had significantly higher prevalence of rotavirus diarrhoea (58.8%, n=47/80) than infants (2-30 months of age) with 31.3% (n=344/1099) and children (31-56 months of age) with 28.6% (n=12/42) (x^2=25.786, p<0.0001). (Table 1).

Trend in rotavirus diarrhoea cases by months during the 8-year study period:

Stool samples were analysed for most patients in 2017 followed by 2021 (Fig 1). However, in terms of the positivity rate, 2014 was the year with the most cases, with positivity rate of 61.7% (n=142/230), 17.6% (n=21/119) in 2015, 27.8% in 2016 (n=32/115), 18.2% in 2017 (n=45/248), 29.3% in 2018 (n=48/164), 35.3% in 2019 (n=12/34), 11.1% in 2020 (n=15/135) and 48.3% in 2021 (n=86/178) (x^2=174.55, p<0.0001).

As regards the distribution by month of the year, the number of cases begins to rise in December, peaks in January, then falls, reaching its lowest level in May (Fig 2).

Table 1: Prevalence of rotavirus diarrhoea in the children over the 8-year period of study with respect to sex and age group

Characteristics	Number of samples	Number of positive samples (%)	Chi square	OR (95% CI)	*p* value
Sex					
Male	726	245 (33.7)		1.113	
Female	497	156 (31.4)	0.6414	(0.8722-1.421)	0.4232
M : F ratio	1.46	1.57			
Age in months					
0-1	80	47 (58.8)			
2-30	1099	344 (31.3)	25.786	NA	<0.0001*
31-56	42	12 (28.6)			
Total	1223	401 (32.8)			

* = statistically significant at p<0.05; NA = Not applicable; OR=Odd ratio; CI=Confidence interval

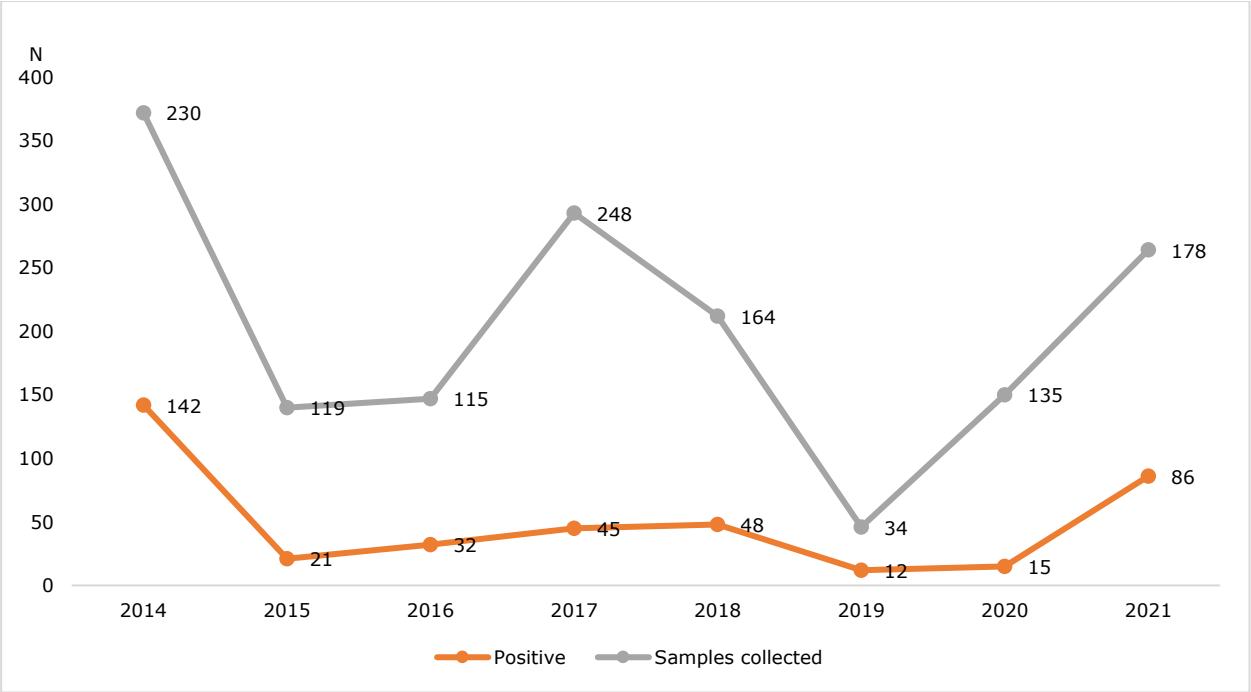

Fig 1: Number of enrolled and positive cases of rotavirus diarrhoea over the 8 years period of study (x^2=174.55, p<0.0001*)

Rotavirus diarrhoea cases by vaccination status:

The distribution of cases according to vaccination status shows that 35.7% of the 401 (n=143) unvaccinated patients, and 69.5% of 177 (n=123) patients with unknown vaccination status were rotavirus positive, compared with 20.9% of the 645 (n=135) vaccinated patients (Fig 3) (x^2=150.85, p<0.0001) .

