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High prevalence and prolonged shedding with enteric viruses among children with acute diarrhea in Franceville, Southeast of Gabon

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ABSTRACT

Background: Diarrheal diseases are a severe public health problem and a major cause of morbidity and mortality in children and young infants, particularly in sub-Saharan African countries.
Objectives: The aim of this study was to detect enteric viruses and to collect data on both viral shedding and new infections by these viruses in children in Franceville.
Study design: A total of 98 stool samples were collected from children aged 0–15 years old living in Franceville and included in this study. These stool samples were collected at the time of acute diarrhea and both 10 and 21 days later. The samples were analyzed by real-time PCR targeting seven viruses: rotavirus A (RVA), adenovirus (HAdV), astrovirus (HAstV), sapovirus (SaV), bocavirus (HBoV), norovirus (GI&GII).
Results: Among the 66 diarrheal stool samples collected at the time of acute diarrhea, 60 (90.9%) samples were found positive for at least one of the enteric viruses tested. HAdV (81.8%) and HBoV (66.7%) were the most frequently detected viruses in diarrheal samples, followed by NoV GII (30.3%), HAstV (10.6%), SaV (10.6%), RVA (7.6%) and NoV GI (1.5%). Viral shedding and new infections by these enteric viruses were observed in the children followed.
Conclusions: Our study provides useful information on both viral clearance and new infections with enteric viruses among children in Franceville, which may help to better control and manage enteric virus infections. Several coinfection and asymptomatic shedding cases suggest an intense community transmission of these enteric viruses and other enteric pathogens in this low-income Gabonese setting.

1. Introduction

Diarrheal diseases are a severe public health problem and a major cause of morbidity and mortality in children and young infants, particularly in sub-Saharan countries [1,2]. The etiology of diarrheal diseases includes bacteria, parasites and viruses, but the latter are considered the main cause of diarrhea in children [3]. Rotavirus and norovirus are the most frequent viruses associated with diarrheal diseases, followed by enteric adenovirus, astrovirus and sapovirus [4,5].

In sub-Saharan African countries, virus prevalence in diarrhea varies according to the context [6–9]. In Gabon, the rate of enteric virus infections associated with diarrhea in children is rarely documented. In 2015, a study reported that among the 317 analyzed diarrheal stool samples, 193 (60.9%) were positive for at least one enteric virus [8]. Recently, another study found that 2.2% of the same 317 diarrheal stool samples were positive for HBoV [10]. A more recent study conducted in Franceville and at the same time as this study reported that 61% of the samples were positive for at least one of the intestinal parasites tested [11].

Abbreviations: RVA, rotavirus A; HAdV, human adenovirus; NoV GI, norovirus GI; NoV GII, norovirus GII; HAstV, human astrovirus; SaV, sapovirus; HBoV, human bocavirus; PCR, polymerase chain reaction; ND, not determined; D0, at time of acute diarrhea; D10, day 10 after the first sampling; D21, day 21 after the first sampling.

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The goal of our study was to detect seven enteric viruses associated with diarrhea in children with diarrhea in Franceville, and to collect the first data on both viral shedding and new infections by these viruses in children by using real-time PCR.

2. Study design

2.1. Ethics approval and consent to participate

This study was approved by the Gabonese National Ethics Committee (PROT N°0020/2015/SG/CNE). Stool samples were collected from children after obtained their parents' or guardians' written informed consent.

2.2. Stool samples collection

In this study, 98 stool samples were collected consecutively from 66 patients with acute gastroenteritis between November 2016 and August 2017. Sixty-six (66) diarrheal stool samples were collected at the time of diarrhea from 66 children. In addition, 32 non-diarrheal stool samples were collected from 16 patients, 16 specimens at day 10 and 16 specimens at day 21 after the initial sampling. The inclusion criteria for the patients were any child aged 0–15 years with diarrhea (several fluid stools) who attended one of the two health facilities (*Centre Hospitalier Régional Amissa Bongo* and *Hôpital de l'Amitié Sino-Gabonaise*) in Franceville. The study only included children for whom the parents or guardians gave their consent to participate. Demographic characteristics (age, sex) and clinical signs were collected. The collected stool samples were stored at 4 °C until their daily transportation to the Centre Interdisciplinaire de Recherches Médicales de Franceville (CIRMF) for molecular tests.

