The dsRNA Electrophoretype of Some Isolated Iranian Calf Rotaviruses

Short Communication

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Summary

A rapid and simple technique for diagnosis of rotaviral diarrhea is polyacrylamide gel electrophoresis (PAGE) of dsRNA extracted from fecal samples. To determine the electrophoretype of Iranian calf rotavirus, 23 rotaviral positive fecal samples from diarrheic Holstein calves at the age of less than one month in Tehran region were examined. The viral dsRNA was extracted with phenol-chloroform, and analyzed by PAGE and silver staining. According to PAGE all of the electrophoretypes were identified as long genome electrophoretypes, characteristic of animal group A rotavirus. There was not any unusual segment rearrangement.

***Key words*:** rotavirus, electrophoretype, PAGE, diarrhea, calf

Introduction

Group A rotaviruses, members of the genus rotavirus within family Reoviridae, are the major cause of acute gastroenteritis in the young mammalian species including calves. The genome of the virus consists 11 segments of dsRNA. Rotaviruses have been classified into seven groups (A to G) according to the antigenic characteristics of Vp6 protein or genomic RNA electrophoretic pattern, namely the electrophoretype (Fukai *et al* 1998). The group A, the most important rotaviruses, is

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the major enteropathogens in the infants of many mammalian and avian species (Estes 2001, Al-Yousif *et al* 2002). Group A rotaviruses, are classified into G and P types on the basis of their outer capsid Vp7 (glycoprotein) and Vp4 (protease sensitive) proteins respectively (Estes 2001). So far there is not any data on occurrence of the G and P types in Iran on a national scale. Prevalence of 28.8% and 31.7% of calf rotaviral diarrhea in dairy farms of Tehran region were reported (Kargar *et al* 1981, Keyvanfar *et al* 2001). The later was also indicated a prevalence of 33.3% and 41.7% for G6 and G10 serotypes in rotaviral diarrhea, respectively. The present study was designed to determine the electrophoretypes of rotaviruses involved in calf diarrhea in Tehran region.

Materials and Methods

Faecal samples. Ninety-four diarrheic faecal samples were collected from Holstein calves in Tehran region from 1998 to 1999. A commercial ELISA kit (Bio-X, Belgium) was used for detection of rotavirus.

Extraction of dsRNA. 23 out of 54 faecal samples, which showed positive reaction for group A rotavirus were used for dsRNA extraction and polyacrylamide gel electrophoresis (PAGE). The rotaviral dsRNA was extracted from the samples according to the Ishizaki *et al* (1995) method. 25µL 1.5M NaCl, 12.5µL 10% sodium dodecyl sulfate and 12.5µL 0.025% Mercaptoethanol (2ME) were added to 200µL of a 10% faecal suspensions in 50mM of Tris. The mixture was incubated for 30min at 37°C and centrifuged at 3000*g* for 5min. The released dsRNA was then extracted by phenol-chloroform method (Parwani *et al* 1992).

Electrophoresis of dsRNA. The dsRNA was dissolved in the PAGE sample buffer consisting of 0.04% bromo thymol blue and 1/10 volume (w/v) of sucrose (Fukai *et al* 1998) and analyzed by PAGE using a 4 and 10% discontinuous polyacrylamide gel system as described by Parwani *et al* (1992). Electrophoresis was performed at 25mA for 6h (Ganga *et al* 1994). Silver staining of the gel was performed by a

modified method of Meril *et al* (1981). In brief the gel was shaken in 1M acetic acid for 15min and then in a 0.8% solution of ammonificated silver nitrate for 20min. Thereafter the gel was washed three times in distilled water and developed in a solution of 3% NaOH containing 0.1% formaldehyde. The reaction was finally stopped by adding 7.5% acetic acid.

Results and Discussion

The results showed that all of 23 samples had a similar long genome electrophoretypes, characteristic of animal group A rotaviruses (Figure 1). There was not any unusual segment rearrangement. Our modified silver staining procedure was found to be fast and sensitive.

Figure 1. *Electrophoretype of some calf rotaviruses isolated from Iran*

Electrophoretype determination of calf rotaviruses is of epidemiological importance and can reveal the introduction of new virus strain in a region. The technique is also simple and rapid and can be used for routine diagnosis and monitoring of calf rotaviruses. A continuous research and identification of electrophoretypes, G serotypes and P genotypes of rotaviruses is necessary to develop a preventive plan against the virus (Fukai *et al* 1998). The electrophoretypes of group A rotaviruses have been classified in three types, namely long, short and

super short, according to the migration pattern of gene segments 10 and 11 of the viral dsRNA. In the short electrophoretypes, the 11th RNA segment migrates more slowly than usual, and thus located between the 9th and 10th segments. In a super-short pattern of RNA migration, the 11th gene segment migrated even more slowly than the short pattern rotaviruses (Estes 2001). So far, the electrophoretype of bovine group A rotavirus has been found to be as either long or super short type (Estes 2001, Ishizaki *et al* 1995), but the long genome electrophoretype is more frequently identified (Hussein *et al* 1995, Fukai *et al* 1998). Accordingly, the electrophoretypes of rotavirus obtained in our study belonged to the long type. Similar studies have been performed in other countries (Hussein *et al* 1995, Ishizaki *et al* 1995). The present study is the first report of the electrophoretype determination of involved rotaviruses in Iran.

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