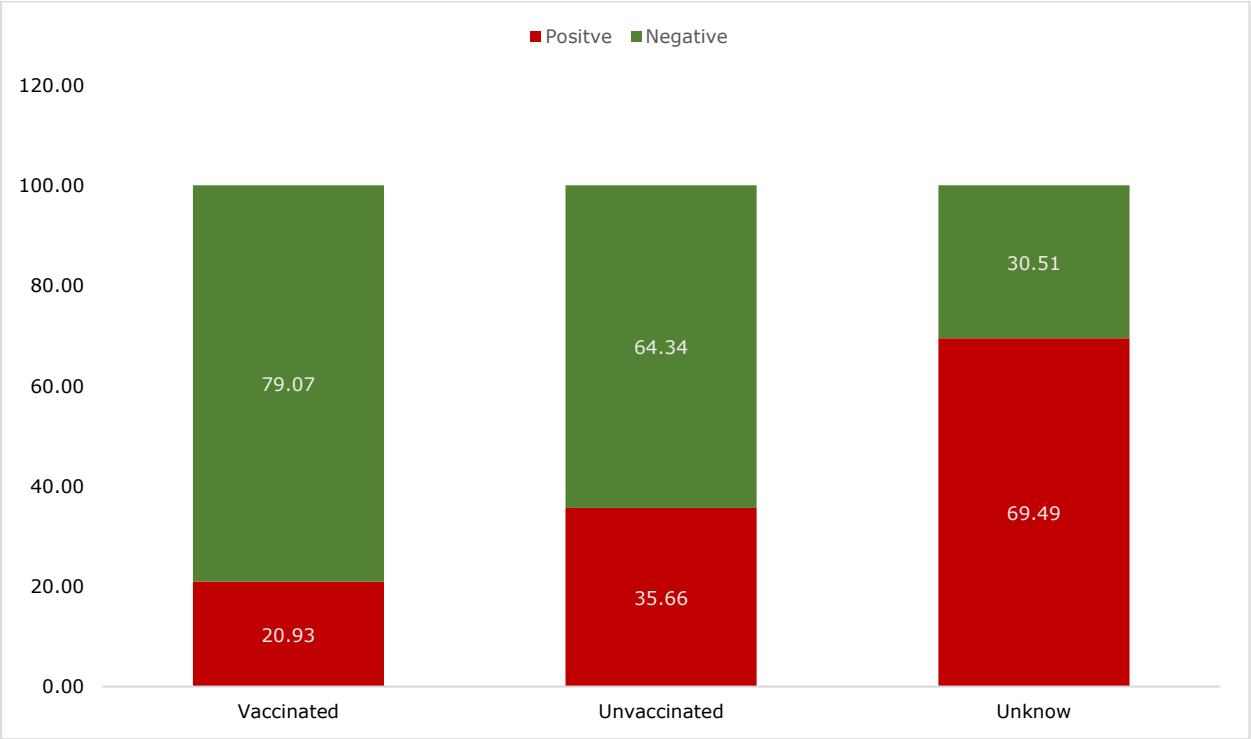

Fig 3: Distribution of rotavirus cases by vaccination status (x^2=150.85, p<0.0001*)

