

Electrophoretotypes of Bovine Rotaviruses Detected in Turkey

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Abstract: A total of 83 faecal samples carrying rotavirus were used in this survey. Five different electrophoretotypes were distinguished according to their migration patterns. All the rotaviruses examined in this research proved to be group A and to have short migration patterns. While local migration patterns were detected in individual herds (e.g. A, B, C, M, P and T), two different migration profiles were detected in herd K during rotavirus enteritis after a two year interval. The greatest variations in electrophoretic mobility in the groups were found in segments 2-4, and 6-9, while segments 1, 10, and 11 showed the most unchanged mobility.

Key Words: Cattle, Rotavirus, Electrophoretotype

Türkiye'de Tespit Edilen Sığır Rotavirus Elektroforetikleri

Özet: Bu çalışmada rotavirus taşıdığı tespit edilen toplam 83 adet dışkı örneği kullanıldı. Migrasyon kalıplarına göre 5 farklı elektroferotik tip tespit edildi. Araştırmada incelenen rotavirusların hepsi A grubu idi ve kısa migrasyon kalıbı sergilediler. Araştırmada takip edilen 7 işletmede kendilerine ilgili rotavirus elektroforetik tipleri tespit edilirken, K işletmesinde 2 yıl ara ile meydana gelen rotavirus enteriti olgularında iki farklı kalıp bulundu. Elektroforetik göçte en büyük farklılıklar 2-4 ve 6-9 segmentlerde gözlenirken, 1, 10 ve 11 nolu segmentler en değişmez migrasyonu sergilediler.

Anahtar Sözcükler: Sığır, Rotavirus, Elektroforetip

Introduction

Rotaviruses cause enteritis in a wide variety of species (1). As members of Reoviridae, they have a double stranded RNA genome consisting of 11 segments ranging in molecular weight from approximately 2.0×10^6 to 0.2×10^6 (2,3). As is well known, there are 3 major antigenic structures in a rotavirus particle. The first, VP3, is encoded by segment 4 and is the minor component of the outer shell. VP6, which is the major component of the inner shell, carries group antigens common for all group A rotaviruses and is encoded by segment 6, while VP7, which is encoded by segment 8 or 9 depending on the strain, is the major component of the outer shell (3). Electrophoretic analysis of the genomic structure has revealed major differences in the mobility of the genome segments between virus isolates from different host species and minor differences between individual isolates from the same species (1,4,5). Therefore, genome electrophoretotyping has been the most common method

for both taxonomic and epidemiological studies (6). On the other hand, it should be remembered that variations in electrophoretotype patterns, particularly in neutralising antigens VP4 and VP7 coding segments, need to be verified using serological techniques, because it has been shown previously that electrophoretotypes do not completely match antigenic variations (7).

In this research, the electrophoretic migration profiles of rotaviruses detected during the epizootics of neonatal enteritis cases between 1995 and 1999 were analysed in order to detect the presence of epidemiologically predominant strain(s) or possible antigenic variations among the viruses.

Materials and Methods

This research was planned for monitoring the electrophoretotypes of bovine rotaviruses detected during acute neonatal gastroenteritis cases in 7 dairy herds

between 1995 and 1999. For this purpose, 83 faecal samples in which rotaviruses had been detected by polyacrylamide gel electrophoresis (PAGE) in previous tests were used in the study. All rotavirus RNA segment carrier faecal extracts was stored at -80°C for further analysis of mobility under standard conditions. The PAGE technique for initial diagnosis was performed as described elsewhere (8). In order to screen electrophoretic mobility patterns, faecal extracts frozen at -80°C were re-extracted using the technique explained by Chomezynski and Sacchi (9). In brief, faecal extracts were mixed with equal volumes of denaturation solution (4 M guanidium thiocyanate, 25 mmol sodium citrate pH 7.0, 0.5% N-laurilsarcosin and 0.1 M 2-mercaptoethanol) and water equilibrated phenol (pH 5.0), 0.1 volume of 2 M sodium acetate (pH 4.0) and 0.2 volume of chloroform:isoamylalcohol (49:1, v/v). The final mixture was vortexed and left on ice for 20 minutes. Following a short spin at 6000 rpm, the upper aqueous phase was removed and precipitated by adding an equal volume of isopropanol at -80°C over two hours. The RNA was then pelleted by centrifugation at 14000 rpm for 10 minutes. Washing with 70% ethanol and subsequent vacuum drying of RNA was followed by resuspension in 20 μl of diethylpyrocarbonate (DEPC) treated distilled water. Samples were run in 1.7% agarose gel containing ethidium bromide and were examined in a UV-transilluminator.