2.3. Nucleic acid extraction and real-time PCR

Fecal samples were suspended in phosphate buffered saline and centrifuged at 1800 rpm for 5 min. Supernatants were either stored at −80 °C or used immediately for nucleic acid extraction with the QIAamp Fast DNA Stool Mini Kit (Qiagen) and QIAamp Viral RNA Mini Kit (Qiagen) according to the manufacturer's instructions. For RNA viruses, RNA was transcribed to complementary DNA (cDNA) using the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems).

For detecting RVA, norovirus GI, norovirus GII, sapovirus (GI, II & IV), all types of human astrovirus, all types of human bocavirus and all types of human adenovirus, we used primers and TaqMan probes previously described [10, 12-15]. Amplification was performed using the 7500 Fast Real-Time PCR system (Applied Biosystems).

2.4. Statistical analysis

Detection frequencies were compared by using Fisher's exact test. The exact McNemar test (binomial test) was used to analyze if the presence of pathogens changed between baseline and follow-up. A p-value below 0.05 was considered as statistically significant. RStudio V1.2.5001 software was used.

3. Results

3.1. Prevalence of viral infection

The median age was 1.17 years. The age of patients ranged from 3 months to 11 years and the male/female ratio was 1.3 (37/29). Prevalent symptoms included diarrhea (100%), fever (56.1%; N = 37/66) vomiting (50%; N = 33/66)), dehydration (16.67%; N = 11/66) and abdominal pain (7.58%; N = 5/66). There was no significant difference in the detection of enteric viruses between male and female patients (p = 0.39). Furthermore, some children with enteric virus infections showed clinical signs while others showed no clinical signs ($p \geq 0.26$; Table 1).

Table 1
Characteristics of diarrheal children with viral infections.

Patient		Infectedn (%)	*P* value
Sex			
Male (N = 37)		35 (94,6)	*0.39*
Female (N = 29)		25 (86.2)	
Clinical sign			
Fever	Yes (N = 37)	33 (91.7)	*0.69*
	No (N = 29)	27 (93.1)	
Vomiting	Yes (N = 33)	30 (90.9)	*1*
	No (N = 33)	30 (90.9)	
Abdominal pain	Yes (N = 5)	5 (100)	*1*
	No (N = 61)	55 (90.2)	
Dehydration	Yes (N = 11)	9 (81.8)	*0.26*
	No (N = 55)	51 (92.7)	

Table 2
Prevalence of enteric viruses in children with acute diarrhea.

Type of enteric viruses*	Prevalence (N = 60)n (%)
Viral monoinfection	**10 (16.7)**
HAdV	7 (11.7)
HBoV	2 (3.3)
No GII	1 (1.7)
Viral coinfection	**50 (83.3)**
HAstV+NoV GII	1 (1.7)
HBoV+NoV GII	1 (1.7)
HAdV+NoV GII	2 (3.3)
RVA+NoV GII	1 (1.7)
HAdV+SaV	1 (1.7)
HAdV+HAstV	1 (1.7)
HAdV+HBoV	20 (33.3)
RVA+HAdV	2 (3.3)
HAdV+HBoV+NoV GII	8 (13.3)
HAdV+HBoV+NoV GI	1 (1.7)
HAdV+HBoV+SaV	2 (3.3)
HAdV+HBoV+HAstV	1 (1.7)
RVA+HAdV+HBoV	2 (3.3)
HAdV+HBoV+SaV+NoV GII	3 (5.0)
HAdV+HBoV+HAstV+NoV GII	3 (5.0)
HAdV+HBoV+HAstV+SaV	1 (1.7)

Abbreviations*: HAdV, human adenovirus; HBoV, human bocavirus; NoV GII, norovirus GII; HAstV, human astrovirus; RVA, rotavirus A; SaV, sapovirus; NoV GI, norovirus GI.

