

Original article

## Enteric Prevalence of Rotavirus and Adenovirus in Under Five Children Predisposed to Acute Diarrhea in Yaounde-Cameroon

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### ABSTRACT:

**Introduction:** Acute diarrhea in tropical countries can be caused by a broad spectrum of viral, parasitic, and bacterial enteropathogens.

The characteristics of the epidemiology of rotavirus and adenovirus worldwide needs to be considered in the process of vaccine development. Acute diarrhea in tropical countries can be caused by a broad spectrum of viral, parasitic and bacterial enteropathogens.

**Objectives:** The main objective of this study was to identify Rotavirus and Adenovirus in under five children predisposed with acute diarrhea in Yaoundé-Cameroon using VIKIA Rota-Adeno rapid test kit (BioMérieux, France).

**Methodology:** A descriptive cross sectional study was carried out in under five children presenting with acute diarrhea in four hospitals in the Yaoundé municipality namely; Yaoundé University Teaching Hospital (YUTH), Chantal Biya Foundation (CIRCB), Gyneco-Obstetric and Pediatrics Hospital (GOPHY), and the District Hospital Biyem-assi, from April 2008 to September 2008.

Stool samples were collected in sterile stool containers following microbiologically approved methods. Wet preparations of the different samples were examined for the presence of parasites, red cells, white cells, and yeast cells. The samples were analyzed for rotavirus and adenovirus by immunochromatographic method using the VIKIA Rota-Adeno rapid test kit.

**Result:** A total of 61 children presenting with acute diarrhoea were recruited into the study during the study period. The mean ( $\pm$  SD) age of the study participants was  $15.02 \pm 13.7$  months (range 2 – 59 months). There were 30 (49.2%) males and 31 (50.8%) females. The mean ( $\pm$  SD) ages of the male and female recruits were  $16.60 \pm 15.07$  and  $13.48 \pm 12.29$  months respectively.

An enteric pathogen incidence of 85.3% was detected in the children. This study showed a rotavirus prevalence of 32.8% in children with

acute diarrhea. A significant difference was observed when the prevalence of rotavirus in males (46.7%) was compared to that of females (19.4%), ( $P=0.04$ ). It was also observed that 41.0% of children 0-12 months were most affected with rotavirus infection, while the age group 13-24 months had a prevalence of 30.7%. Three children (4.9%) were positive for both rotavirus and adenovirus. Adenovirus was detected in 6.6% of the children. The age distribution of adenovirus infection showed 7.7% prevalence in the age group 0-12 months and one case of adenovirus in the age group 37-48 months. 6.5% of females and 6.7% of males were infected with adenovirus. There was a significant incidence of viral infection, bacterial/parasitic infection and Rota virus among the children, with a low prevalence recorded for adenovirus. The males were more predisposed to Rota virus than males

*Co-infection could cause difficulties for pediatricians and health care workers in terms of the diagnosis, treatment and prophylaxis of diarrhea in children. More studies are necessary in order to evaluate this area so as to further elucidate this problem in Cameroon.*

**Key words:** Enteric prevalence, Rotavirus, Adenovirus, *E. coli*, *E. histolytica*, Candida, Salmonella, acute diarrhea,

### RESUME:

**Introduction:** La diarrhée aigue dans les pays tropicaux peut être cause par un spectre élargi de virale, parasite et bactérie entéro pathogène.

La prévalence des infections virale, bactérie/parasitique et Rota virus étaient significatif avec une faible incidence de Adénovirus chez les enfants. Les males étaient plus prédisposé que les femelle avec la rota virus.

Dans la plus part de pays sous-Saharien, l'étude sur la prevalence et l'importance Clinique de different pathogene diarheique sont soit incomplete ou inexistent. Les caracteristiques de l'epidemiologie de rota et adeno virus dans le mode entire doivent etre considerer dans les processus de developpement de vaccine.

