Original article

Usage of Silver-Stained Polyacrylamide Gels Electrophoresis (PAGE) for Detection of Rotavirus Infection by Direct Identification of Viral Nucleic Acid

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Abstract:

Background: Rotavirus infects almost all children by the age of five. More than 180,000 annual deaths due to rotavirus, occurs in Bangladesh. ***Aims***: This study aimed to determine the incidence of rotavirus infection in children by a modified polyacrylamide gel electrophoresis (PAGE) in stool samples. ***Materials and Methods***: In this descriptive type cross sectional study, a total of 400 stool samples were examined for the presence of rotavirus by a modified PAGE analysis of viral genome. Stool culture was done for common enteric pathogens. The study was carried out from November 2012 to July 2013 in the department of Microbiology, Mymensingh Medical College, Mymensingh. ***Results***: PAGE results were found in 365 of 400 (91%) specimens. Maximum incidence of rotavirus infection was seen in age group of 6 months - 24 months (67.25%). All 151 rotavirus positive cases did not show infection with bacterial pathogens. ***Conclusion***: The modified PAGE technique for the detection of viral RNA was found to be rapid, simple, reliable and less expensive technique.

Keywords: rotavirus; polyacrylamide gel electrophoresis (page) technique; diarrhoea

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Introduction:

Rotavirus infects almost all children by the age of five, both in the developing and developed countries[1]. Rotavirus is composed by 11 double-stranded RNA segments surrounded by three concentric protein layers. The outer capsid consists of VP7 (a glycoprotein) and VP4 (a protease-sensitive protein) which carry independent neutralization and protective antigens[2]. In temperate climates, rotavirus is most often detected in the winter and rarely in the summer, whereas in the tropics it is found all year round, with less-defined seasonal variation[3]. Of the approximately 600,000 annual deaths due to rotavirus (RV) worldwide, more than 180,000 occur in Bangladesh. Also, 20 to 30 percent hospitalized cases of diarrhoea are due to rotaviruses[4].

Clinically rotavirus gastroenteritis is characterized by profuse diarrhoea, mild fever and vomiting leading to mild to severe dehydration. The clinical manifestations of rotavirus diarrhoea alone are not sufficiently distinctive to permit diagnosis[1]. The laboratory diagnosis of Rota virus infection is done mainly by ELISA, which require expensive

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commercial kits and reagents as also expensive instruments. Hence, not many laboratories are able to diagnose rotavirus infection. In view of this we undertook to evaluate the reliability of the Polyacrylamide gel electrophoresis (PAGE) technique as developed by Herring et al[5].

Materials and methods:

During the period of November 2012 to July 2013, all patients with acute diarrhoea irrespective of sex admitted in Mymensingh Medical College Hospital and S.K hospital, Mymensingh were included in this study. Information about children and their consent were taken from their parents/guardians. This study was approved by ethical Committee of Mymensingh Medical College Hospital and S.K hospital.

Inclusion Criteria

- Children not older than 5 years of age group.
- Children with profuse watery diarrhoea
- Diarrhoea less than 10 days duration.

Exclusion Criteria

- Children above 5 years.
- Diarrhoea more than 10 days.

Stool samples were investigated for rotavirus by PAGE (Polyacrylamide gel electrophoresis). PAGE and silver staining technique were performed as per the method of herring et al[5] and Merill et al[7]. Briefly a 0.5ml of 0.1 M sodium acetate solution containing 1 percent sodium dodecyl sulphate and 0.5ml phenol chloroform mixture was added to 100 mg of fecal sample. This was vortexed and centrifuged at 7000rpm for 2 minutes. The aqueous upper layer containing the double stranded RNA was removed for electrophoresis and run on gel of size 14×16cm and 0.75mm thickness with 7 wells. Ten percent polyacrylemide gels with 3 percent stacking gel were used. Each well was loaded with 40μl of RNA extract to which 10 μl of sample buffer containing 0.5 M Tris base, 1 percent bromophenol blue and 20 percent glycerol were added. The running buffer consisted of Tris glycine pH 8.8. Discontinuous electrophoresis was carried out as described by Laemlli at 30 mA for 3 hrs at room temperature[8]. Finally, the double stranded RNA was visualized by silver staining. The gel was gently lifted of the glass and the stacking gel was cutoff and bottom gel was placed in washing solution consisting of 200 ml ethonol (95 %) and acetic acid (5%) and continuously rocked for 25 to 30 minutes. Next washing solution was drained of and 0.011 silver nitrate added for 50 minutes and then drained off. The gel was then briefly rinsed twice with distilled water. Developing solution (NaOH 15 gram, 3.8ml formaldehyde dissolved in 500ml distilled water) was added for 5 to 10 minutes. This was replaced with stopping solution namely 5% acetic acid for 5 min and examined for the eleven bands. Total time for PAGE and silver staining was approximately 5 hours which included 15 min for RNA extraction, 3h for run and 2h for staining.