Among the 66 diarrheal stool samples collected from patients at the time of acute diarrhea, 90.9% (60/66) samples were detected to be positive for at least one enteric virus. The most frequently detected viruses in children with diarrhea were HAdV (81.8%; n = 54/66) followed by HBoV (66.7%; n = 44/66), NoV GII (30.3%; n = 20/66), HAstV (10.6%; n = 7/66), SaV (10.6%; n = 7/66), RVA (7.6%; n = 5/66) and NoV GI (1.5%; n = 1/66) (Fig. 1).

3.2. Monoinfection and coinfection by enteric viruses in diarrheal children

Among the 60 samples which tested positive for enteric viruses, 10 (16.7%) contained one virus (HAdV, HBoV or NoV GII) (Table 2) and 50 (83.3%) contained more than one virus with two viruses detected in 29 samples (48.3%), three viruses detected in 14 samples (23.3%) and four viruses detected in 7 samples (11.7%). Among the seven viruses detected in this study, only HBoV was significantly associated with coinfections (p = 0.00017) (Table 3).

Table 4 shows the patients' age distribution for each enteric virus detected. The vast majority of etiological viruses were represented by HAdV, HBoV and NoV GII, which were the most frequently detected in

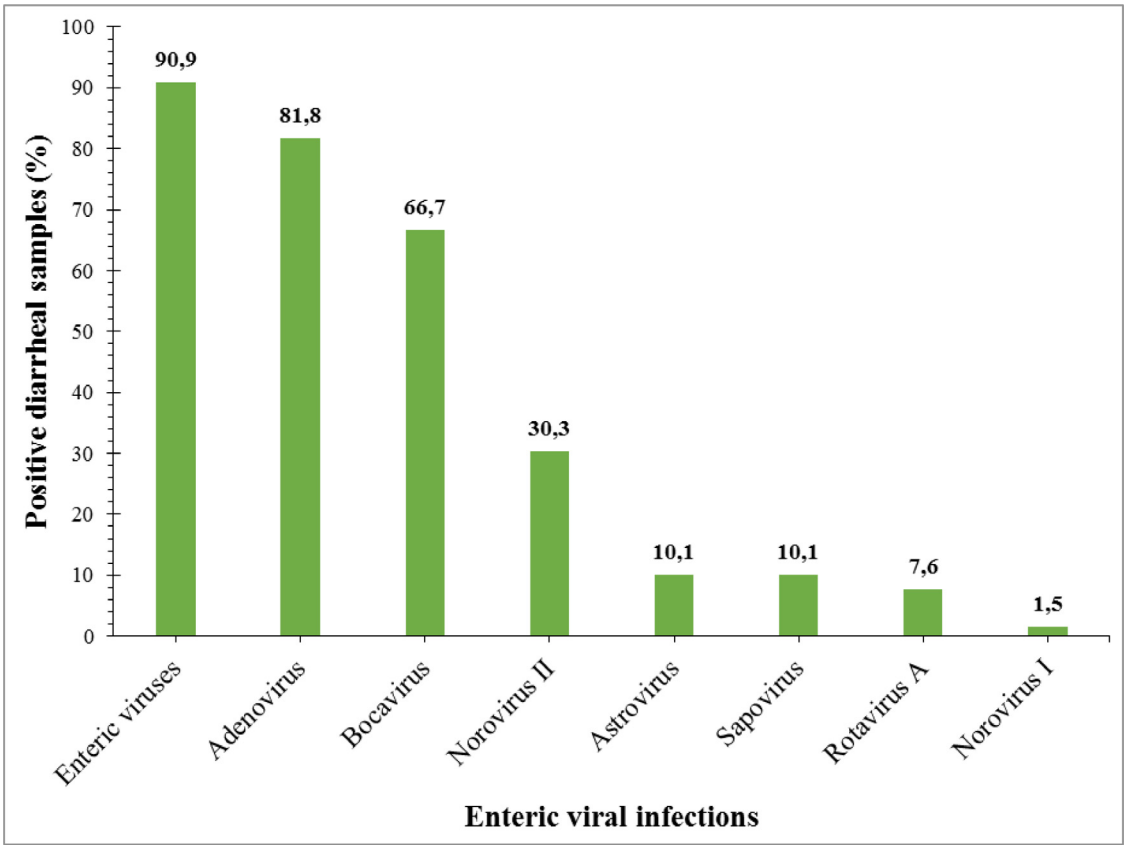

Fig. 1. Molecular detection rate of each enteric virus infection in 66 analyzed diarrheal stool samples.