**Objectifs :** L'objectif principal de cette étude était pour identifier les rota et adénovirus chez les enfants a moins de cinq ans manifestants la diarrhée aigue a Yaoundé Cameroun en utilisant le test rapide VIKIA Rota-Adeno (Biome rieux, France).

**Méthodologie :** Une étude trans-sectionné descriptif a été mené avec les enfants moins de cinq ans manifestants la diarrhée aigue dans quatre l'hôpital dans la ville de Yaoundé notamment ; Centre Hospitalier Universitaire (CHU), la fondation Chantal Biya (FCB), L'hôpital gynéco-obstétrique et pédiatrique (HGO), et L'hôpital de district de Biyem-assi, a partir de Avril 2008 jusqu'au Septembre 2008. Les selles étaient collecte dans les boites stériles selon les processus standard en microbiologie et examine pour la présence des parasites, globule rouges, globule blancs, et levures. Les échantillons étaient analysée pour la présence de rota et adéno virus par la méthode immuno chromatographique, en utilisant les kits de test rapide Rota-Adeno.

**Résultat :** Un total de 61 enfants moins de cinq ans étaient recrute dans l'étude et le moyen âge de participation étaient de 15.02 ±13.7 mois (range de 2-59 mois). Il y'avait 30 (49.2%) males et 31 (50.8%) femelles. L'incidence de pathogène entérique de 85.3%, étaient détectée chez les enfants. Une différence significative était observée chez les males. On a note que 41.0% des enfants 0-12 mois étaient plus infecte avec rota-virus alors que les 13-24 ans avaient un prévalence de 30.7%. Trois enfants (4.9%) étaient positif avec rota et adénovirus. L'âge de distribution de l'infection adénovirus montrée une prévalence de 7.7% chez les enfants 0-12 mois et un cas de adéno virus dans the âges de 37-48 mois. Il y avait une incidence significatif de infection viral, bactérie/infection parasitique et rota virus parmi les enfants, avec une faible prévalence pour adénovirus.Co-infection peuvent causée une difficulté pour le pédiatre et responsable de soins et de la sante pour le diagnostiques, traitement et prophylaxie de diarrhée chez les enfants. Il faut encore beaucoup de études pour évaluer ce sujet pour élucider le problème au Cameroun.

**Mot Clés :** prévalence entérique, Rotavirus, Adénovirus, *E. coli*, *E. histolytica*, Candida, Salmonelle, diarrhée aigue.

## INTRODUCTION

Diarrhea is a major cause of childhood morbidity and mortality in poor countries. More than one billion episodes of diarrhea occur every year among children under five years of age causing approximately 2.5 million deaths [1, 2]. The WHO Child Health Epidemiology Reference Group estimates that 16 percent of deaths in African children younger than five years are directly attributed to diarrheal diseases [3, 4].

Acute diarrhea in tropical countries can be caused by a broad spectrum of viral, parasitic, and bacterial enteropathogens. For many sub-Saharan countries, studies on the prevalence and clinical significance of different diarrheal pathogens are incomplete or not available [4, 5].

The characteristics of the epidemiology of rotavirus and adenovirus worldwide will need to be considered in the process of vaccine development. Fecal specimens from children in developed countries show a single rotavirus strain from one of the four common serotypes found globally, specifically P(8)G1, P(8)G3, P(4)G2, and P(8)G4 [6, 7, 8]. In contrast, in developing countries the rate of mixed infections of at least two strains can be 30%, and viruses can include uncommon geographically specific serotypes [5, 6]. The first study from Cameroon showed that of the eight hundred and ninety diarrhea stools collected from children under the age of 5 years in Western Cameroon between 1999 and 2000. Rotaviruses were detected in 21.9 per cent of the stools [9], therefore Rotavirus infection was shown to be an important component of diarrheal disease in young children in Cameroon. The results of this study in Cameroon reinforce the need to continue with surveillance programs in Africa [10].

