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## Electrophoretotypes Characterization of Human Rotavirus in Two Pediatric Services, Ouagadougou, Burkina Faso

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The aim of this study was to determine the prevalence of rotavirus infection and characterized group A rotavirus in stool by immunochromatographic test and polyacrylamide gel electrophoresis. Then 150 specimens of stools were collected from patient children between December 2006 and April 2007 and analyzed. The antigenic detection of rotaviruses carry out by immunochromatographic has revealed the presence of group A rotavirus antigen in 21 (14%), adenovirus were also found in 8 (5.33%) and a co-infection rota-adenovirus in 2 (1.33%) of the 150 stool samples tested. Infants were most frequently affected, 15 (71, 43%) of the 21 children rotavirus-positive, were infants  $\leq$  1 year of age. Based on migration patterns of RNA segments of 21 rotavirus isolates, two distinct groups of electropherotypes of group A rotaviruses were identified: 7 (36.84%) isolates were Long (L) and 12 (63.15%) were Short (S) electropherotypes but any co-infection by both was identified. This first study in the Burkina-Faso has shown the main genomic patterns of rotavirus. There is a need for further detailed studies on the molecular characterization of rotavirus which would have important implications in vaccine evaluation programs.

**Key words:** Acute diarrhea, rotavirus, immunochromatographic, PAGE, electrophoretotypes

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## INTRODUCTION

Diarrhea is a major cause of childhood morbidity and mortality in socioeconomic developing countries (Parashar *et al.*, 2003a, 2006). Annually, more than 1 billion episodes of diarrhea occur among children under 5 years of age, causing approximately 2.5 million deaths, particularly in tropical regions (Kirkwood *et al.*, 2006; Kosek *et al.*, 2003; Logan *et al.*, 2006; O'Ryan *et al.*, 2001; Parashar *et al.*, 2003a). Acute childhood diarrhea is commonly caused by viruses, notably rotavirus (Pang *et al.*, 2004).

Acute diarrhea in tropical countries can be caused by a broad spectrum of parasitic, bacterial enteropathogens and viral gastroenteritis. Rotavirus diseases are the most childhood important diarrheic diseases (Parashar *et al.*, 2003b). Rotavirus, a member of the Reoviridae family is a non enveloped virus with a double-stranded RNA (dsRNA) genome of 11 segments enclosed in triple-layered protein coat (Bishop *et al.*, 1973). For many sub-Saharan countries, studies on the prevalence and clinical significance of different diarrheic pathogens are incomplete or not available at all. However it is estimated that 500, 000-870, 000 deaths occur annually because of severe dehydration caused by human rotavirus in developing countries with 150, 000-200, 000 deaths in Africa (Steele and Ivanoff, 2003). The WHO Child Health Epidemiology Reference Group estimates that 16% of deaths in African children younger than five years are directly attributable to diarrheic diseases (Bryce *et al.*, 2005). This makes rotavirus-vaccine development a high priority for Africa. In Burkina Faso the occurrence of rotavirus infection has been reported by Sanou *et al.* (1999). They represented about 15-41% of diarrheic diseases, but the circulating strains and electrophoretotypes have not been studied. Electrophoresis of the rotavirus RNA genome allows detection and classification of the viruses into two majors groups, the Long (L) and the Short (S) electrophoretotypes based on the migration patterns of gene segments 10 and 11. Presently six groups (A-F) and 14 G serotypes have been identified, with group A rotaviruses found to be the predominant cause of infection in humans (Estes, 1996). Underlying reasons for the spread of diarrheic diseases are found in poor hygiene and sanitation, limited access to safe drinking water as well as in inadequate education of health care providers and recipients (Barro *et al.*, 2002; Barro *et al.*, 2007; Curtis *et al.*, 2000; Koopmans and Duizer, 2004; Thapar and Sanderson, 2004). This research deal with occurrence of human rotavirus group A from children under five years stool samples using immunochromatographic (ICG) test and their electrophoretotypes characterization by the polyacrylamide gel electrophoresis (PAGE).

