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Molecular epidemiology of norovirus infections in symptomatic and asymptomatic children from Bobo Dioulasso, Burkina Faso

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ABSTRACT

Background: Noroviruses (NoV) are a leading cause of gastroenteritis worldwide. Few epidemiological data regarding the NoV strains circulating in African countries are available.
Objectives: To determine the prevalence of NoV in Bobo Dioulasso (Burkina Faso) in both symptomatic and asymptomatic gastroenteritis patients.
Study design: Patients both with and without gastro-intestinal disorders were selected. Clinical and epidemiological data, as well as stool samples, were collected through March to April 2011.

NoV molecular detection (genogrouping and genotyping) and viral load quantification were also performed for all samples.
Results: NoV were detected in 22.2% of the 418 collected stool samples (21.2% and 24.8% from the 293 symptomatic patients (SP) and the 125 asymptomatic patients (ASP) respectively).

Genogroup (G) distribution was 7.5%, 10.2% and 3.4% for GI, GII and both GI/GII respectively among SP and 12.0%, 11.2% and 1.6% for GI, GII and both GI/GII, respectively, among ASP.

Average viral load values were higher in SP than in ASP for GI ($p = 0.03$) but not for GII.

Phylogenic analysis showed a high degree of genotype diversity in SP and ASP. One recombinant GII.7/GII.6 sequence was, to the best of our knowledge, detected for the first time.
Conclusions: This study enabled identification of the specific molecular epidemiology of NoV strains circulating in a representative country in Eastern Africa, and additionally showed that ASP could play an important "reservoir" role. A high strain diversity was detected with a surprisingly high proportion of NoV GI compared to the common genotypes usually reported in comparable epidemiological studies.

1. Background

In developing countries, diarrheal diseases are a major cause of morbidity and mortality, causing more than 1 billion cases of diarrhoea and with 2.5 million deaths occurring in children under 5 years-old between 2000 and 2003 [1]. Epidemiological studies carried out in a range of (mostly industrialised) countries have highlighted Noroviruses (NoV) as the leading non-bacterial agent of gastroenteritis outbreaks in both children and adults, and as an important cause of sporadic gastroenteritis [2]. NoV are single-stranded RNA, non-enveloped viruses belonging to the family *Caliciviridae*, genus *Norovirus*. The genus *Norovirus* has been separated into five genogroups (GI to GV) on the basis of genetic analysis. Genogroups I, II and IV include strains that infect humans [3]. Each genogroup is further divided into several genotypes that in turn comprise several variants, reflecting the high genetic diversity of NoV driven by quick viral evolution (mutations, recombination) typical of such RNA viruses.

NoV are transmitted through the faecal-oral route, either by direct person-to-person spread or indirect transmission routes. The clinical course is characterised by short incubation period (12–48 h), acute-onset vomiting, watery non-bloody diarrhoea with abdominal cramps, fever and nausea [4]. Recovery is usually complete after 2 or 3 days. However, more prolonged courses of illness and virus shedding can occur, particularly among young

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Table 1
Results for molecular detection of noroviruses in symptomatic/asymptomatic populations from different African countries.

Country	Sampled population (number of included patients)	Enteric symptomatology (S[a]/AS[b])	Percentage of positive samples	Note	Reference
Cameroon	5–15 y (54)	AS	29.6	GI.3, GII.4, 8, 17	[23]
Libya	<5 y (239)	S	15.5		[24]
Tanzania	<5 y (280)	S	13.7		[25]
South Africa	<2 y (252)	S	14.3		[26]
Botswana	Children (100)	S/AS	24	S:22%; AS:31%	[18]
Egypt	<18 y (169)	S	26	GII.4≫	[12]
Tunisia	<12 y (788)	S	16.2	GII.4 ≫ GI.2, 4, GII.1, 4,	[27]
	Children (632)	S	17.4	14, b/2, b/3	[28]
Ghana	<11 y (367)	S/AS	7	GII ≫ GI	[29]
	Children (367)	S/AS	10		[19]
	Children (82)	S	15.9	GII ≫ GI	[30]
Madagascar	Children (237)	S	6	GII ≫ GI(.1, 3, 4)	[31]
Malawi	<5 y (398)	S	6.5		[32]

G: genogroup.
[a] Symptomatic.
[b] Asymptomatic.

children, elderly and immunocompromised [5] patients. In these specific populations, increased morbidity and mortality has been demonstrated [6–8].

