



Astrovirus Gastroenteritis in Children Younger than 5 Years in Ouagadougou (Burkina Faso): Prevalence and Risks Factors Influencing Severity

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Authors' contributions

This work was carried out in collaboration among all authors. Authors ZG, CB and LWN designed the study, managed the literature searches, wrote the protocol and conducted the bench work. Author ZG wrote the first draft of the manuscript. Authors CB and LWN performed the statistical analysis. Author JS supervised all the work. Authors BD, TMZ, IT, TRC, FWD, DO, JN and VP corrected the manuscript.

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ABSTRACT

Aims: The aim of this study was to investigate the prevalence of astrovirus infections and associated risks factors.

Methods: A prospective study was undertaken from May 2009 to March 2010, covering the rainy and dry seasons, at the Saint Camille Medical Center in Ouagadougou, Burkina Faso. A total of 213 non hospitalized children less than 5 years of age with diarrhea were enrolled and examined for astrovirus, others enteropathogens, and clinico-epidemiological aspects.

Results: Astroviruses prevalence among the enrolled children was 14.6%. Astrovirus infections were common during the cold dry season from December to February (38.7%), during the rainy season from June to September (54.8%), also during dry season in March (3.2%) and May (3.2%). Children younger than 11 months of age were most affected by astroviruses (16%). Moderate and severe malnutrition influenced more severe symptoms of astrovirus related diarrheas.

Conclusion: The present study shows that astroviruses have an important role in pediatric viral-associated diarrhea in Burkina Faso. Diarrhea is more severe in malnourished children.

Keywords: Gastroenteritis; Astrovirus; Diarrhea; Malnutrition; Burkina Faso.

1. INTRODUCTION

Gastroenteritis constitutes the main cause of morbidity and mortality among children younger than 5 years and are also a major cause of malnutrition and diminished growth [1]. Diarrheal diseases cause 1.6 million deaths, and constitute about 27% of all deaths among children aged less than 5 years annually [2,3].

Infantile diarrheas are caused by viruses, parasites, bacteria, and some toxins produced by fungi [4,5]. Human astroviruses (HAstVs) are a major cause of viral gastroenteritis after rotaviruses and noroviruses [6] in children, adults and elderly [7].

Human astroviruses (HAstVs) are non-enveloped RNA virus belonging to *Astroviridae* family and subdivided into two distinct genera: *Mamastrovirus* and *Avastrovirus* [8]. They induce moderate or severe diarrhea, fever, loss of appetite, abdominal pain, nausea and vomiting [9]. Human astroviruses are recognized as an endemic cause of acute gastroenteritis globally [10]. Serological studies have shown that up to 90% of individuals in certain populations have antibodies against HAstVs [11,12]. These viruses are transmitted by the fecal-oral route, from person to person and from animals to humans [10]. Studies conducted around the world have shown a wide range of prevalence, with prevalences varying from 8.6% in Thailand to 4.3% in Australia and 3% in Iran [1,13,14]. In Brazil, astroviruses were found in 33% of the children younger than 5 years with diarrhea [15].

In Africa, the few studies conducted on HAstVs reported prevalence of 7% in South Africa [16], 7% in Nigeria [17], 6.3% in Gabon [18], and 4% in Ivory Coast [19]. These studies have not showed specific seasonal pattern for HAstVs infections.

Prior to 2009 in Burkina Faso, the prevalence of astroviruses in children with gastroenteritis was unknown and factors influencing disease such as malnutrition and seasonality had not been investigated.

The aim of this work was to establish the prevalence of astrovirus in children younger than 5 years with diarrhea in the city of Ouagadougou and to assess risks factors for infection and severity.