## MATERIALS AND METHODS

**Study area and population:** The centre area of Burkina Faso has in all parts savannah-type vegetation and climate with rains from May to October. Ouagadougou, the capital, has a population of approximately 1, 200, 000 inhabitants. Between December 2006 and April 2007, corresponding to the dry season and cold period, 150 children with acute diarrhea fulfilled the inclusion criteria were consecutively enrolled in the study at the pediatric Health Centre CHU CDG and the pediatric service of Medical Centre of the sector 30. Patients met the following inclusion criteria: acute diarrheas according WHO definitions ( $\geq 3$  watery or loose stools within 24 h for  $< 14$  days, age  $\leq 5$  years).

**Stool samples collect:** From December to April, fecal specimens were collected from sick children between 1 and 60 months of age using these health services laboratory facilities. Whole stool specimens collected into sterile jar were transported and stored at 4°C until processing for analysis. For detection of rotavirus antigen, individual stool samples were mixed with 10% (wt/vol) of cold PBS (pH 7.4). The samples were homogenized by vortex mixing and were centrifuged at 1,500 x g at 25°C for 10 min and the supernatants were used or stored at -70°C until use. Each month, specimens were analyzed by an immunochromatographic method and the PAGE for enteric rotaviruses identification as previously described by Roman *et al.* (2003).

**Immunochromatographic (ICG) detection of rotavirus antigen:** Fresh aliquot processing as indicated above was used to perform the Rota-Adeno immunochromatographic test (VIKIA® Rota-Adeno, Biomerieux®, Nancy l'Etoile, France) as previously described (Roman *et al.*, 2003; Wilhelmi *et al.*, 2001) and recommended by manufacturer. About 70-80  $\mu$ L of processed stool samples was putted into ICG well. After 10 min incubation at room temperature, the results of test were read by observation of colored indicator. Stool samples rotavirus positive are indicated by the blue color in front of letter R and adenovirus positive by the red color in front of letter A.

**Viral ribonucleic acids extraction:** The RNA was extracted by phenol-chloroform method as previously (Steele and Alexander, 1987) with slight modifications. Fecal samples were diluted 1:10 by weight with PBS pH 7.4 and the crude fecal extracts were centrifuged at 3,000 x g, 20 min to sediment any macroscopic debris. The clarified stool suspensions were mixed with 1/10 volume of 1 M sodium acetate containing 1% (wt/vol) sodium dodecyl sulfate and incubated for 15 min at 37°C. Viral RNA was extracted by deproteinization with 1 volume of

a 1:1 phenol-chloroform, mixture at 56°C for 15 min and centrifugation at 10,000 x g for 3 min. The RNA was precipitated from the aqueous phase with 1/10 volume of 3 M sodium acetate and 3 volumes of ethanol at -20°C. The RNA was pelleted by centrifugation at 10,000 x g for 10 min and the pellet was carefully dried and resuspended in 40 µL of sample buffer (0.12 M Tris-HCL, 0.1% sodium dodecyl sulfate, 15% glycerol, 0.001% bromophenol blue).

**Polyacrylamide gel electrophoresis of genomic RNAs:** PAGE was performed on 21 ICG rotavirus positive stool specimens, from 12 children admitted to the hospital and 9 children in community with diarrhea, to screen for the rotavirus electrophoretotypes identification. Electrophoresis of the extracted RNA was carried out in 10% polyacrylamide slab gels, [acrylamide-to bis-acrylamide ratio of 37.5: 1, in TBE 1X, which were polymerized with 0.01% (vol/vol) N', N', N', N'-tetramethylethylenediamine and 0.05% (wt/vol) ammonium persulfate] with a 3% stacking gel, using the discontinuous buffer system as previously described (Laemmili, 1970) without sodium dodecyl sulfate. Approximately 30 µL of each sample was loaded into gel wells and electrophoresis was performed at room temperature for 18 h at 40 mA and 100 V. The gel was stained with ethidium bromide (1 µg mL<sup>-1</sup>) and then visualized with a UV transilluminator.

**RESULTS**

**Prevalence and age distribution of diarrheic viral enteropathogens:** Table 1 indicates viral enteropathogens identified in stool samples. Rotaviruses were detected in 21 of 150 (14%) and adenoviruses were detected in 8 of 150 (5.33%) stool samples by immunochromatographic (ICG). Mix infections by both viruses were observed with 2 (1.33%) stool samples.