In each run a control strain i.e., SA-11 (Simian rotavirus strain) was run which was obtained from NIV Japan[6].

Culture of stool samples were done to know the association of common enteric pathogen with rotavirus positive cases by using standard culture techniques [9].

Results:

During the study, PAGE results were found in 365 of 400 (91%) specimens. Children belonging to the study group were in relation to their ages in months as <6 months, 6-12 m, 13- 24 m, 25-36 m, 37-48 m, and ≥ 49 m [Table:1].

Maximum incidence of rotavirus infection was seen in age group of 6 m-24 m (67.50%), whereas age groups <6 and >24 months showed an incidence of 23.75%. The study shows a statistically significant difference ($Z = 4.27$, $P = 0.001$) in the incidence

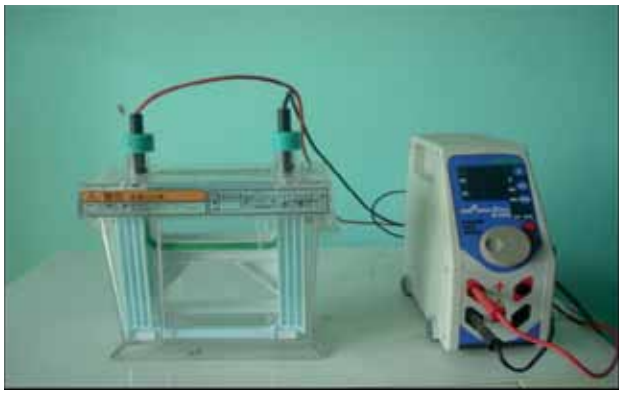

Figure 1: Polyacrylamide gel electrophoresis Chamber

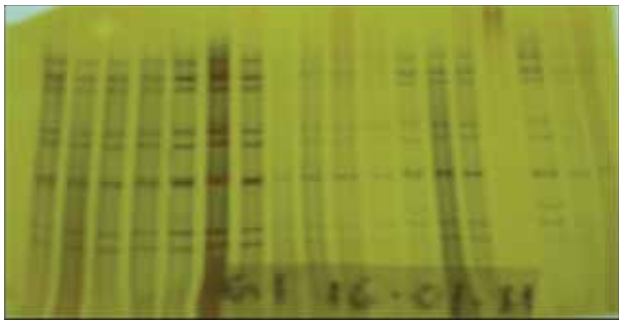

Figure 3: Photograph of polyacrylamide gel electrophoresis of rotavirus showing segments(11) of RNA genome.

