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ANALYSIS OF ELECTROPHORETYPES OF ROTAVIRUS FROM DIARRHOEIC FAECES OF NEONATAL BUFFALO CALVES IN INDIA

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Summary. — Two distinct rotavirus RNA electrophoretypes were revealed by polyacrylamide gel electrophoresis followed by silver staining (PAGE-SS). The rotavirus was sampled from the faeces of buffalo calves in India from October, 1986 to January, 1987. In the buffalo farm under study one electrophoretype was prevalent first and then the second appeared after the disappearance of the first electrophoretype. For the detection of rotavirus in the diarrhoeic faeces of buffalo calves PAGE-SS was found more sensitive when compared with agar gel immunodiffusion and discontinuous counter immunoelectrophoresis tests.

Key words: rotavirus; RNA electrophoretypes; buffalo calves

Rotavirus has been recognised as an important cause of acute viral diarrhoea in neonatal bovine calves (Flewett and Woode, 1978; McNulty, 1978). Rotavirus genome consists of 11 segments of double-stranded RNA which can be revealed by polyacrylamide gel electrophoresis. Because of the segmented nature of the genome, genetic reassortment among different rotavirus strains probably results into great genetic heterogeneity of rotaviruses. In fact, considerable genome profile variations have been observed within strains of the same rotavirus subgroup and even the same serotype (Beards, 1982). However, the molecular basis of variations in rotavirus genome profiles is still not clearly understood.

Although changes in rotavirus RNA electrophoretypic pattern do not necessarily indicate the change in antigenic domains, electrophoretyping has become an excellent method for studying genomic variation, locating mixed infections, identifying atypical rotaviruses lacking group A antigen and isolating rotavirus strains with varying virulence (Pocock, 1987). Published reports on buffalo calf rotavirus electrophoretypes are lacking. In the present study, we attempted to investigate the RNA migration profile of rotavirus strains collected from diarrhoeic faeces of neonatal calves in an organised farm by polyacrylamide gel electrophoresis followed by silver staining (PAGE -SS). Presence of distinct RNA electrophoretypes of buffalo calf rotavirus during the period from October, 1986 through January, 1987 were thus

recorded on the basis of changes in the RNA migration pattern in the polyacrylamide gels. Agar gel immunodiffusion (AGID) and discontinuous counterimmunoelectrophoresis (DCIE) tests were also used for screening of buffalo calf rotavirus in the course of investigation.

Faecal samples from diarrhoeic calves (1—60 days old) were collected from Central Buffalo Research Institute, Hisar, during the period between October, 1986 and January, 1987. A 20 per cent suspension of each faecal sample was prepared in phosphate buffered saline pH 7.2. The mixture was centrifuged at 4,000 g for 20 min at 4 °C. The supernatant was then centrifuged at 12,000 g for 30 min at 4 °C. The faecal supernatant obtained was used in all tests.

Nebraska strain of calf diarrhoea virus (NCDV) of faecal origin was received in freeze-dried form from Dr. M. B. Rhodes, Institute of Agriculture and Natural Resources, Nebraska, Lincoln, U.S.A. An Australian strain of calf rotavirus of faecal origin in freeze-dried form was received from Dr. S. Tzipori, Attwood Veterinary Research Laboratory, Department of Agriculture, Victoria, Australia.

Rabbit standard antisera to calf rotavirus were the generous gift from Dr. S. Tzipori, Attwood Veterinary Research Laboratory, Department of Agriculture, Victoria, Australia. Rabbit anti-calf rotavirus serum made specific by absorption with rotavirus negative faecal sample provided by Dr. S. Khattar from this laboratory was also used for serological testing.

Agarose immunodiffusion (AGID) was performed according to the method described by Kwapinski (1972) on microscopic slides coated with 1% agarose (Hi-Media, Bombay, India) prepared in phosphate buffer saline, pH 7.2 containing 0.01% merthiolate .The wells in the agar gel (3 mm dia) were 3 mm apart from each other. The immunodiffusion was allowed to proceed for 24—72 hr at 4 °C.

Discontinuous counterimmunoelectrophoresis (DCIE) test was performed essentially according to the method of Wallis and Melnick (1971). Briefly, each glass slide (7.5 cm × 5.0 cm) was coated with 9—10 ml of 1% Noble agar (Difco, Detroit, Michigan, U.S.A.) in 0.025 mol/l barbiturate buffer, pH 8.6. Two parallel rows of 6 wells each (3 mm diameter) were punched 5 mm apart. Anodal wells contained antiserum and cathodal the faecal extracts. The electrophoresis was carried out for 45 min at a constant current of 10 mA per slide in an immuno-electrophoresis apparatus both the anode and cathode reservoirs containing 0.05 mol/l barbiturate buffer, pH 8.6. After the electrophoresis, the slides were incubated at 4 °C for 2 hr.