2. MATERIALS AND METHODS

2.1 Setting

The study was conducted from May 2009 to March 2010 at the Saint Camille Medical Centre (CMSC) and at the Biomolecular Research Center Pietro Annigoni (CERBA/LABIOGENE) in Ouagadougou, Burkina Faso. Ouagadougou is located in the intertropical zone marked by two seasons: a rainy season from June to September and a dry season from October to May, in which a colder period is observed between December to February. The CMSC is a social and humanitarian medical center with several services including: a maternal and child health service (SMI), and a Center for Recovery and Nutritional Education (CREN) that receives

malnourished children. The biomolecular analyses were performed at the Biomolecular Research Pietro Annigoni Center (CERBA).

2.2 Patients

Two hundred and thirteen (213) non-hospitalized children younger than 5 years with diarrhea attending the CMSC were recruited after obtaining written consent from their parents or guardians. They responded to a questionnaire on the epidemiological and clinical characteristics of their children. The study was approved by the CMSC (CERBA/CMSC) Ethics committee.

2.3 Clinical Characteristics and Nutritional Status

Information including gender, age, ethnicity, residence and various clinical signs such as fever ($\geq 38^{\circ}\text{C}$), nausea, vomiting, loss of appetite, duration and number of stools in the last 24 hours were recorded. Diarrhea was defined as the passage of unusually loose or watery stools, usually at least three times in a 24 hour period [20]. The dehydration status in children was also assessed. Severe, some and no dehydration were measured using the childhood diarrhea management guidelines developed by World Health Organization (WHO) [20].

Children's nutritional status was assessed by evaluating the weight, height and age according to the WHO criteria [21]. The children were classified according to the Z-score (or SD-Score: standard deviation score). This classification recommended by WHO and UNICEF uses the nutritional indices weight/height (WHZ), weight/age (WAZ), height/age (HAZ) [21].

Three malnutrition indicators were used: underweight ($Z < -2$ SD; weight-for-age score), growth stunting or chronic malnutrition ($Z < -2$ SD; length-for-age score), and wasting or acute malnutrition ($Z < -2$ SD; weight-for-length score).

Severity score for diarrhea episodes was calculated by using 14-point score system proposed by Ruuska and Vesikari [22] and adapted by Nakagomi et al. [23] and Nitiema et al. [4].

2.4 Collection and Microbiological Examination of Stools

Fecal samples were collected in sterile containers early in the morning by mothers,

transported and stored at 4°C during 24 hours in the laboratory for parasitological and bacteriological analysis. A portion of each stool sample was diluted 1/10 in phosphate saline (PBS) and stored at -20°C for astrovirus and others enteric virus screening. In previous studies samples had been screened for rotavirus, norovirus, pathogenic bacteria, parasites, and yeasts [4, 24]. Bacteria (*Escherichia coli*, *Shigella* spp., *Salmonella* spp.) were investigated by conventional culture procedures [25]. Parasites and yeasts were investigated using conventional microscopy and staining methods [26]. The presence of mucus or blood in stool, as well as the characteristics of the stool was determined.

2.5 Viral RNA Detection

Fecal samples (1/10 diluted) were centrifuged at $12000 \times g$ for 5 min and the supernatant was used for RNA extraction using the Ribo-Sorb kit (Sacace Biotechnologies, Como, Italy).

The viral genome was amplified using the RT-PCR astrovirus kit 175 V-19-50R with electrophoretic detection (Sacace Biotechnologies, Como, Italy) and the GeneAmp PCR system 9700 (Applied Biosystems, USA).

2.6 Statistical Analysis

Statistical analysis was performed using SPSS software version 17.0 (SPSS, Chicago, IL, USA). Malnutrition was evaluated using Nutrition StatCalc from Epi Info 3.5.1 (US Center for Disease Control and Prevention (CDC), 2008) and the CDC/WHO 1978 growth reference data [27]. Data were analyzed by using Chi-square test. A $P < 0.05$ was considered significant.

3. RESULTS

3.1 Epidemiological Characteristics of Children Enrolled in the Study

The children enrolled in the study were composed of 114 (54%) boys and 99 (46%) girls. The majority of children (93%) were from the city of Ouagadougou. The mean age was 15.7 ± 9 months of age. Children's average height was 74 ± 9 and weight was 7.8 ± 2 kg, respectively. The distribution of children in age ranges was 41% in the 0-11 month age group, 45% in the 12-23 age group and 14% in the 24-60 month age group (Table 1).