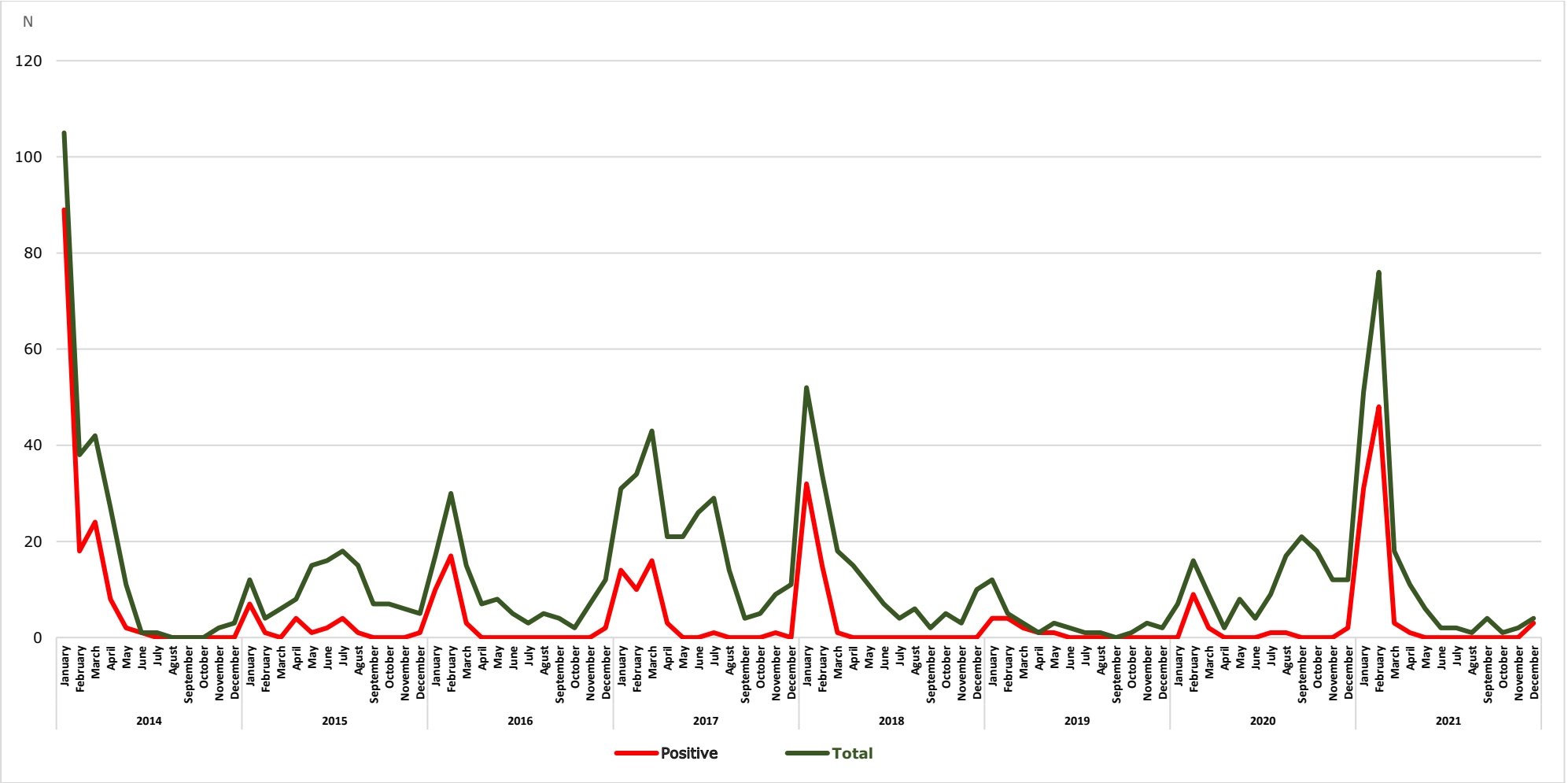

Fig 2: Number of enrolled and positive cases of rotavirus diarrhoea with respect to months during the 8-year period of study

Table 2: Frequency of G (VP7) and P (VP4) rotavirus serotypes and different combinations from RT-PCR assay of stool samples of children with rotavirus diarrhoea

Types	Frequency	Percent
VP7 (n = 47)		
G1	2	4.3
G2	1	2.1
G3	11	23.4
G9	2	4.3
G12	31	66.0
VP4 (n = 47)		
P6	28	59.6
P8	19	40.4
Combination genotypes (n = 7)		
G1P[8]	2	4.3
G2P[6]	1	2.1
G3P[6]	5	10.6
G3P[8]	6	12.8
G9P[8]	2	4.3
G12P[6]	22	46.8
G12P[8]	9	19.2

Results of RT-PCR genotyping:

A total of 47 strains were genotyped by RT-PCR. Within the G genotype, G1, G2, G3, G9 and G12 genotypes were identified in proportions of 4.3%, 2.1%, 23.4%, 4.3% and 66,0% respectively (Table 2). For genotype P, P6 and P8 were identified in 59.6% and 40.4% of cases respectively (Table 2). The genotype combinations identified were G1P[8] (4.3%), G2P[6] (2.1%), G3P[6] (10.6%), G3P[8] (12.8%), G9P[8] (4.3%), G12P6 (46.8%) and G12P[8] (19.2%) (Table 2).

Discussion:

This study showed a prevalence rate of 32.7% for rotavirus infection in children with gastroenteritis over the 8 years of the study. Other studies in Burkina Faso prior to the introduction of the vaccine found an approximate prevalence rate of 33.8% between 2008 and 2010 (13), and a much higher rate of 63.5% between 2011 and 2012 (14). This high prevalence of 63.5% corroborates the prevalence of 61.7% in 2014 in our study, which fell to 17.6% in 2015. This rate remained low in 2017 (18.2%) and 2020 (11.1%), in contrast to 2016 (27.8%), 2018 (29.3%), 2019 (35.3%) and 2021 (48.3%), the year in which the highest rate since the introduction of the vaccine was reported. However, all the rates in our study with the exception of 2014, are lower than the rates before the year in which the vaccine was introduced. This drop in the proportion of positive cases of rotavirus infection can be seen in certain African countries such as Mauritania, which saw a 24% drop in cases of hospitalisation, with a sharp fall (74%) in children aged 2 to 5 years (15).