Currently, diagnosis of NoV infection mainly relies on the detection of viral RNA sequences in the stools of affected persons, by means of RT-PCR assays. Commercial ELISA detecting NoV antigens are very specific tests, but currently exhibit inadequate sensitivity [9,10] to be useful for the diagnosis of sporadic cases.

Many studies describe molecular NoV epidemiology worldwide. However, few data are available about the specific NoV strains circulating in African countries. Up until now, NoV have been molecularly detected in (usually children's) stool samples from both symptomatic and asymptomatic populations from some African countries, with detection rates ranging from 6% to 29.6%. Moreover, studies including non-diarrhoeic patients were very rare (Table 1). When genotyping was performed, the large majority of these studies identified a higher prevalence of GII NoV. However, NoV from both genogroups were also detected in urban and rural river waters as well as in sewage waters from some African countries (namely Ghana, Kenya, Tunisia and Egypt) [11–14]. No epidemiological study has previously been performed in the Sahelian region.

2. Objectives

The aims of this study were: (1) to clarify the molecular prevalence of NoV in both rural and urban areas of Bobo-Dioulasso (Burkina Faso's second city), in children both exhibiting and not exhibiting gastro-enteritis symptoms; (2) to quantify the viral load excreted; and (3) to perform genotyping of the circulating strains.

3. Study design

3.1. Sampled population

To be included in the study patients had to meet the following inclusion criteria: children, either (a) with diarrhoea or liquid stool samples, or (b) without gastro-intestinal disorder for at least 15 days before the sampling. One stool per patient was collected.

Patients (n = 418) were recruited from across Bobo-Dioulasso, Burkina Faso, over an 8 week period (March–April 2011). Sampling was initially performed during medical consultations in one of the four health care centres (Accart-Ville, Colsama, Colma and Dô). However, footfall was low at the Care Centres, and as a result there were not enough samples collected (n = 4). The remaining specimens were collected during a campaign against child malnutrition in two areas of Bobo-Dioulasso (Belleville and Colsama) (n = 231), and during medical consultations in private homes organised for this survey (n = 183).

3.2. Clinical and epidemiological data

A case report form gathering clinical and general information was filled in by each study participant or his/her parents before sampling. This case report form included personal data and usual gastro-intestinal and/or general symptoms observed during the previous 2 weeks, and aimed to differentiate symptomatic from asymptomatic patients.

3.3. Sample treatment and analysis

One gram of stool sample was 10-fold diluted in a PBS–azide 0.01% solution and centrifuged (3000 rpm – 5 min). The supernatant was transferred into new tubes for storage at 4 °C until their transport to Belgium. The specimens were transported to Belgium at room temperature.

Molecular detection of NoV in stool samples was performed in the Medical Microbiology Department of the University of Liège, Belgium. The RNA was automatically extracted on a Maxwell instrument (Promega, Leiden, The Netherlands) using the 16 Cell LEV Total RNA Purification Kit (Promega, Leiden, The Netherlands), following manufacturer instructions. The extracted RNA was

Table 2
Primers and probes used for NoV detection by real time RT-PCR.

Primers/probes	Sequence (5′–3′)	Polarity	Final conc.	Fluorophore (5′)
NoV GI/IV				
QNIF4	CGCTGGATGCGNTTCCAT	+	500 NM	
NV1LCR	CCTTAGACGCCATCATCATTTAC	–	900 nM	
NVGG1p	TGGACAGGAGAYCGCRATCT	+	100 nM	6-FAM
NoV GII				
QNIF2	ATGTTCAGRTGGATGAGRTTCTCWGA	+	500 nM	
COG2R	TCGACGCCATCTTCATTCACA	–	900 nM	
QNIFS	AGCACGTGGGAGGGCGATCG	+	100 nM	JOE