The population-based incidence of rotavirus gastroenteritis in children < 5 years of age in Ouagadougou, Burkina Faso, in five months is shown in (Table 2). The incidence of rotavirus gastroenteritis is 71.43% in the class of children ≤ 1 year of age.

**Electrophoretotypes identification:** Before electrophoresing genomic RNA extraction yield was assess by spectrophotometer. Nineteen stool samples exhibited a higher RNA extract. Of the total 21 immunochromatographic rotavirus-positive stool samples, 19 (90.48%) exhibited the typical human rotavirus group A electrophoretotype profile. The major electrophoretic patterns are indicated in Fig. 1.

The migration patterns of RNA segments of rotavirus isolated from stool samples of diseased children in Burkina Faso, exhibited the group A genomic RNA profiles. While, two distinct groups of electropherotypes

of group A rotaviruses were identified: 7 (36.84%) strains were long, and 12 (63.15%) were short electropherotypes. Short and Long electropherotypes co-circulated during study period but any co-infection and atypical electrophoretic migration patterns were identified.

**RNA segments diversity between various rotavirus electrophoretotypes:** Two methods are usually used to detect rotavirus segment varieties. The coelectrophoresis of mixed different electropherotypes (Rodger *et al.*, 1981) and standard electrophoresis comparison with a molecular ladder or the reference strain RNA. The second method was used in present study. Genomic RNAs electrophoretic migration pattern shown in Fig. 1 analysis shown that both long and short electropherotypes exhibited few varieties. Observation of migration pattern of bands corresponding to RNA segments has shown clearly that short and long electropherotypes are different in segments group 4. The result in (Table 3) indicated the electropherotypes which exhibited the slightly difference or same profile regarding the Lourenco *et al.* (1981) group of segment classification. Electrophoretotype S1 is different to S2, S3, S4 and S5 by segments 7, 8, 9; S4 is different to S5 by segments 5 and 6 and electrophoretotype S3 is different to S4 by segment 4. Looking long electropherotypes, L1 is different to L2, L3, L4 and L5 by segment 4. L3, L4 were identical. In other, S2, S3, S4 and S5 group 4 segments 10 and 11 exhibited the same position. S3 and S4 group 3 segments 7, 8 and 9 occupied the same position with S2.

Table 1: Occurrence of rotavirus and adenovirus, in stool samples from 150 children attending a health services CHU-CDG and CMA sect. 30 in Ouagadougou, Burkina Faso

Virus	Prevalence
Rotavirus only	21 (14%)
Adenovirus only	8 (5.33%)
Rota-adenovirus	2 (1.33%)

Table 2: Distribution of children with less 5 years of age and those excreting Rotavirus

Age (month)	Diarrheic children (n = 150)	Rotavirus positive (n = 21)
0-6	53 (35.33%)	10 (47.61%)
7-12	46 (30.66%)	5 (23.80%)
13-18	20 (13.33%)	none
19-24	14 (9.33%)	3 (14.28%)
25-30	2 (1.33%)	none
31-36	5 (3.33%)	1 (4.76)
37-60	10 (6.66%)	2 (9.52)

Table 3: Diversity of segments among various genomic RNA electrophoretotypes

Group of segments <sup>a</sup>	Electrophoretotypes exhibiting differences in the group of segments	
Group 1	S3 and S4	L1 and L2, L3, L4, L5
Group 2	S4 and S5	-
Group 3	S1 and S2, S3, S4, S5	-
Group 4	-	-

<sup>a</sup>Group of segments according Lourenco *et al.* (1981) classification; S1 to S5 and L1-L5 represent the major electropherotypes identified; - = No difference