Table 1. Summary of anthropometric parameters based on age groups

Age group months	Number	Mean weight kg (IQR)	Mean size cm (IQR)	Mean age months (IQR)
0-11	88	6.812 (2.17)	68.407 (20)	8.6256 (8.32)
12-23	95	7.899(2)	74.842(6,5)	17.012(5.38)
24-60	30	10.43(2.409)	86.91(10.5)	32.002(10.41)
Total	213	7.811(2.253)	73.909(10)	15.6851(10.35)

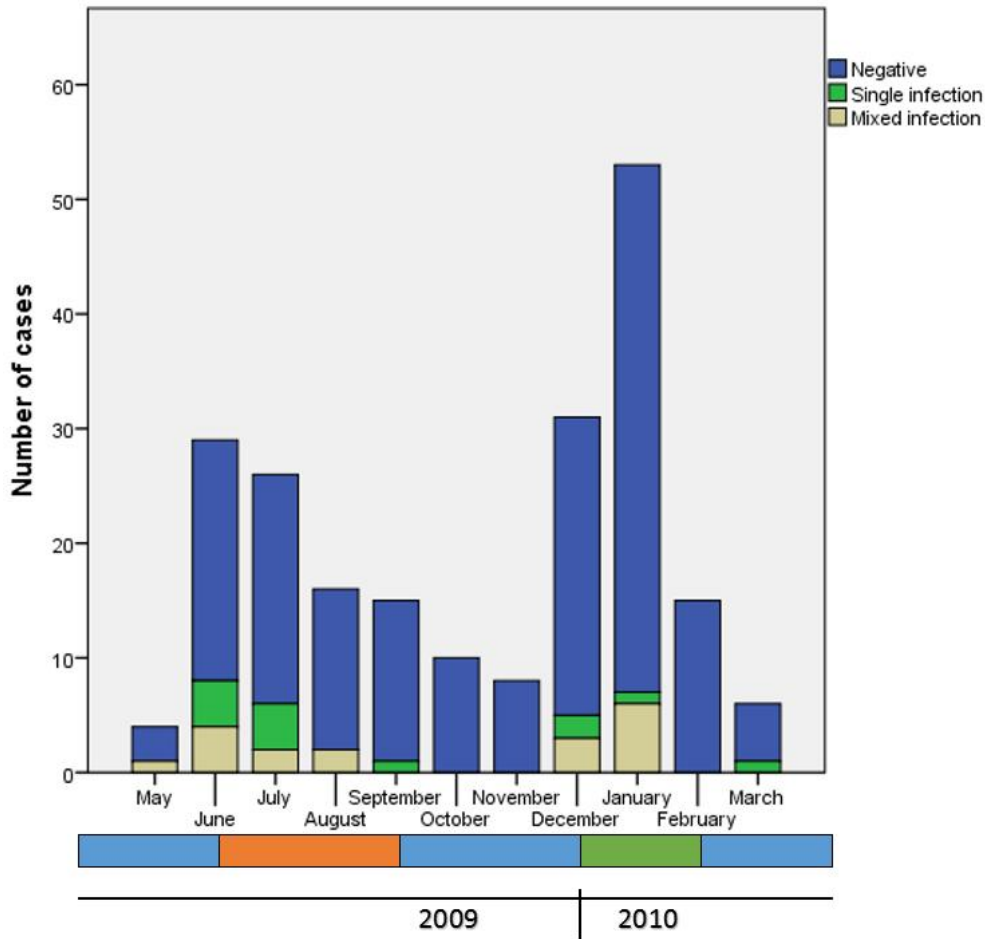


Fig. 1. Seasonal distribution of astrovirus infections

Legend: Dry season (blue line); Rainy season (orange line); Cold dry season (green line)