The drop in the number of Rotavirus diarrhoea illnesses between 2014 and 2015 is probably linked to the introduction of the vaccine on 31 October 2013 in Burkina Faso. This drop is estimated to be 73.8% effective against rotavirus gastroenteritis, but could fall to around 60% in high-mortality regions (7,16–18). In 2016, Burkina Faso achieved an estimated vaccination coverage rate of 91% throughout the country, which could contribute to this drop in the positivity rate (5). The reduction in cases due to the vaccine is confirmed by the results according to vaccination status that we observed in this study.

In fact, 64.3% of stool samples from unvaccinated children were negative for rotavirus antigen, compared to 79.1% of samples from vaccinated children. Although the negativity rate in vaccinated children was significantly higher than in unvaccinated children (x^2=150.85, p<0.0001), 20.9% of rotavirus infections were reported despite vaccination. Rotavirus infections despite vaccination have been noted in certain countries where a weak protective effect was reported in low-income countries compared with industrialized countries (19). The reason for this weak protective effect could be linked to various co-morbidities such as malnutrition, variations in the genotypes circulating in different countries and individual variations in the vaccine response (6,20). Examples of inter-individual variations

in vaccine response include variations in human blood group antigens, which are antigens that bind the VP4 protein of the P genotype. It has been shown that this variation could be the cause of reduced vaccine sensitivity in Lewis-negative children, a very common group in Africa (21).

Another factor that could favor rotavirus infection is the seasonal cycle of the year. In this study, the number of cases increases between December and April, peaking between January and February. The incidence of gastroenteritis increases during the dry, cold period of the year, and in Burkina Faso it could be said that the number of cases increases in December and declines from April onwards, corresponding to the dry, cold season (13,22,23). Climate is certainly a factor in the outbreak of cases, but there are also factors linked to the virus, such as the VP6 protein, which defines the different groups of rotaviruses, of which group A is the main group responsible for more than 90% of cases of gastroenteritis in humans (24). Epidemics linked to certain group B viruses have been observed in certain countries such as China and India, and group C viruses have been sporadically isolated on almost every continent. In this study, the diagnosis of diarrhoea on suspicion of rotavirus concerned the group A virus.

Of the P (VP4) genotypes, P6 predominated, accounting for 59.6% of cases, and P8 for 40.4%. These two genotypes are the majority circulating worldwide, as is the P4 genotype, which was not identified in this study (25,26). In genotype G (VP7), G12 was the majority genotype identified with 66%, followed by G3 genotype with 23.4%. Genotypes G12 and G3, along with genotypes G1, G2 and G9, which were only identified in a minority of cases in this study, are the most frequently incriminated genotypes in humans (25). We did not identify G4 genotypes, which declined dramatically since 2003, or G8 and G6 genotypes, which were reported in other studies in Burkina Faso and other African countries (27–29).

This predominance of G12 corroborates the results of other studies in other African countries (28). The G12 genotype was first identified in sub-Saharan Africa in 2004, and since then it has emerged as the most important phenotype in several African countries, even after the introduction of the vaccine (28, 30). Among the genotype combinations notified, G12P[8] accounts for almost half of all genotype combinations, followed by G12P[6]. These two combinations are the most commonly encountered in Africa, but previous studies showed the emergence of the G6P[6] genotype in Burkina Faso and other African countries (27,31), but in our study we did not identify this genotype.

Conclusion:

This study highlighted the involvement of a variety of group A rotavirus genotypes in diarrhoea in children aged 0-5 years. The P genotypes implicated in this study are P6 and P8, and the G genotypes implicated are G1, G2, G3, G9 and G12. The genotypic combinations are dominated by G12P[6] followed by G12P[8].

What is remarkable is the considerable drop in the proportion of positive cases in the year following the introduction of the vaccine into the EPI programme in Burkina Faso on 31 October 2013. However, cases are still being recorded, even though the proportions remain lower than before the vaccine was introduced, thus demonstrating the efforts that still need to be made in fighting against rotavirus diarrhoea in children.

Contributions of authors:

IT conceptualized the study and was involved in data curation, formal analysis, and writing of original manuscript draft; DCD was involved in data curation; HS, AKB, JTD, MT, MK, MS, ASO, IS were involved in review and editing of manuscript; and ROT was involved in review/editing of the manuscript and supervision of the project.

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Conflict of interests:

Authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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