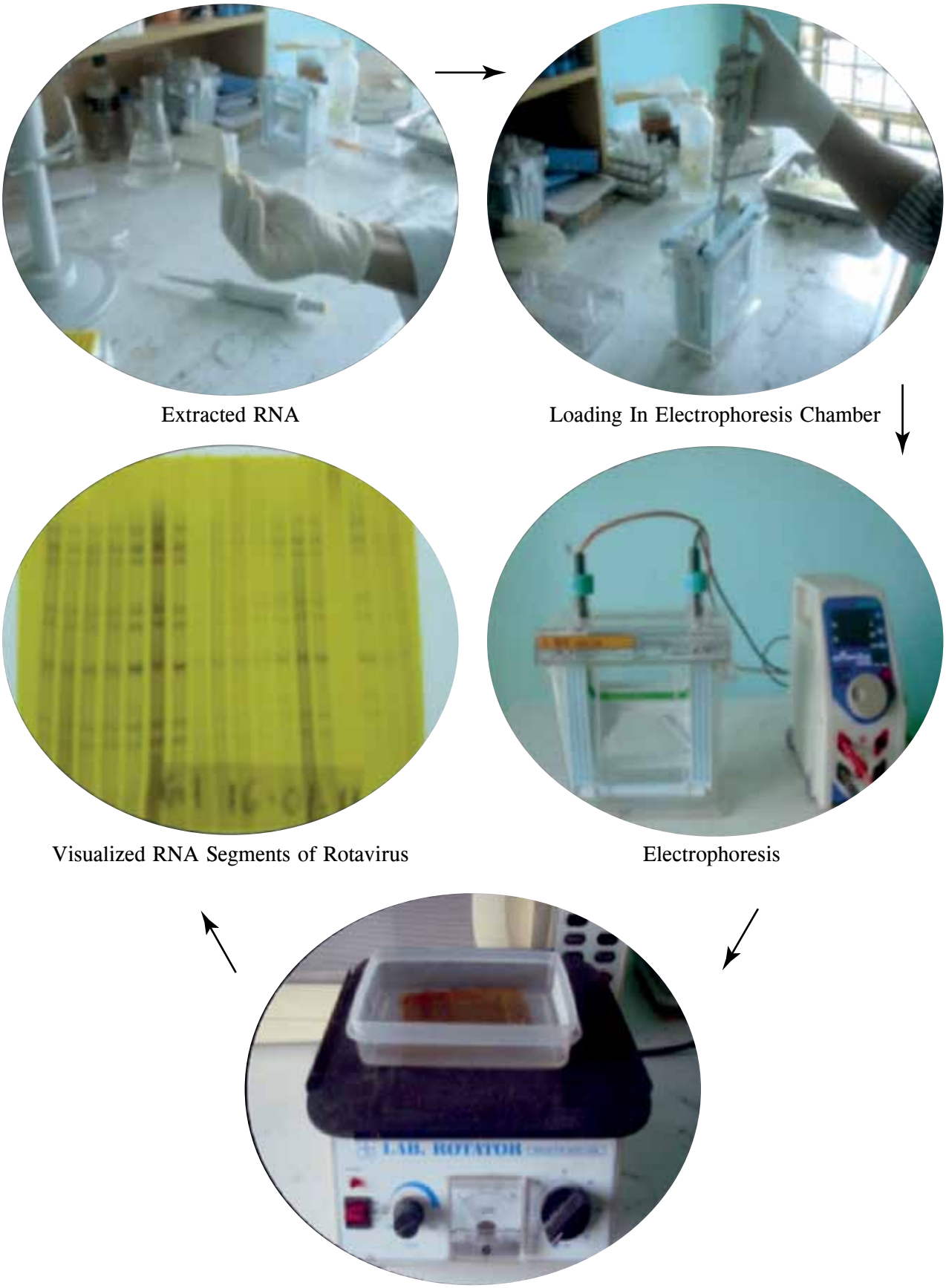

Figure 2: Pictorial presentation of major steps of PAGE

Table 1: Incidence of Rota virus in children of different age groups

Age (Month)	PAGE +VE (%)	Negative (%)	GRAND TOTAL (%)
6	13	1	14
6 - 12	158	4	162
13 - 24	111	5	116
25 - 36	57	-	57
37 - 48	14	2	16
49 - 60	12	23	35
Total	365(91)	35(9)	400(100)

Table 2: Seasonal distribution of Rota virus positive cases

Month	PAGE + ve	Total cases (400)
November	26	28
December	34	36
January	63	64
February	70	72
March	55	62
April	47	50
May	47	48
June	23	28
July	–	12

All 151 rotavirus positive cases did not show infection with bacterial pathogens.