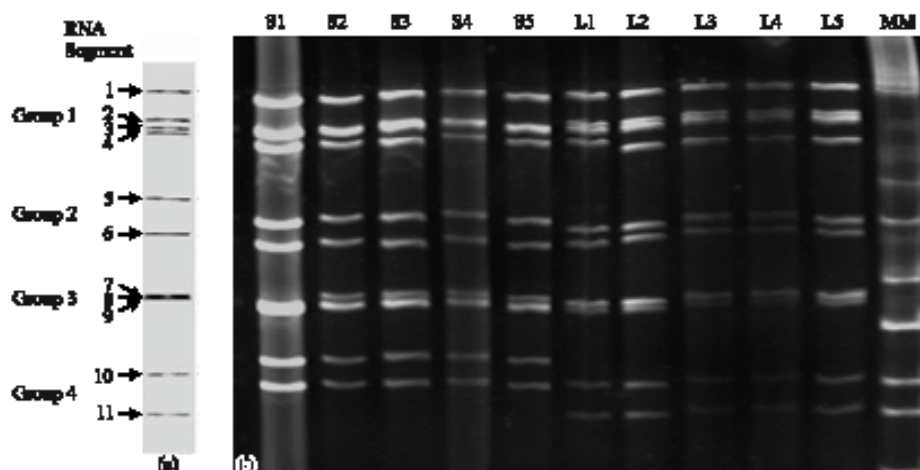


Fig. 1: (a) Representative 4-2-3-2 pattern of group A rotaviruses (Jayaram *et al.*, 2004). (b) Polyacrylamide gel electrophoresis (10%) analysis of human rotavirus. Major samples that exhibited the typical group A genomic RNA profiles after 18 h migration at 100 V (40 mA). The long profiles are shown by letter L and the short profiles by letter S. Ladder (New England Biolabs® Inc, USA)

Table 4: Electrophoretotypes distribution during the study period

Electrophoretotypes	Study period			
	Dec.-Jan.	Feb.	Mar.	Apr.
Short	S1*, S2*, S3*	S2*, S3*	S4*, S5*	S4*, S5*
Long	L3*	L2*, L3*, L4*	L1*, L2*	L2*

\*From Hospitalized children, \*From community children

**Temporal distribution of electrophoretotypes:** Each month, electrophoretic profiles were observed with the collected stool samples. As shown in (Table 4) both majors classes of rotavirus electrophoretotypes were observed (long and short RNA profiles). The long electrophoretotypes were observed only in hospitalized children stool samples. Each month a number of different rotavirus strains, as defined by PAGE RNA electrophoretotypes circulated in the community and hospitalized children.

## DISCUSSION

Rotavirus gastroenteritis represents a major part of diarrheic diseases among the children less 5 years of age in the world (Bryce *et al.*, 2005; Carlin *et al.*, 1998; Glass *et al.*, 1996; Parashar *et al.*, 2003a) and in West African countries (Armah *et al.*, 2003; Audu *et al.*, 2002; Kasule *et al.*, 2003; Parashar *et al.*, 2003b; Steele and Alexander, 1987; Steele and Ivanoff, 2003). In Burkina Faso data report a rate of 14-41% of rotavirus gastroenteritis prevalence in pediatric services (Sanou *et al.*, 1999; Ihapar and Sanderson, 2004). Viral gastroenteritis data are very scarce in Burkina Faso because of lack of diagnosis tools. The current study was carried out with the technical assistance of Multi-Diseases Surveillance Centre

(MSDC/WHO) located in Burkina Faso. Results of this study were among the first researches on molecular characterization of rotavirus in Burkina Faso.

Although preliminary, our findings clearly indicated the major importance of rotavirus in the etiology of gastroenteritis in our region. The incidence (Table 1) of rotavirus diarrheas in Ouagadougou, Burkina Faso (14%) is comparable to those of other studies conducted in the world (Díez-Domingo *et al.*, 2006; Parashar *et al.*, 2003b). Data indicated that adenoviruses are also cause of children less five years of age diarrheas. As indicated in Table 2, rotavirus infection was predominant in children of 0-6 months of age with the high percentage (47.61%) following by the group of 7-12 months of age (23.80%). This trend has been observed in many studies and means that the rotavirus diarrheic gastroenteritis's occur early in human life (Adah *et al.*, 2001; Georges-Courbot *et al.*, 1988; Kasule *et al.*, 2003; Sanou *et al.*, 1999).