Results

The results of electrophoresis revealed 5 different types of mobility among the rotaviruses detected in the field cases during 1995 - 1999 and they were referred to as type I to type V (Figure). A single type of rotavirus segment mobility was monitored in the herds, except in herd K where two types of migration patterns (Type III and IV) were screened during consecutive diarrhoea cases. The type III migration pattern was detected in 1996, whereas the type IV was in 1998. The remaining ds-RNA segment migration patterns monitored belonged to individual herds located in various geographical areas as listed in the Table. Type I migration pattern was detected in four herds (herds A, B, M and P), while type II and type V patterns were found only in herd T and herd C, respectively (Table).

Table. Frequency of the electrophoretotypes detected among the herds with which the study was performed.

Herd Code	Electrophoretotypes				
	I	II	III	IV	V
A	X				
B	X				
C					X
K			X	X	
M	X				
P	X				
T		X			

With respect to their electrophoretic mobility, all the rotaviruses examined in this research proved to be members of group A and to have short migration patterns. The greatest variations in electrophoretic mobility in the groups were found in segments 2-4, and 7-9, while segments 1, 10, and 11 showed the most unchanged mobility (Figure).

Discussion

In this research, the electrophoretic migration profiles of rotaviruses detected in livestock herds between 1995 and 1999 were analysed in order to evaluate possible antigenic differences during diarrhoea epidemics in calves. A total of 83 faecal samples carrying rotavirus were used in the survey. Five different electrophoretotypes were distinguished according to their migration patterns.

Electrophoretotype I is regarded as the most common migration pattern among the 83 viruses infecting herds. Therefore, the rotavirus strain having this type of RNA migration pattern is considered to be the predominant strain in this research. Meanwhile, the remaining electrophoretotypes, except type IV, are believed to be modified from type I, showing small-scale alterations in terms of the migration of segments 2 to 4 and 5 to 9.

An antigenic shift is highly possible among the rotaviruses, resulting in more virulent progeny viruses. These antigenic alterations can be indicated easily by means of the electrophoretic migration patterns of isolates recovered at different time points. Differences in