Table 3
Distribution of enteric viruses detected in diarrheal children with mono- and coinfection (N = 60).

Viral infection	Monoinfection$N$ = 10n (%)	Coinfection$N$ = 50n (%)	P-value
adenovirus	7 (70.0)	47 (94.0)	*0.052*
bocavirus	2 (20.0)	42 (84.0)	***0.00017***
norovirus GII	1 (10.0)	19 (38.0)	*0.142*
astrovirus	0 (0.0)	7 (14.0)	*0.588*
sapovirus	0 (0.0)	7 (14.0)	*0.588*
rotavirus A	0 (0.0)	5 (10.0)	*0.578*
norovirus GI	0 (0.0)	1 (2.0)	*1*

Table 4
Distribution of enteric viruses detected in children with diarrhea according to age group.

Viral infection	0–2 years$N$ = 51n (%)	>2–5 years$N$ = 8n (%)	>5–15 years$N$ = 7n (%)	P-value
adenovirus	45 (88.2)	6 (75.0)	3 (42.9)	***0.011***
bocavirus	36 (70.6)	4 (50.0)	4 (57.1)	*0.443*
norovirus GII	16 (31.4)	2 (25.0)	2 (28.6)	*1*
astrovirus	6 (11.8)	1 (12.5)	0 (0.0)	*1*
sapovirus	7 (13.7)	0 (0.0)	0 (0.0)	*0.482*
rotavirus A	3 (5.9)	0 (0.0)	2 (28.6)	*0.122*
norovirus GI	1 (2.0)	0 (0.0)	0 (0.0)	*1*

all three age groups (Table 3). Only HAdV detection in diarrheal stool samples varied according to the age group (p = 0.011).

3.3. Viral detection and viral clearance

Diarrheal stool samples were collected in 66 children at the time of acute diarrhea (D0). However, non-diarrheal stool samples could only be collected from 16 of these 66 children at day 10 (D10) and at day 21 (D21) after the first sampling. The detection frequencies for enteric viruses were high at D0 with rates of 81%, 69% and 44% for HAdV, HBoV and NoV GII, respectively. The rates were similar at D10 (56%, 56% and 31%, respectively) and at D21 (81%, 88% and 38%, respectively). Long shedding and new infections by HAdV or by HBoV were observed in the children followed (Fig. 2 and Table 5). In total, 48% (n = 16/33) of the enteric viruses detected at baseline were no longer detected at D10 (clearance rates ranged from 36% to 100%), and 28% (n = 7/25) of the viruses detected at D10 were no longer detected at D21, corresponding to clearance rates ranging from 11% to 100% (Table 5).