PAGE analysis (Fig. 1) revealed that prevalence of both long L (36.85%) and short S (63.15%) electrophoretotypes. However the rates of L and S electrophoretotypes differ from findings in many studies: Armah *et al.* (2003) in the East of Ghana isolated only a short electrophoretotypes, Kasule *et al.* (2003) reported high prevalence of long electrophoretotypes (70%) in Botswana; Szczepaniak *et al.* (1996) and White *et al.* (1984) found 83% of long profile in Venezuela. Although some segments were stacked up, the general genomic RNAs segments pattern was 4-2-3-2 as described by Lourenco *et al.* (1981) classification. Short and long electrophoretotypes are also different by segments group IV. The co-circulation of short and long electrophoretotypes

was reported in many countries (Gault, 1998) and this situation was confirmed by our findings. In this study few cases 2 (1.33%) of co-infection with other gastroenteritis viruses such as Adenovirus were identified. But, in contrast with data from Ali *et al.* (1993) in Bangladesh rural areas, none rotavirus typical electrophoretic migration exhibiting a feature of mixed infections pattern were observed. In addition, higher variations of electrophoretotypes have been detected with increasing frequency in studies (Armah *et al.*, 2003; Kasule *et al.*, 2003). Genomic RNAs electrophoretic migration pattern analysis has shown slightly variations among both long and short electrophoretotypes (Table 3). Most long electrophoretotypes are similar to the LB electrophoretotype and electrophoretotypes short are similar to electrophoretotypes SB identified in Ga-Rankuwa Hospital (Steele and Alexander, 1987). This distribution of RNA patterns for the five months surveyed in this study was not unusual, although the percentage of short patterns was greater than in most studies (White *et al.* 1984; Szczepaniak *et al.*, 1996; Armah *et al.*, 2003; Kasule *et al.*, 2003; Logan *et al.*, 2006).

The temporal distribution study between December 2006 and April 2007 has shown that both electrophoretotypes short and long were existed and circulated (Table 4). Several studies shown that high peak of rotavirus gastroenteritis are observed at the period of January to March (Diez-Domingo *et al.*, 2006; Georges-Courbot *et al.*, 1988). Two short electrophoretotype S1 and S4 and all the long profiles were detected in hospitalized children stool samples. The S3 pattern was detected in non-hospitalized children stool samples. The electrophoretotypes short and long were identified in the two health services. A number of different rotavirus strains as revealed by PAGE RNA electrophoretotyping circulated. Steele and Alexander (1987) shown that they are a constant change in numbers and types of RNA electrophoretotypes and some strains ceased to exist with a new strains emerging within the community. It was reported that most of the rotavirus strains existed for short periods and disappeared within the space of few months (Steele and Alexander, 1987). In present study S2 and L3 have circulated within three months interval (December-January-February).

In the light of the above described findings, assessing the importance of rotavirus infection epidemiological study should be emphasized to well know the sources, sociological, hygienic aspects as previously suggested (Barro *et al.*, 2008). The findings of this study confirmed the occurrence of group A human rotavirus in stool samples of children with gastroenteritis. We also confirmed some of the circulating long and short electrophoretotypes in Burkina Faso. The sample size and

distribution of temporal sampling are slight limitations of this study. It has however demonstrated some fundamental features of rotavirus molecular epidemiology in Burkina Faso high incidence of short electrophoretotypes. The findings highlight the need for continuous monitoring of serotypes identification and distribution in West Africa to provide information essential for rotavirus vaccine development.

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#### REFERENCES

- Adah, M.I, A. Wade and K. Taniguchi, 2001. Molecular epidemiology of rotaviruses in Nigeria: Detection of unusual strains with G2P [6] and G8P [1] specificities. *J. Clin. Microbiol.*, 39: 3969-3975.
- Ali, A., L. Unicomb, F. Bingnan and A. Hossain, 1993. Molecular heterogeneity of human group A rotavirus in rural Bangladesh as determined by electrophoresis of genomic ribonucleic acid. *Trop. Med.*, 35: 53-63.
- Armah, G.E., A.D. Steele, F.N. Binka, M.D. Esona and R.H. Asmah *et al.*, 2003. Changing patterns of rotavirus genotypes in Ghana: Emergence of human rotavirus G9 as a major cause of diarrhea in children. *J. Clin. Microbiol.*, 41: 2317-2322.
- Audu, R., S.A. Omilabu, M. De Beer, I. Peenze and A.D. Steele, 2002. Diversity of human rotavirus VP6, VP7 and VP4 in Lagos state Nigeria. *J. Health Popul. Nutr.*, 20: 59-64.
- Barro, N., P. Nikiéma, C.A.T. Ouattara and A.S. Traoré, 2002. Evaluation de l'hygiène et de la qualité microbiologique de quelques aliments de la rue et les caractéristiques des consommateurs dans les villes de Ouagadougou et de Bobo-Dioulasso (Burkina-Faso). *Rév. Sci. Tech. Santé*, 25: 7-21.
- Barro, N., A.R. Bello, Y. Itsiembou, A. Savadogo and C.A.T. Ouattara *et al.*, 2007. Street-vended foods improvement: Contamination mechanisms and application of food safety objective strategy: Critical review. *Pak. J. Nutr.*, 6: 01-10.