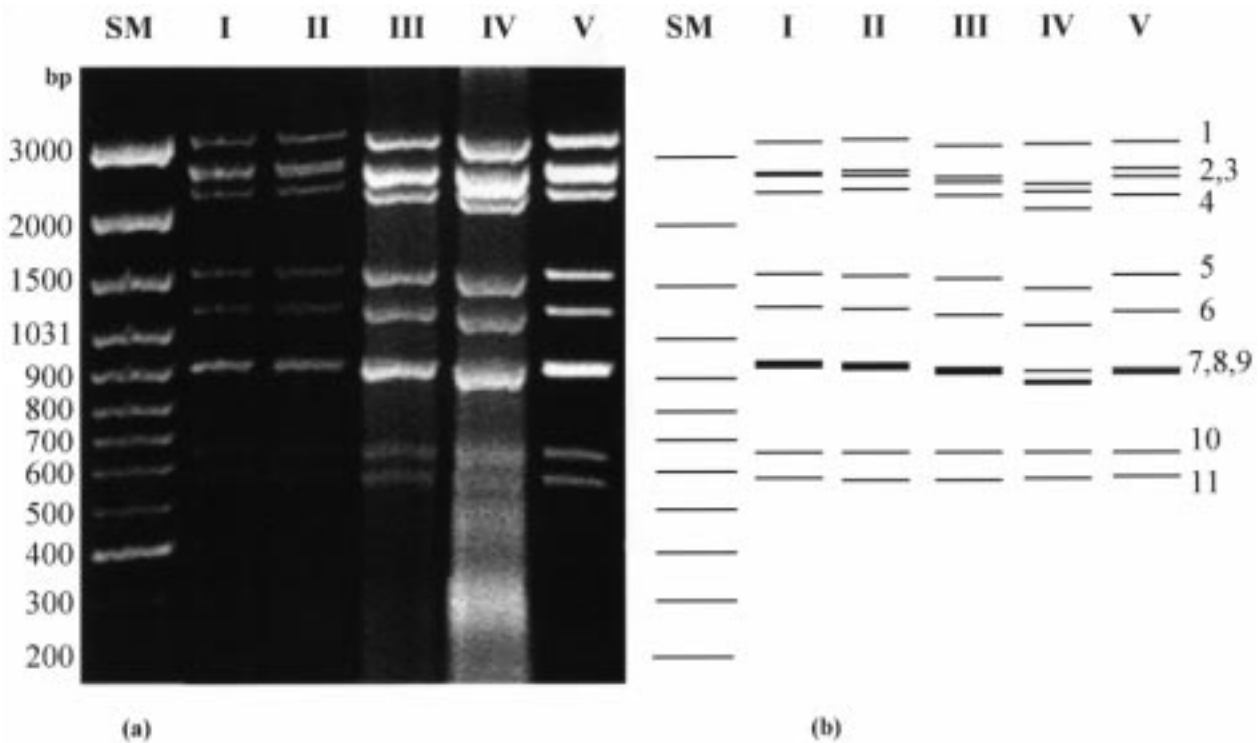


Figure. Electrophoretotypes of rotavirus ds-RNA after separation by electrophoresis through 1.7% agarose gel. (a) Electrophoresis and the bands visualised by UV-illumination of the gel after staining with 1 µg/ml ethidium bromide. (b) diagrammatic representation of the genome profile of rotaviruses detected during the study.

the RNA segment patterns of circulating rotaviruses during sequential epidemics have been reported in detail by numerous investigators (7,10,11). Accordingly, in this study, two different RNA segment patterns (Figure, lanes III and IV) were found in herd K during subsequent outbreaks after a two year interval. Evaluation of the segment migration profiles on this farm revealed that significant changes occurred in segments 4-6, 8 and 9. Elevation of the virulence observed in recently emerged viruses and follow-up enteritis cases with high mortality in the herd might be related to massive changes in the composition of shell proteins (e.g. VP3, VP6 and VP7), which are encoded by the segments concerned. Similarly, the results of da Costa Mendes et al. (11) indicated that within 8 months of the preliminary detection, a new electrophoretic pattern emerged on one farm where mortality rate of enteritis cases increased dramatically, whereas the pattern remained unchanged on the other farm where the cases were mild.

Bukrinskaia et al. (12) have determined various mobility patterns of rotavirus RNA of both long and short migration types during epidemics in children in Russia during the winter of 1988-1989. This situation readily reflects the abundance of antigenic variants of the rotaviruses in one epidemic. Unfortunately, we could not detect mixed infections caused by antigenically different rotaviruses in the same herd.

In this study, the detection of variations in segment profiles of rotaviruses and comparison of certain patterns between individual herds were carried out for the first time in Turkey. As important epidemiologic data, it might be emphasized that the occurrence of genomic variations and consequently their antigenic reflection in the circulating rotaviruses could often be encountered among dairy herds after different intervals. However, the antigenic basis of variations postulated here remains to be confirmed by serological techniques.

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