3.2 Seasonal Distribution of Astrovirus

An increase in diarrhea cases in the cold-dry and rainy seasons coincided with an increase of astroviruses infections. Astroviruses were detected in 14.6% (31/213) of the children enrolled in the study. During the study, we observed a substantial increase in astrovirus diarrhea cases during the rainy season from June to September 54.8% (17/31) with a peak in June 25.8% (8/31). The cases of astrovirus diarrhea also increased during the cold

dry season from December to mid-February 38.7% (12/31) with a peak in January 22.6% (7/31). Astrovirus diarrhea cases were also found during dry season in March 3.2% (1/31) and May 3.2% (1/31). During the rainy season and May, cases of astrovirus-associated gastrointestinal infections were mixed with bacteria and parasites in 29% (9/31). Also, during the cold dry season cases of astrovirus-associated gastrointestinal infections were mixed with rotavirus, bacteria and parasites in 29% (9/31) (Fig. 1).

Table 2. Astrovirus infections in relation to sex, age-group and breastfeeding

Parameters	Number	Total HAstVs (n=31) n(%)	P-value	Mixed infection (n=18) n(%)	P-value
Gender					
Male	114	16 (14.0)	0.818	11 (9.6)	0.500
Female	99	15 (15.2)		7 (7.1)	
Age groups^a					
0-11	88	14 (16)	0.398	4 (4.5)	0.940
12-23	95	13 (13.7)		9 (9.5)	
24-60	30	4 (13.3)		5 (16.7)	
Type of breastfeeding^b					
Exclusively breastfed	63	9 (14.3)	0.557	6 (4.5)	0.234
Non-exclusively breastfed	20	1 (5)		0 (0)	
Not breastfed	130	21 (16.2)		12 (9.2)	

Legend: a. Chi-square test (0-11 and 12-23 vs 24-60); b. Chi-square test (breastfed (exclusively and non-exclusively) vs. not breastfed)

Table 3. Association between astrovirus infections and clinical symptoms

Clinical feature	N 213	HAstV single infection (n=13) n(%)	P-value	Mixed infection (n=18) n(%)	P-value
Fever	74	3 (4.1)	0.628	7 (9.5)	0.628
Vomiting	107	2 (1.9)	0.017	7 (6.5)	0.017
Loss of appetite	146	9 (6.2)	0.463	10 (6.8)	0.463
Nausea	37	5 (13.5)	0.099	2 (5.4)	0.099
Abdominal pain	188	13 (6.9)	0.337	15 (8.0)	0.337
Stools^a					
Watery	131	6 (4.6)	0.241	9 (6.9)	0.295
Loose	82	7 (8.5)		9 (11.0)	
Duration of diarrhea (days)^b					
1-3	104	7 (6.7)	0.274	7 (6.7)	0.004
4-5	45	2 (4.4)		5 (11.1)	
≥ 6	64	4 (6.3)		6 (9.4)	
Loose stools in the past 24 h^c					
1-3	67	3 (4.5)	0.034	5 (7.5)	0.047
4-5	84	5 (6.0)		7 (8.3)	
≥ 6	62	5 (8.1)		6 (9.7)	
Dehydration status^d					
Severe dehydration	72	3 (4.2)	0.467	4 (5.6)	0.237
Some dehydration	101	4 (4.0)		10 (9.9)	
No dehydration	37	5 (13.5)		3 (8.1)	
Severity score		7		7.53	0.110

a: Fisher's exact test Watery vs Loose Stools; b: Fisher's exact test 1-3 vs (4-5; ≥ 6); c: Fisher's exact test 1-3 vs (4-5; ≥ 6); d: Fisher's exact test (Severe dehydration & Some dehydration) vs No dehydration

3.3 Astrovirus Infections in Relation to Sex, Age-group and Breastfeeding

Prevalence of astrovirus was 16% (14/88) in children younger than 11 months, 13.7% (13/95) among children aged between 12 and 23 months and 13.3% (4/30) among those between 24 and 60 months. No significant association was observed between gender, age-group and

astrovirus prevalence. Astrovirus diarrhea was similar in exclusively breastfed children (9/63) compared to non-exclusively breastfed children (1/20) and to those who were not breastfed (21/130). Mixed infections (astrovirus, rotavirus, bacteria and parasite) were observed (18/31) in the same trend with total astrovirus infected cases (Table 2).