For PAGE-SS, the technique of Svensson *et al.* (1986) was followed with minor modifications. In brief, 10 ml of each 20% faecal extract in PBS was used for RNA extraction. Faecal supernatants were vortex mixed with 1 : 10 volume 1 mol/l sodium acetate with 1% SDS, pH 5.0. After incubation for 15—30 min at 37 °C the suspension was treated with equal volume of phenol-chloroform-isoamyl alcohol (25 : 24 : 1) mixture for 15 min at 56 °C and centrifuged at 10,000 rev/min for 5 min. The aqueous layer was then treated with 1 : 5 volume chloroform-isoamyl alcohol (24 : 1) mixture and centrifuged at 10,000 rev/min for 3 min. To the aqueous phase thus obtained was added 1 : 10 volume 3 mol/l sodium acetate and RNA was precipitated by 3 volumes of ethanol at —20 °C. After incubation overnight at —20 °C, the RNA was sedimented at 10,000 rev/min for 15 min. The pellet was dissolved in Laemmli's sample buffer (0.12 mol/l Tris-HCl, 0.1% SDS, 15% glycerol, 0.001% Bromophenol blue, pH 6.8). Electrophoresis was performed in 7.5% polyacrylamide slab gels with 3.0% stacking gel by using Laemmli's discontinuous buffer system without SDS (Laemmli, 1970), at 100 V for 6—8 hr at room temperature in a vertical slab gel electrophoresis apparatus (Pharmacia Biotechnology International AB, Uppsala, Sweden). Silver staining of polyacrylamide gels was done strictly according to the method described by Svensson *et al.* (1986).

The prevalence of rotavirus in diarrhoeic faeces of buffalo calves during the four months employing AGID, DCIE and PAGE-SS is shown in Table 1. PAGE-SS was found more sensitive than AGID, and DCIE tests. A comparative picture of the RNA electrophoretypes of NCDV, Australian strain, a local cow-calf rotavirus and two representative buffalo calf rotaviruses is shown in composite photograph of the silver stained polyacrylamide slab gels (Fig. 1). Differences in the migration pattern were observed in class I and

Table 1. Comparison of agar gel immunodiffusion (AGID), discontinuous counterimmunoelectrophoresis (DCIE) and RNA polyacrylamide gel electrophoresis (PAGE-SS) tests for the detection of buffalo calf rotavirus.

Year and month	No. of faecal samples tested	AGID	DCIE	PAGE-SS
1986				
Oct.	6	3	3	3
Nov.	16	5	5	9
Dec.	16	4	4	6
1987				
Jan.	21	2	3	4
Total:	59	14 (23.56%)	15 (25.43%)	22 (35.59%)

class III segments and not in the clas II and class IV segments. Segments 2 and 3 (class I) migrated closely in cow-calf rotavirus (lane 3) and buffalo calf rotavirus (lane 4) as compared to that of another buffalo rotavirus (lane 5). RNA segments 7, 8 and 9 (class III) moved as a single band in case of NCDV and the Australian strain (lane 2) while in case of cow-calf rotavirus (lane 3) and buffalo calf rotavirus (lane 4) the segments 7 and 8 comigrated and 9 moved as a separate band. In contrast, segments 7, 9 and 8 migrated separately in case of the buffalo rotavirus (lane 5).

Two distinct electrophoretypes were detected in buffalo calf rotavirus during the four months in question. The first electrophoretype (lane 4) was prevalent during October-November, 1986 and the second from end of November till January, 1987. Only one electrophoretype circulated at one time among the buffalo calves in this farm. We could neither find mixed infection with both the electrophoretypic strains nor the both electropherotype strains contemporarily in different animals. The second electrophoretype seems to have emerged probably after the first had disappeared from the calves in this farm. Difference in the migration profile of segment 8 of the two electrophoretypes may be important in that the segment 8 or 9 was responsible for coding the neutralizing antigen of rotaviruses.

The role of rotavirus as pathogen of gastrointestinal tract has been widely recognised. However, the reports about the prevalence of rotavirus in diarrhoeic samples of buffalo calves are meagre. In the present study, detection of rotavirus strains with differences in the electrophoretic mobility of gene segment 3 and 8 is reported. Analogous to the situation with influenza virus, rotavirus population present on a farm may change genetically even though differences in the electrophoretic mobility of RNA segments of rotavirus do not compulsarily denote antigenic variation. Further studies are in progress to obtain more data on buffalo calf rotavirus RNA electrophoretypes and molecular epidemiology of rotavirus infection on buffalo farms in India.

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Legend to the Figure (Plate XXIII):

Fig. 1. A composite photograph of PAGE-SS of rotavirus RNA from a Nebraska strain (lane 1) an Australian strain (lane 2), a local cow-calf strain (lane 3) and two representative buffalo calf strains (lanes 4 and 5).