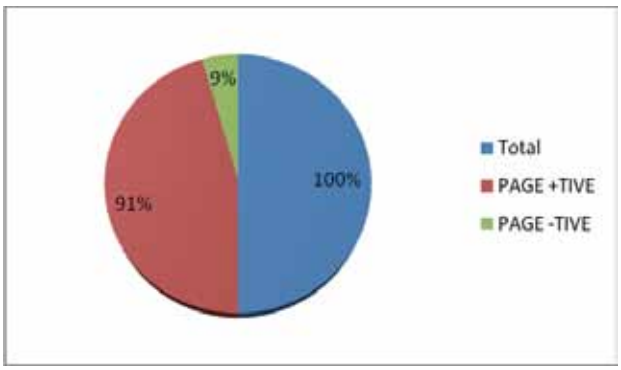

Figure 4: Incidence of Rota virus in children of different age groups

of rotavirus infection between the age groups 6-24 months and < 6 months and >24 months. The youngest patient found to be positive for rotavirus infection in this was 4 months old and the oldest was 60 months (5 years).

Rotavirus positive samples were found throughout the study period from November to July, except in the month of July where no cases were detected. Maximum incidence of rotavirus positive samples was noted in January and February. The incidence showed a declining trend between March to June [Table: 2].

Discussion:

During the current study 365 out of 400 samples (91%) were positive for rotavirus infection by PAGE methods. The available data highlights the importance of rotavirus as a cause of diarrhea in children, which is severe enough to deserve specialized care. The observed proportion of 91% of all diarrhea cases being associated with rotavirus falls within the range of values reported by workers from Bangladesh. The reported positivity varies from 30.5% to 90.7% [4,10-12]. The positivity rates also vary between various settings, i.e. hospitalizations, symptomatic and asymptomatic infections and nosocomial infections[13]. In this study majority of children who showed evidence of rotavirus infection belonged to the age group of 6 months to 24 months(67.50%), whereas other children <6 and >24 months accounted for only in 23.75%. Many investigators from different parts of Bangladesh expressed their similar views about more prevalence of rotavirus infection occurring in the age group of 6-24 months[13-16]. It appeared that infants below 4 months of age were initially protected to some extent by maternal antibodies against severe diarrhoea due to rotavirus[4]. The greater risks of infants and young children in the interim period of 6 to 12 months with declined levels of maternal antibodies to rotavirus infection have been documented[12-14].

Analysis of seasonal variation pertaining to rotavirus revealed that cooler months had increased rate of rotavirus associated diarrhea than the hotter months. Similar observations were made by some reports from Bangladesh and other countries[4,18-20]. It has been observed that temperature influences the stability of human and animal rotavirus that contributes to the efficient transmission of the human rota virus[4,17]. Moreover the influence of low relative humidity in the home has been suggested as a facilitating factor for the survival of rotaviruses on surface. This is suggestive of the indirect but important influence of meteorological factors on the complex epidemiology of human rotavirus infection [4,17].

In our study we did not find simultaneous infection with bacterial pathogens in rotavirus positive cases. Some of the authors [14,21] showed an association of bacterial pathogens with rotavirus positive cases. Various enteropathogens isolated in their study

were *E coli, Salmonella, Shigella* and *V. cholera* and the isolation of these bacterial pathogens was higher in rota virus negative cases.

In our study a complete PAGE results were observed in 365 (91%) of the 400 tested specimens. This finding closely correlates with the findings of other authors who found a 96.7% to 97.14%[22-24] results by PAGE methods in different developing countries.

The remaining 35 (9%) samples showed conflicting results. Negative result of the same sample in PAGE method is difficult to explain, the possibility of presence of lot of empty virus particles or due to low concentration of viral RNA in the fecal specimen and insufficient extraction of viral RNA could be possible[25,26].

The PAGE system used in this study was very simple to perform and the results were available on the same day. The main requirement was of trained personnel and proper standardization of the technique. Most reports states that the greatest advantage of PAGE and silver stain method are its lack of ambiguity and the fact that it provides information about viral electropherotypes. More over it generated epidemiological data regarding the circulation of strains in the community.

Conclusion:

The modified PAGE system was thus found to be reliable, rapid, no expensive reagents were required and simple enough to establish in small laboratories, in which facilities and budgets are limited. A locally produced slab gel electrophoresis system with power pack was the only equipment required. This method could be used for the routine diagnosis of rotavirus infection in the laboratory.

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Conflict of interest: None declared

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