Table 4. Malnutrition indicators in relation to the presence of the different enteropathogens and severity of diarrhea

Z score ^a	Number	HAstV single infection (n = 13) n(%)	Severity score	Mixed infection (n= 18) n(%)	Severity score
Underweight status (WAZ)					
Z >-2	87	5 (5.7)	4.8 ± 1.643	5 (5.7)	7.6 ± 1.342
Z= -2 to -3	58	2 (3.4)	7.5 ± 0.707	8 (13.8)	7.5 ± 2.726
Z <-3	67	6 (9.0)	9 ± 1.871	4 (6.0)	7.5 ± 1.291
		<i>P</i> = 0.125		<i>P</i> = 0.003 ^c	
Stunting status (HAZ)					
Z >-2	107	5 (4.7)	4.80 ± 1.643	9 (8.4)	7.67 ± 1.658
Z= -2 to -3	64	2 (3.1)	8	4 (6.3)	6 ± 2.449
Z <-3	41	6 (14.6)	8.80 ± 2.049	4 (9.8)	8.75 ± 1.708
		<i>P</i> = 0.002 ^b		<i>P</i> = 0.084	
Wasting status (WHZ)					
Z >-2	142	8 (5,6)	6 ± 2.138	13 (9.2)	7.85 ± 1.951
Z= -2 to -3	42	4 (9,5)	9 ± 2.646	1 (2.4)	4
Z <-3	28	1 (3,6)	9	3 (10.7)	7.33 ± 1.528
		<i>P</i> = 0.069		<i>P</i> = 0.406	

a: Fisher's exact test [(Z<-3) & (Z=-2 to -3)] vs (Z >-2); b & c: significant difference

3.4 Relationship between Clinical Symptoms and Astrovirus Infections

The children infected only with HAstVs (6.1%, 13/213) presented the following symptoms: fever ≥ 38.0 °C (4.1%), vomiting (1.9%), Nausea (13.5%), Abdominal pain (6.9%), watery stool (4.6%), duration of diarrhea ≥ 6 (6.3%), ≥ 6 loose stools in the past 24h (8.1%), and severe dehydration (4.2%). Astrovirus infections were common in children with vomiting ($P = 0.017$), the number of stools per day more than 3 ($P = 0.034$). In contrast, no associations were observed between astrovirus presence and clinical signs such as fever, loss of appetite, nausea and abdominal pain among children. Mixed infections were observed in 8.5% (18/213) of the diarrhea cases. The same statistical trend was notified with single and mixed HAstVs infection. One difference was the non-significant difference of duration of diarrhea for single infection.

Highest clinical severity scores were observed in mixed infection than single infection. However these differences were statistically non-significant when comparing HAstVs single and mixed infections (Table 3).

3.5 Relationship between Malnutrition and Astrovirus Severity

We observed that 58.7% (125/213) of the enrolled children were underweight ($Z < -2$ SD; weight-for-age score), 49.3% (105/213) suffered from stunting ($Z < -2$ SD; length-for-age score) and 32.9% (70/213) were wasting ($Z < -2$ SD; weight-for-length score). Astrovirus infections were observed in 16% (20/125) underweight children, 15.2% (16/105) in growth stunted children and in 12.9% (9/70) of children with wasting. Single HAstVs is associated to stunting status ($P = 0.002$) and mixed infection to Underweight status ($P = 0.003$). The clinical severity score for diarrhea increased with malnutrition (moderate and severe) for all malnutrition indicators in single infection (Table 4).