- Barro, N., L. Sangaré, M.C. Tahita, O. Traoré, C.A. De Souza and A.S. Traoré, 2008. Risk Associated with Practices, Procedures and Processes of Street-Vended Foods that Lead to Foodborne Viral Contamination: Critical Review. In: Hygiene and its Role in Health. Patrick, L.A. and J.P. Lachan (Eds.). Nova Science Publishers Inc., Hauppauge, NY 11788, USA., ISBN: 978-1-60456-195-1 (In Press).
- Bishop, R.F., G.P. Davidson, I.H. Holmes and B.J. Ruck, 1973. Virus particles in epithelial cells of duodenal mucosa from children with acute non-bacterial gastroenteritis. *Lancet*, 2: 1281-1283.
- Bryce, J., C. Boschi-Pinto, K. Shibuya and R.E. Black, 2005. WHO estimates of the causes of death children. *Lancet*, 365: 1147-1152.
- Carlin, J.B., P. Chondros, P. Masendycz, H. Bugg, R.F. Bishop and G.L. Barnes, 1998. Rotavirus infection and rates of hospitalisation for acute gastroenteritis in young children in Australia, 1993-1996. *Med. J. Aust.*, 169: 252-256.
- Curtis, V., S. Cairncross and R. Yonli, 2000. Domestic hygiene and diarrhea-pinpointing the problem. *Trop. Med. Int. Health*, 5: 22-32.
- Díez-Domingo, J., I.O. Martín, A.B. Sanz, A.G. López and C.C. Martínez *et al.*, 2006. Rotavirus gastroenteritis among children under five years of age in Valencia, Spain. *Pediatr. Infect. Dis. J.*, 25: 455-457.
- Estes, M., 1996. Rotaviruses and their Replication. In: *Fields Virology*, 3rd Edn. Fields, B.N., D.M. Knipe and P.M. Howley (Eds.). Lippincot-Raven Publisher, Philadelphia Pa, pp: 1625-1655.
- Gault, E., 1998. Genetique des rotavirus. *Virol*, 2: 139-148.
- Georges-Courbot, M.C., A.M. Beraud, G.M. Beards, A.D. Campbell and J.P. Gonzalez *et al.*, 1988. Subgroups, serotypes and electrophoretotypes of rotavirus isolated from children in Bangui, Central African Republic. *J. Clin. Microbiol.*, 26: 668-671.
- Glass, R.I., P.E. Kilgore and R.C. Holman, 1996. The epidemiology of rotavirus diarrhea in the United States: Surveillance and estimates of disease burden. *J. Infect. Dis.*, 174: 5-11.
- Jayaram, H., M.K. Estes and B.V. Prasad, 2004. Emerging themes in rotavirus cell entry, genome organization, transcription and replication. *Virus Res.*, 101: 67-81.
- Kasule, M., T.K. Sebuya, B.A. Gashe, G. Armah and A.D. Steele, 2003. Detection and characterization of human rotavirus among children with diarrhoea in Botswana. *Trop. Med. Int. Health*, 8: 1137-1142.
- Kirkwood, C.D., N. Bogdanovic-Sakran, D. Cannan, R.F. Bishop and G.L. Barnes, 2006. National rotavirus surveillance program annual report. *Commun. Dis. Intell.*, 30: 133-136.
- Koopmans, M. and E. Duizer, 2004. Foodborne viruses: An emerging problem. *Int. J. Food Microbiol.*, 90: 23-41.
- Kosek, M., C. Bern and R.L. Guerrant, 2003. The global burden of diarrhoeal disease, as estimated from studies published between 1992 and 2000. *Bull. World Health Organ.*, 81: 197-204.
- Laemmli, U.K., 1970. Cleavage of structural proteins during the assembly of head of bacteriophage T<sub>4</sub>. *Nature*, 227: 680-685.
- Logan, C., J.J. O'Leary and N. O'Sullivan, 2006. Real-time reverse transcription-PCR for detection of rotavirus and adenovirus as causative agents of acute viral gastroenteritis in children. *J. Clin. Microbiol.*, 44: 3189-3195.
- Lourenco, M.H., J.C. Nicolas, J. Cohen, R. Scherrer and F. Bricout, 1981. Study of human rotavirus genome by electrophoresis: Attempt of classification among strains isolated in France. *Ann. Virol. Inst. Past*, 132: 161-173.
- O'Ryan, M., I. Pérez-Schael, N. Mamani, A. Peña and B. Salinas *et al.*, 2001. Rotavirus-associated medical visits and hospitalizations in South America: A prospective study at three large sentinel hospitals. *Pediatr. Infect. Dis. J.*, 20: 685-689.
- Pang, X.L., B. Lee, N. Boroumond, B. Leblanc, J.K. Preiksaitis and C.C. Yulp, 2004. Increased detection of rotavirus using reverse transcription-polymerase chain reaction (RT-PCR) assay in stools specimens from children with diarrhea. *J. Med. Virol.*, 72: 496-501.
- Parashar, U.D., E.G. Hummelman, J.S. Bresee, M.A. Miller and R.I. Glass, 2003a. Global illness and deaths caused by rotavirus disease in children. *Emerg. Infect. Dis.*, 9: 565-572.
- Parashar, U.D., J.S. Bresee and R.I. Glass, 2003b. The global burden of diarrhoeal disease in children. *Bull. World Health Organ.*, 81: 236-236.
- Parashar, U.D., C.J. Gibson, J.S. Bresee and R.I. Glass, 2006. Rotavirus and severe childhood diarrhea. *Emerg. Infect. Dis.*, 12: 304-306.
- Rodger, S.M., R.F. Bishop, C. Birch, B. McLean and I.H. Holmes, 1981. Molecular epidemiology of human rotaviruses in Melbourne, Australia, from 1973 to 1979, as determined by electrophoresis of genome ribonucleic acid. *J. Clin. Microbiol.*, 13: 272-278.
- Roman, E., I. Wilhelmi, J. Colomina, J. Villar and M.L. Cilleruelo *et al.*, 2003. Acute viral gastroenteritis: Proportion and clinical relevance of multiple infections in Spanish children. *J. Med. Microbiol.*, 52: 435-440.