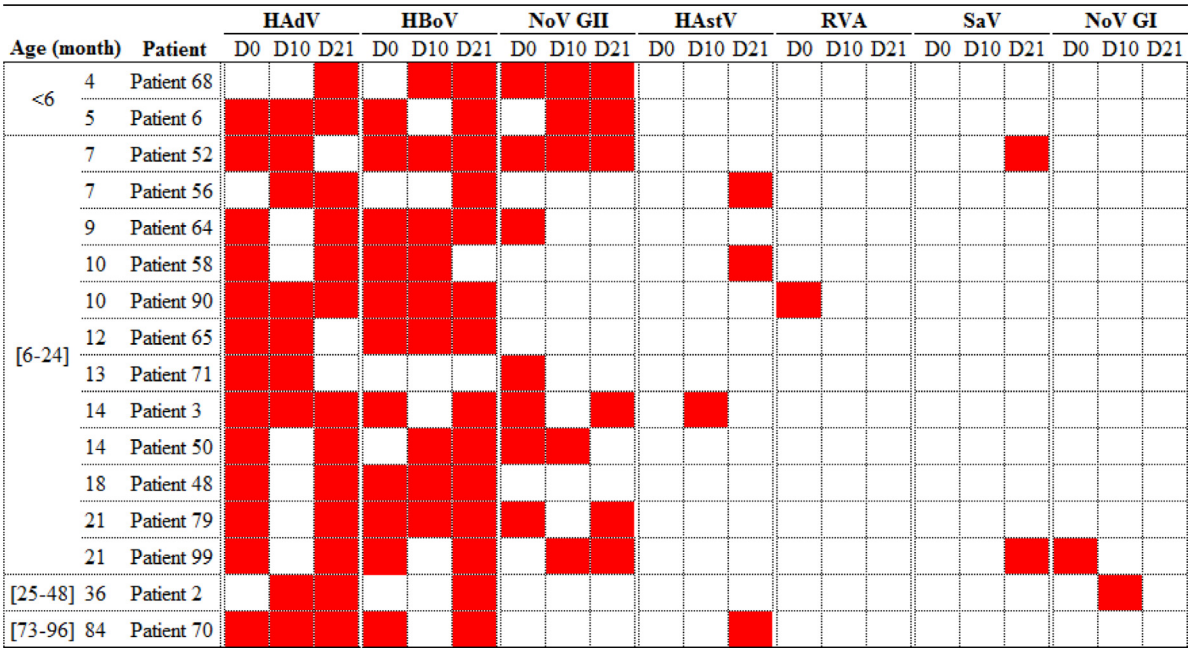

Fig. 2. Overall visualization of enteric virus infections during the medical follow-up of 16 children (N = 16) in Franceville: prolongation of viral shedding, new infections and reinfections.
The boxes colored in red represent cases of infection.
Abbreviations*: HAdV, human adenovirus; HBoV, human bocavirus; NoV GII, norovirus GII; HAstV, human astrovirus; RVA, rotavirus A; SaV, sapovirus; NoV GI, norovirus GI.

Table 5
Changes in detection frequency between day 0, day 10 and day 21, N = 16.

Virus*	Positive at			Cleared at		New at		Change (%)		p^a	p^b
	D0 n (%)	D10 n (%)	D21 n (%)	D10 n (%)	D21 n (%)	D10 n (%)	D21 n (%)	D0 to D10	D10 to D21		
HAdV	13 (81)	9 (56)	13 (81)	6 (46)	3 (33)	2 (13)	7 (44)	−31%	+44%	0.29	0.34
HBoV	11 (69)	9 (56)	14 (88)	4 (36)	1 (11)	2 (13)	6 (38)	−18%	+56%	0.68	0.13
NoV GII	7 (44)	5 (31)	6 (38)	4 (57)	1 (20)	2 (13)	2 (13)	−29%	+20%	0.68	1
HAstV	0 (00)	1 (06)	3 (19)	0 (00)	1 (100)	1 (06)	3 (19)	ND	+200%	1	0.62
RVA	1 (06)	0 (00)	0 (00)	1 (100)	0 (00)	0 (00)	0 (00)	−100%	ND	1	ND
SaV	0 (00)	0 (00)	2 (13)	0 (00)	0 (00)	0 (00)	2 (13)	ND	ND	ND	0.48
NoV GI	1 (06)	1 (06)	0 (00)	1 (100)	1 (100)	1 (06)	0 (00)	0%	−100%	1	1

Abbreviations*: HAdV, human adenovirus; HBoV, human bocavirus; NoV GII, norovirus GII; HAstV, human astrovirus; RVA, rotavirus A; SaV, sapovirus; NoV GI, norovirus GI.
[a] P value by McNemar's exact sign test analyzing whether there was a significant net change from D0 to D10 in the detection of each pathogen.
[b] P value by McNemar's exact sign test analyzing whether there was a significant net change from D0 to D21 in the detection of each pathogen.