This work was done to bring to light the prevalence of rotavirus and adenovirus associated with acute gastroenteritis in the Yaoundé municipality in order to serve as a base for continuous monitoring, and to provide complementary data on the pathogens that are frequently encountered in diarrheic samples in our context and thus help the physician better in prescription and management of patients with viral and/or bacterial/parasitic diarrhea infections in Cameroon. We determined the prevalence of Rotavirus and Adenovirus in under 5 children presenting with acute diarrhea in Yaoundé-Cameroon.

## METHODOLOGY:

A descriptive cross-sectional study was conducted in the Yaoundé University Teaching Hospital (YUTH), Gyneco-Obstetric and Pediatrics Hospital Yaoundé (GOPHY), The Mother and Child center of the Chantal Biya Foundation (CBF), and the District Hospital Biyem-Assi were used as study site for recruitment; while the Yaoundé University Teaching Hospital Bacteriology Laboratory. The study lasted for 6 months, from April 2008 to September 2008. The inclusion criteria was all in and out-patients less than 5 years who presented with acute diarrhea at YUTH, GOPHY, CBF and the District Hospital Biyem-Assi, and whose parents gave their informed consent to participate in the study. Those excluded were children between 0 to 5 years consulted in the above hospitals for other reasons than diarrhea.

**Sampling and Sample collection.** The study sample was obtained by a conservative sampling method. After informed consent was obtained, a pediatrician assigned to the study examined each patient and filled out the demographic data and information on clinical symptoms and illness onset on a standardized questionnaire form. The different stool samples were collected following microbiologically approved aseptic methods and/or techniques. The samples were transported to the laboratory within 2 hours after collection for analysis.

### Principles and Procedure of Tests:

VIKIA Rota-Adeno kit is a qualitative test based on the association of monoclonal antibodies specific to rotavirus and adenovirus respectively. The test uses immunological reactions (antigen-antibody) performed on a test strip by migration (Immunochromatographic test or Lateral flow). About 50mg or 50ul of the stool was diluted in the diluant provided by the manufacturer and homogenized. Two drops of the diluted stool sample were then transferred to the sample well of the test cassette. After 10 minutes, a blue line indicated the presence of rotavirus while a red line indicated the presence of adenovirus; a blue and a red line indicated the presence of the two viruses. The integrity of the test was indicated by a control line in the absence of which the test was considered invalid. Data were entered and analyzed using EPI Info version 3.2, Word, and Excel for Microsoft Windows. Rates and proportions were calculated and the results were presented using frequency tables and Bar charts. The Chi square test and the Yates correction test where appropriate were used to analyze categorical

variables. A 95% confidence interval was calculated and values of  $P < 0.05$  were considered significant. Ethical clearance for this study was obtained from National Ethical Clearance Commission the Hospitals involved in the study. An Authorization was obtained from the Faculty of Medicine and Biomedical Sciences. Subjects' parents and heads of study units were informed of the objectives of the study and their signed consent obtained. All participants who fulfilled the inclusion criteria were recruited.

## RESULTS

A total of 61 children less than 5 years presenting with acute diarrhoea were recruited into the study during the study period. The mean ( $\pm$  SD) age of the study participants was  $15.02 \pm 13.7$  months (range 2 – 59 months). There were 30 (49.2%) males and 31 (50.8%) females (Table 1). The mean ( $\pm$  SD) ages of the male and female recruits were  $16.60 \pm 15.07$  and  $13.48 \pm 12.29$  months respectively. Forty of the 61 children (65.6%) were 0-12 months old (Table 2).

Rotavirus had the highest prevalence (46.7%) in males, while *E. coli* had the highest prevalence (22.6%) in females (Table 1). All the pathogens identified were found in females whereas in males, *Salmonella* was not identified (Table 1). A significant difference was observed in the prevalence of rotavirus in males (46.7%) when compared to that of females (19.4%), ( $P=0.04$ ); there was no significant difference of adenovirus infection in the two sexes ( $P = 0.6$ ).