- Sanou, I., K.L. Kam, A. Tougouma, L. Sangare and J.H.P. Nikiema *et al.*, 1999. Diarrhées aiguës de l'enfant: Aspect épidémiologiques, cliniques et évolutifs en milieu hospitalier pédiatrique à Ouagadougou. *Méd. d'Afr. Noire*, 46: 21-26.
- Steele, A.D. and J.J. Alexander, 1987. Molecular epidemiology of rotavirus in black infants in South Africa. *J. Clin. Microbiol.*, 25: 2384-2387.
- Steele, A.D. and B. Ivanoff, 2003. Rotavirus strains circulating in Africa during 1996-1999: emergence of G9 strains and P [6] strains. *Vaccine*, 21: 361-367.
- Szczepaniak, Z., W. Gut and Z. Jarzabek, 1996. Characterization of rotaviral RNA isolated from children with gastroenteritis in Poland. *Arch. Immunol. Ther. Exp. (Warsz.)*, 44: 303-308.
- Thapar, N. and I.R. Sanderson, 2004. Diarrhoea in children: An interface between developing and developed countries. *Lancet*, 363: 641-653.
- White, L., I. Perez, M. Perez, G. Urbina, H. Greenberg, A. Kapikian and J. Flores, 1984. Relative frequency of Rotavirus subgroups 1 and 2 in Venezuelan children with gastroenteritis as assayed with monoclonal antibodies. *J. Clin. Microbiol.*, 19: 516-520.
- Wilhelmi, I., J. Colomina, D. Martín-Rodrigo, E. Roman and A. Sánchez-Fauquier, 2001. New immunochromatographic method for rapid detection of rotaviruses in stool samples compared with standard enzyme immunoassay and latex agglutination techniques. *Eur. J. Clin. Microbiol. Infect. Dis.*, 20: 741-743.