Otherwise, the detection frequencies were very low at D0 for RVA and NoV GI, and were zero at D0 for HAstV and SaV. Moreover, low detection rates were found, 6% at D10 for both HAstV and NoV GI, and 13% and 19% at D21 for SaV and HAstV, respectively (Fig. 2 and Table 5).

4. Discussion

Enteric viruses remain a major causative agent of gastroenteritis in children suffering from diarrhea in developing countries.

In this study, we found that 90.9% of the children with diarrhea were infected by enteric viruses in Franceville, Gabon. This prevalence of enteric viruses was higher than the figures previously reported in Franceville (60.9%) [8], and in previous studies conducted in Burkina Faso (85.6%) [6], West Africa (36%) [7] and Tanzania (32.2%) [9]. The high percentage of enteric viruses might be caused by poor hygiene practices and inadequate sanitation infrastructures.

In Gabon, vaccination against RVA is not currently included in the routine immunization schedule, but it is recommended by the Gabonese Pediatric Association [16]. Data on rotavirus vaccination rates in Franceville are unavailable, but a previous data collected at Libreville (Gabon's capital city) showed that the vaccination coverage against RVA was very low (about 5%) [17], due to the cost of this vaccine. Therefore, the low rate of RVA-positive samples reported in this study is all the more surprising compared to previous data in Gabon in which RVA was the most prevalent etiology of childhood diarrhea [8]. This can be due to the small size of diarrheal samples.

Human adenoviruses are particularly pathogenic in both immunocompetent and immunocompromised individuals, and currently, no adenovirus vaccine is available for the general public [18, 19]. In this study, we used broad-range primers for real-time PCR amplification which were able to detect all known HAdV serotypes. The HAdV detection rate found here (81.8%) was higher than figures previously reported in Gabon [8]. This high detection rate may be explained in part by the fact that some non-enteric adenovirus can shed in the stool [19]. Unfortunately, primers specific to enteric adenoviruses known to be associated with diarrhea could not be used. Further research, including genotyping

and phylogenic analysis, is needed to identify and understand the role of enteric and non-enteric adenoviruses in these patients with diarrhea.

Bocavirus was, after adenovirus, the most frequently detected virus in diarrheal children with a detection rate of 60.6%. This is the second study on the detection of bocavirus among diarrheal children in Gabon, especially in Franceville. This rate was higher than the rate previously found in Gabonese children (2.2%) [10], and in other studies [20–22].

NoV GII, one of the two norovirus genogroups (GI and GII) that are known to infect humans, was more frequently detected than NoV GI in our study. Similar findings were previously reported in Gabon [8] and in other studies [23, 24].

For some of the monitored children, stool samples collected at the time of acute diarrhea and samples collected at day 21 after the first sampling were positive for HAdV, HBoV or NoV GII, whereas samples collected at day 10 after the first sampling were negative. These results could be due to prolonged shedding that could not be detected at day 10 because of the low viral load that was below the threshold for detection by real-time PCR. However, it may also be due to a reinfection with the same virus because some subjects may have a short period of viral shedding after infection with an enteric virus [25]. However, to determine the cause, whether prolonged shedding or a reinfection, further epidemiology studies or sequencing are needed.

This study has several limitations. First, the small sampling size might lead to a bad estimation of viral prevalence in the stool samples. Second, molecular tests for the genotyping of certain enteric viruses such as rotavirus, norovirus, adenovirus and astrovirus were not performed in this study. Third, the stool samples were only tested for a limited number of enteric pathogens.

Our study provides useful information on enteric viruses among children in Franceville, Gabon, which may improve the control and management of enteric virus infections.

Frequent coinfection and asymptomatic shedding suggest intense community transmission of these enteric viruses and other enteric pathogens in this low-income Gabonese sitting.

Declaration of Competing Interest

The authors declare no conflict of interest.

The 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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Authors' contributions

NKN: Investigation, Writing – Original draft. SELD: Writing – Original draft. GML: Investigation. OBME: Investigation. SLOL: Investigation, supervision. JBLD: supervision, funding acquisition.

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