**Table 1: Distribution of the different pathogens by sex group.**

Sex	Frequency (%)	Rotavirus (%)	Adenovirus (%)	<i>E. coli</i> (%)	<i>E. histolytica</i> (%)	Candida (%)	Salmonella (%)
<b>Female</b>	31(50.8)	49.4	6.5	22.6	6.5	0.7	3.2
<b>Male</b>	30(49.2)	46.7	6.7	23.3	46.7	6.7	0
<b>Total</b>	61	96.1	13.2	45.9	53.2	7.4	3.2

The age distribution showed that rotavirus and *E.coli* infections were predominant in children between the age group 0-12months and 13-24months. Most of the pathogens were identified in children less than 24months. Candida was the only pathogen identified in the age group 25-36months, while

*E.histolytica* was the only pathogen identified in the age group 49-60 months. Adenovirus and *E. coli* were identified in the age group 37-48 months (Table 2).

**Table 2: Distribution of identified pathogens in different age groups.**

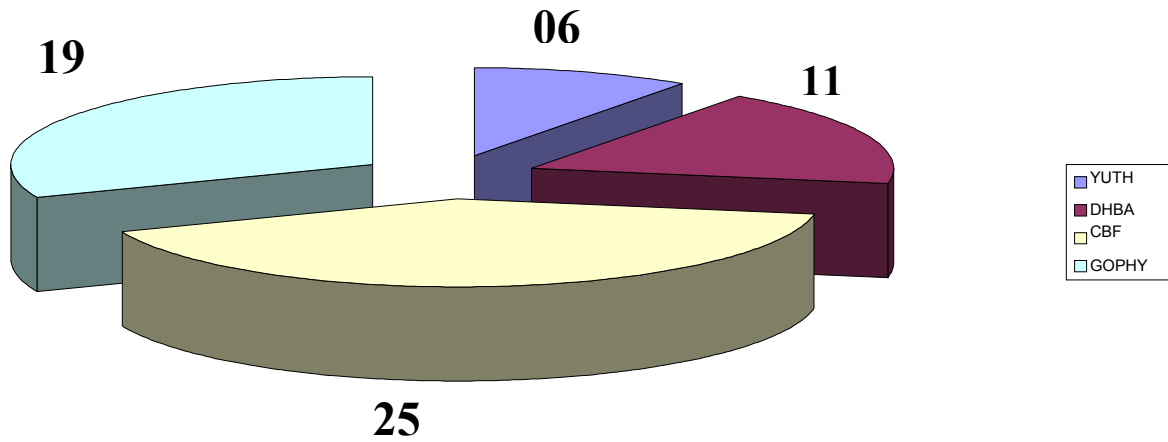
Age group (months)	Frequency (%)	Rotavirus	Adenovirus	<i>E. coli</i>	<i>E. histolytica</i>	Candida	Salmonella
<b>0-12</b>	40 (65.6)	41	7.7	23.1	10.3	5.1	0.0
<b>13-24</b>	13(21.3)	30.7	0.0	30.8	15.45	15.4	7.7
<b>25-36</b>	03(4.9)	0.0	0.0	0.0	0.0	0.0	32.3
<b>37-48</b>	03(4.9)	0.0	0.0	33.3	0.0	0.0	0.0
<b>49-60</b>	02(3.3)	0.0	33.3	0.0	33.3	0.0	0.0
<b>Total</b>	61(100)	71.7	41.0	87.2	59.05	20.5	40.7

Rotavirus presented with the highest prevalence (32.8%) while Adenovirus prevalence (6.6%) was least of the identified pathogens.

