Molecular epidemiology of norovirus in symptomatic and asymptomatic population in Burkina Faso

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BACKGROUND

Noroviruses (NoV), belonging to the family *Caliciviridae,* are now recognized as the leading cause of gastroenteritis outbreaks worldwide, and represent an important cause of sporadic gastroenteritis in both children and adults.

Many studies describe NoV epidemiology. However, few data are available about the NoV strains circulating in most of African countries, in particular in Burkina Faso. The population of Burkina Faso is characterized by the young age of it's habitants, and most are living in rural environment.

Fig1. Geographical regions of Burkina Faso (source: www.guidemondialdevoyage.com)

OBJECTIVES

The purpose of this epidemiological study was to determine the prevalence of NoV infection in the area of Bobo Dioulasso (Southern part of Burkina Faso) by molecular diagnosis methods in patients presenting or not gastroenteritis symptoms, to quantify the excreted viral load, and to genotype the circulating strains.

MATERIAL AND METHODS

Patients with and without gastro-intestinal disorders were selected in several Health Care Centres of Bobo Dioulasso (Fig.2) and during a campaign against malnutrition (Fig.3). Clinical and epidemiological data, as well as stool samples (n=453), were collected during 8 weeks through March to April 2011.

Fig.2: Health Care Centres of Bobo Dioulasso. 1: Dô ; 2: Colma ; 3: Accarville ; 4: Colsama ; 5: Belleville.

Fig.3: Campaign against malnutrition, Colsama (photo: C.Martin)

Viral genomic RNA was automatically extracted with a Maxwell® (Promega) instrument. Molecular detection of genogroups (G) I, II and IV NoV in stool samples was performed by a home-made real-time RT-PCR (1), targeting the ORF1-ORF2 polymerase junction region.

For each positive sample, viral load was estimated by using standard curves (successive dilutions of recombinant GI and GII plasmids). Molecular characterization was performed on 27 detected strains, using both polymerase and capsid regions.

RESULTS

Clinical and epidemiological data

Table I: Epidemiological data for the symptomatic (SP) and the asymptomatic patients (AP).

	SP	AP	Proportion of SP
Age			
0-6 m	24	3	0.89
7-12 m	75	13	0.85
13-36 m	116	45	0.72
3-5 y	65	62	0.51
6-10 y	13	2	0.87
>11 y (11-68y)	26	9	0.74
Sex			
Male	146	56	0.72
Female	173	78	0.69
Health Care Centre			
Colsama	101	53	0.68
Belleville	142	81	0.63
Accart Ville	38	0	1
Colma	34	0	1
Dô	4	0	1

Most of the selected patients in both symptomatic (SP) and asymptomatic patients (AP) were younger than 10 years, representing 91.9 and 93.3% respectively (Table I).

Table II: Frequency of reported symptoms in the symptomatic patients.

Symptoms	N	%
Diarrhoea	319	100
Abdominal pain	138	43
Fever	88	28
Vomits	64	20
Muscular pain	57	18
Shiver	39	12

Molecular detection of NoV and genogrouping

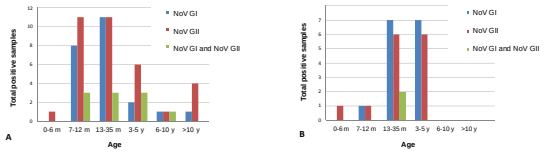
NoV were detected in 21.6% of the 453 collected stool samples, with a distribution of 21.0% and 23.1% in the samples from the 319 symptomatic and the 134 asymptomatic patients respectively.

Genogroup distribution was 34.3% for GI, 50.7% for GII and 15% for both GI and GII among SP's samples, and was 48.4% for GI, 45.2% for GII and 6.4% for both GI and GII among AP's samples (Table III).

Table III. Results of norovirus detection and genogrouping by real time RT-PCR in stool samples of symptomatic (SP) and asymptomatic patients (AP) from Bobo Dioulasso (Burkina Faso).

		NoV detection		Genogrouping		
Patients	Total	Negative	Positive	GI/IV	GII	Mixed
SP	319	252	67	23	34	10
AP	134	67	31	15	14	2
Total	453	355	98	38	48	12

Fig 4. Repartition of the NoV genogroups detected by real time RT-PCR among the different age categories in symptomatic (A) et asymptomatic (B) patients from Bobo Dioulasso (Burkina Faso).



There is neither statistically significant difference between the rate of positive samples in SP and AS (chi2 test) nor in the different age categories (Kolmogorov-Smirnov test).

Average viral load values were higher for GI NoV in SP than in AP (p=0.02), when they were higher for GII NoV in AP than in SP (p=0.04) (Table IV).

Table IV. Descriptive statistics of NoV viral loads obtained by real time RT-PCR in stool samples of habitants from Bobo Dioulasso (Burkina Faso).

		Viral load (copies/ml) (*)		
	N Samples	Average value	Min	Max
SP				
GI/IV	33	3 053 564	16.2	46 300 000
GII	44	177 376	<10	5 104 758
AP				
GI/IV	17	191 397	11.8	1 764 338
GII	16	890 641	12.1	13 300 000

(*) 95% confidence interval

Phylogenic analysis showed a high degree of genotypical diversity in both groups of patients (Fig.5).

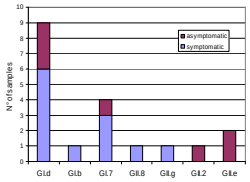


Fig 5. Distribution of NoV genotypes in stool samples of 12 symptomatic and 7 asymptomatic patients from Bobo Dioulasso (Burkina Faso) obtained from PCR performed on the polymerase region.

One recombinant strain GII.7/GII.6 was also detected, to our knowledge, for the first time (Fig.6).

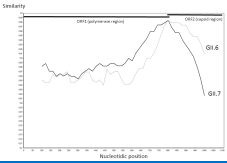


Figure 6. Sliding window nucleotide identity plot of a partial coding region for the RNA-dependent RNA polymerase (3' end of open reading frame 1) and the 5' extremity of ORF2 coding the single capsid protein of the Hu/NV/T209/11/BF genome compared with GII.6 (Hu/NV/SU16/97/JP, gi: AB039778) and GII.7 (Hu/NV/SU4/96/JP, gi: AB020556) reference strains. Analysis performed with the Simplot software. Nucleotide positions are reported on the X-axis and percent similarity on Y-axis.

CONCLUSION

Even if a true pathogenic role of NoV could not be shown from the study design, it allowed to precise the molecular epidemiology of NoV strains prevalent in a representative country of the East African region. It also showed that asymptomatic patients could play an important role as a NoV "reservoir". Despite the fact that GII strains, and more precisely those belonging to GII.4 genotype, are nowadays highly reported worldwide, the surprising proportion of NoV GI detected in this study suggests that GI and GII strains should be excreted in equal proportion in the environment. The origin of this epidemiologic difference, even if partially explained by the difference in immunity and genetic sensitivity of the population, is still to be solved.

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Bibliography

(1) A. Stals *et al*, Journal of Virological Methods 161 (2009) 247–253

Ethic statement: The Medical Ethics Committee of the Faculty of Medicine (University of Liège, Belgium) has accepted the performance of this study (B707201111959, ref. 2011/179).

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