4. DISCUSSION

In this study, we investigated the impact of astroviruses in children younger than 5 years old with diarrhea in the city of Ouagadougou, Burkina Faso. The distribution of diarrhea was 14.1% (30/213) among children 24-60 months, 44.6% (95/213) among children 12-23 months,

and 41.3% in children less than one year. A previous study in Burkina Faso showed a diarrheal prevalence of 47% in children in the 0-11 month age group [28]. The average weight (8 ± 2 kg) and height (74 ± 9 cm) of children were undersized as it relates to their ages given their high rates of malnutrition (58.7%, 125/213) and dehydration (81.2%, 173/213).

This study reports a total pediatric HAstVs prevalence of 14.6% (31/213) among diarrhea cases. Comparing HAstVs positive results with others enteropathogens (rotavirus, bacteria and parasites) found in the same patients by Nitiema et al. [4]; we had 8.5% (18/213) of mixed infection and 6.1% (13/213) of single infection. However, another subsequent study in Burkina Faso on patients with and without diarrhea disorders showed HAstVs prevalence of 4.9% [29]. Others prevalence rates between 2 and 6% was reported by several studies in Iran, Gabon and Ivory Coast [1,18,19]. Higher prevalence has been reported in Nigeria (19.4%) [30] Brazil (33%) [15] and in South Africa (37%) [31].

We have found a distribution of astroviruses mostly in cold dry (9/31 for single HAstV and 12/31 for mixed infection) and rainy season (8/31 for single HAstV and 17/31 for mixed infection) in Burkina Faso. One case of HAstV infection was also found in the dry season in March (1/31 for single HAstV) and May (1/31 for mixed infection). In others studies, no pattern of seasonality between the HAstVs positivity rate and climate variables was observed [29,32]. However, astrovirus infections have been found in both hot and cold weather [29,33]. The high prevalence of astroviruses during the dry season might be explained, by a decrease in humidity and precipitation favoring the drying of soil, increasing the transport of dry fecal contaminated particles through the air [34].

The astrovirus infections in children with diarrhea were slightly higher (16%, 14/88) in the 0-11 month age group compared to the 12-23 month age group (13.7%, 13/95). The prevalence was also 13.3% (4/30) for children over 24 months. Our results corroborate those reported in Thailand, Australia and France [13,35,36]. These high rates can be explained by a massive contamination through food, through drinking water and a resistance of astroviruses to environmental factors [37,38].

In this study, 48.2% (104/213) of children went to consultation 1-3 days after onset of diarrhea.

Indeed, 50.2% (107/213) rate of consulting after three (03) days of diarrhea reflects the normalization of diarrhea and may be due to the lack of knowledge of their consequences by mothers. The same observation was reported by Sanou et al. [28] in Burkina Faso.

Breastfeeding has been associated with a decreased incidence of diarrhea in infants in high-, middle- and low-income countries [39,40]. No significant difference was observed between the astrovirus infections and the type of feeding. This can be explained by the low percentage of breastfed children (29.6%; 63/213) in our study, as well as those who benefited from mixed feeding (9.4%; 20/213). Exclusive breastfeeding for a period of six months has many advantages for the infant including the clear advantage to protect against gastrointestinal infections [41].

The Astrovirus infections were associated with the children's nutritional status in our study. Similarly, previous studies have shown a strong correlation between infections with enteropathogens and children's nutritional status [39]. Increased malnutrition could extend the duration and severity of the impact of childhood diarrhea [42].

5. CONCLUSION

In this study, we report the prevalence of astroviruses in children younger than 5 years and risks factors influencing severity of diarrhea-induced in Burkina Faso. This study establishes the impact of malnutrition in the duration and severity of astrovirus gastroenteritis among children younger than 5 years. The study was limited as only for 11 months and no comparisons were made to diarrhoea-free control groups. A long period surveillance should be necessary in future study to clarify HAsV seasonality in Burkina Faso.

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CONSENT AND ETHICAL APPROVAL

Two hundred and thirteen (213) non-hospitalized children younger than 5 years with diarrhea attending the CMSC were recruited after obtaining written consent from their parents or guardians. They responded to a questionnaire on the epidemiological and clinical characteristics of their children. The study was approved by the CMSC (CERBA/CMSC) Ethics committee.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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