**Table 3: Summary Table of Results**

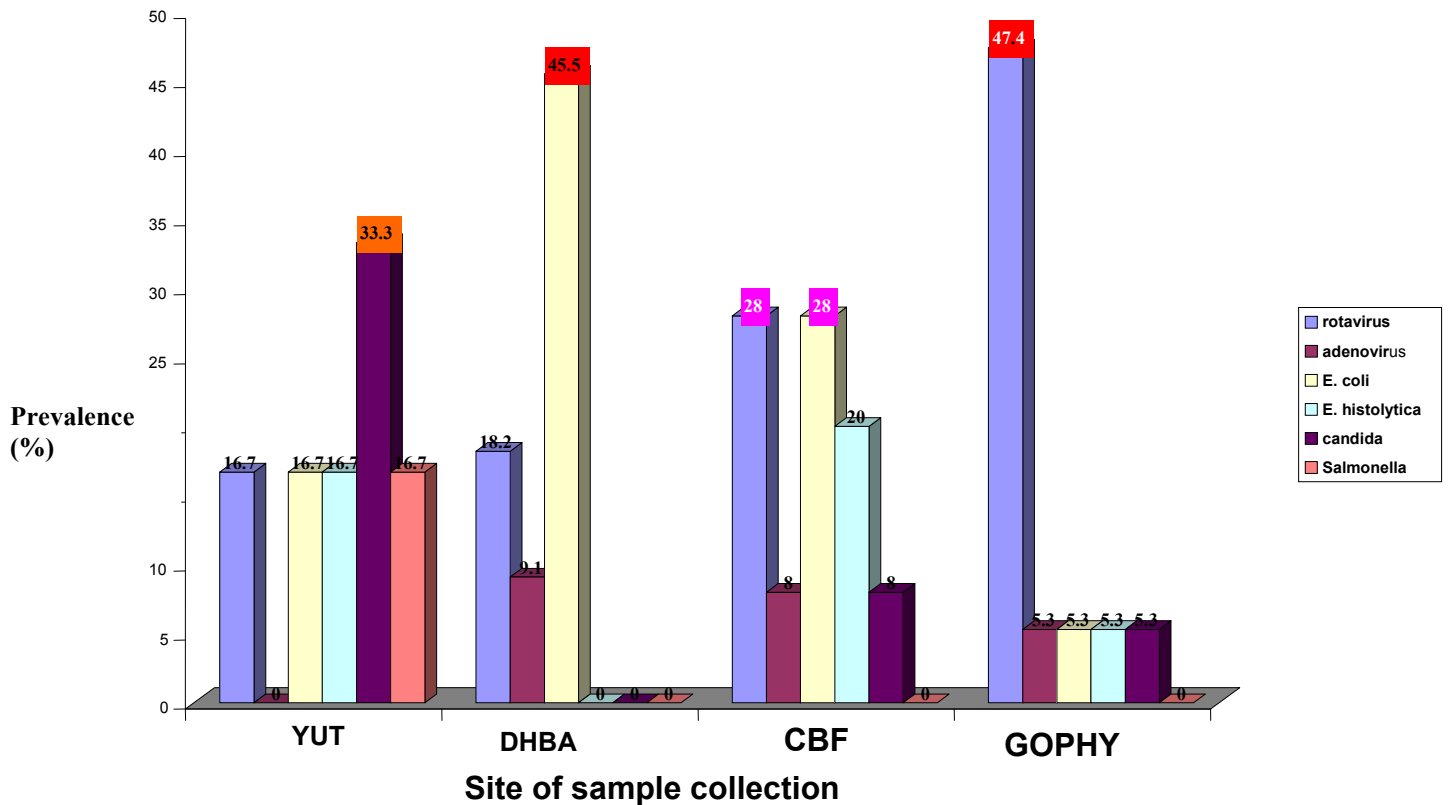
Infection	Study Population	Positive Cases	Total	Prevalence (%)
<b>Rotavirus</b>	Children <5 yrs with diarrhoea	20	61	<b>32.8</b>
<b>Adenovirus</b>	Children <5 yrs with diarrhoea	4	61	6.6

**Proportion of samples collected from different sites**



**Figure 1: Proportion of samples collected from different sites.**

The highest proportion of samples was collected from CBF (41.0%), followed by GOPHY (31.0%), then DHBA (18.0%) and the lowest from YUTH as shown in figure 1.



**Figure 2: Site distribution of identified pathogens.**

The pathogens detected in the stool samples included rotavirus, adenovirus, *E. coli*, *E. histolytica*, *Candida*, and *Salmonella*, with frequency varying at each study site (figure 2). Specifically *Candida* (33.3%) was the predominant pathogen identified in YUTH, *E. coli* (45.5) in DHBA, rotavirus and *E. coli* (28.0% each) in CBF, and rotavirus (47.4%) in GOPHY.

#### DISCUSSION:

A total of 61 stool samples from children under 5 years (60 months) old with acute diarrhea (mean  $\pm$ SD= 15.02  $\pm$  13.7 months and range 02 – 59 months) consulting in four hospitals in Yaounde (YUTH, District hospital Biyem-assi, CBF, and HGOPY) were collected and analyzed for some enteric pathogens (Rotavirus, Adenovirus, some bacteria, parasites and fungi).

Of the 61 children studied, enteric pathogen were detected in 52 children (85.3%). This prevalence was much higher than those reported for some foreign studies as [11] , 11, 12, 13,[4] 66% [12], 50% [13], 54% [5], 78% [14, 23], and 77% [15]. To our knowledge the prevalence reported in this study is the highest ever reported. However, these different studies are not all directly comparable as the microbiological methods were different, and the range of pathogens studied varied.

Many studies have shown the important role of rotavirus as a cause of diarrhea in children in both developed and developing countries [6, 7, 12, 15, 16, 17, 18]. Most of the cases occurred in children less than 5 years of age. Overall, the prevalence of rotavirus-positive children with diarrhea ranges from 30 to 50% [18]. Our study showed a rotavirus prevalence of 32.8% in children with acute diarrhea.

A similar result (32%) was obtained in an urban hospital in Mexico [19]; these results are higher than the 21.9% obtained in Western Cameroon [10]. A significant difference was observed when the prevalence of rotavirus in males (46.7%) was compared to that of females (19.4%), ( $P=0.04$ ). We also observed that 41.0% of children from 0 to 12 months old were the most affected, or had the highest number of cases with rotavirus infection (16 out of 40%), while the age group 13-24 months had a prevalence of 30.7% (4 out of 13). This decrease in trend of the prevalence of enteric rotavirus with increasing age is in accordance with results obtained in Hanoi Vietnam [12]. This might partly be explained by the fact that older children acquired protective immunity during previous exposures and are therefore more resistant to infection with this pathogen, or the infection is less symptomatic [18, 19]. In addition to the age distribution of rotavirus infection, many studies have indicated a higher ratio of infected males to infected females [7, 18,

20, 21, 22]. The ratio in our study was 2.4. No reasonable explanation has yet been advanced for this distribution. Three children were positive for both rotavirus and adenovirus. Adenovirus was detected in 4 children (6.6%) with acute diarrhea. These results are similar [23], lower [5, 12, 24], or higher [19, 22, 24] than other results reported in the literature.

#### CONCLUSION

The prevalence of viral infections was 39.3% while that of Bacteria/Parasite infections was 36.06%. Males were more likely to contract rotavirus infection than females. Most children under 24 months were more susceptible to both rotavirus and adenovirus infections. Co-infection rate was low at 5.7% within the children population under study. The co-infection could cause difficulties for pediatricians and health care workers in terms of the diagnosis, treatment and prophylaxis of diarrhea in children. More studies are necessary in order to throw more light on this problem in Cameroon.

**ACKNOWLEDGEMENTS:** Our great appreciation to the management and Staff of the four hospitals in the Yaoundé municipality namely; Yaoundé University Teaching Hospital (YUTH), Chantal Biya Foundation (CIRCB), Gyneco-Obstetric and Pediatrics Hospital (GOPHY), and the District Hospital Biyem-assi, for accepting us to collect data from their patients. MULTIPPA-Cameroon for the technical support of the project.

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