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Molecular G Typing of Bovine Rotaviruses in Iran

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Abstract: Fifty rotavirus-positive fecal samples, selected from 500 ELISA tested diarrheic specimens were used in this study. Viral RNA was extracted from each sample and reverses transcribed to cDNA. The cDNA was then amplified by oligonucleotide primers specific for RNA segment 9, coding for VP7. After the first amplification, PCR products were subjected to a multiplex semi-nested PCR to investigate the presence of bovine rotavirus serotypes: G6, G8 and G10. The results indicated prevalence of 48 and 26% for G6 and G10 serotypes, respectively. Twenty four percent of the samples showed a mix infection by G6 and G10 serotypes and no sample was found positive for the type G8. With the best of our knowledge this is the first report of molecular typing of bovine rotaviruses in Iran.

Key words: Bovine rotavirus, typing, PCR, Iran

INTRODUCTION

Group A rotaviruses, the most commonly found rotaviruses in animal and human diarrhea, are classified into G and P types, according to antigenic properties of viral proteins VP7 and VP4, respectively (Glass *et al.*, 2006; Pisanelli *et al.*, 2005; Estes *et al.*, 2001). At least, 15 G types and 26 P types have been recognized so far (Kapikian *et al.*, 2001). Although G types 1, 3, 5-8, 10 and 15 have been described in cattle, only G6, G8 and G10 are the most common group A rotaviruses of cattle (Adah *et al.*, 2003; Garaicoechea *et al.*, 2006). Research into the G and P types of group A rotaviruses is very important for preventive veterinary medicine and more specifically, for the development of a vaccine. It is also important from the point of view of ecology and public health, because interspecies transmission from cattle to humans and from humans to cattle has been reported (Fukai *et al.*, 1998).

So far there has not been any report of molecular typing of bovine rotaviruses from Iran. Therefore, the present study was under taken to determine the prevalent group A rotaviruses of cattle in a major region of dairy cattle industry in Iran.

MATERIALS AND METHODS

Fecal samples: A total of 50 rotavirus-positive fecal samples from diarrheic calves of up to one month of age

were used in this study. The samples have been selected from 500 ELISA tested specimens, collected from Tehran region, a major region of dairy cattle industry in Iran. All the samples have been collected in 2000 and were tested at 2006, in the virology laboratory of faculty of veterinary medicine, Shahid Chamran university of Ahwaz.

RNA extraction: Extraction of rotaviral dsRNA was based on the methods described previously (Garaicoechea *et al.*, 2006; Fukai, 1999), with some modifications. A 20% (V/V) suspension was prepared from each sample and extracted by Tripure isolation reagent (Roche), as per the manufacturer protocol. In brief, the fecal suspension was mixed with three volumes of Tripure isolation reagent, shaken for 15 sec and incubated at room temperature for 5 min. Chloroform was added (0.2 mL chloroform/1 mL Tripure isolation reagent), the sample was shaken for 15 sec and placed at room temperature for 7 min. After centrifugation at 12000 g for 15 min at 4°C, the colorless upper aqueous phase was transferred to a new tube and RNA was precipitated by adding isopropanol. RNA was washed with 75% ethanol, dissolved in diethyl pirocarbonate (DEPC) treated water and stored at -70°C until use.

PCR- G typing: Procedure for the PCR-typing was similar to the method described by Gouvea *et al.* (1994). The first amplification of the full-length VP7 gene (1062 bp) was followed by a multiplex semi-nested amplification, using

serotype-specific primers. Briefly, viral dsRNA was denatured with dimethylsulfoxide at 97°C for 5 min and then immediately cooled on ice. Denatured dsRNA was reverse transcribed at 42°C for 60 min with murine leukemia virus reverse transcriptase (Fermentas) and primers sBeg 9 and Ehd 9, specific for a full-length copy of VP7 gene. The first PCR was carried out under PCR conditions of 30 cycles of 94°C for 1 min, 42°C for 2 min and 70°C for 1 min.

A 1:10 or 1:100 dilution of the first round PCR products were used as template in a multiplex semi-nested PCR, with a pool of G6, G8 and G10 serotypes-specific primers (DT6, HT 8, ET 10). Amplification was performed under PCR conditions of 25 cycles of 94°C for 1 min 55°C for 2 min and 72°C for 1 min. Reaction mixtures consisted of 20 mM Tris HCl (pH 8.3), 50 mM KCl, 1.5 mM MgCl₂, 0.2 mM dNTPs, 0.5 μM of each primer and 2.5 U of Taq DNA polymerase (Cinagene, Iran).

The primers used in this study, namely sBeg 9, Ehd 9, DT6, HT8 and ET10 have been previously designed by Gourvea *et al.* (1994) and Fukai *et al.* (1998)

RESULTS

In the first round of PCR for amplification of segment 9, a DNA band of about 1062 bp was produced only in 25 samples (Fig. 1). The second semi-nested PCR for identification of G6, G8 and G10 serotypes, resulted in the

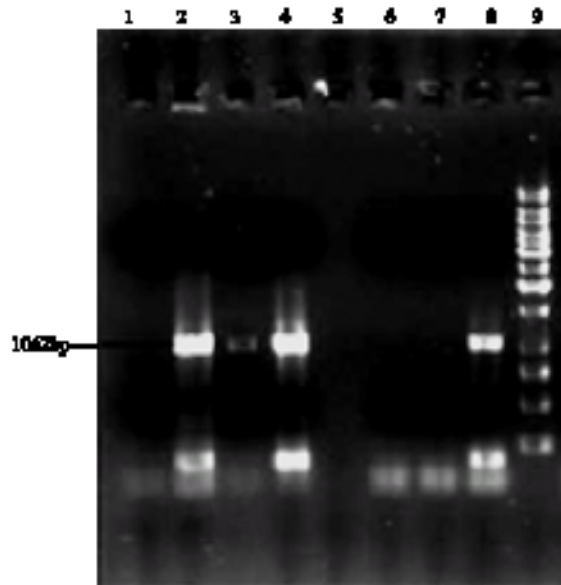


Fig. 1: RT-PCR products of 1062 bp (lanes 2, 3, 4 and 8), produced from some samples by using sBeg and Ehd 9 primers. Lanes 1 and 9 represent the negative control and 1 Kbp DNA Ladder, respectively

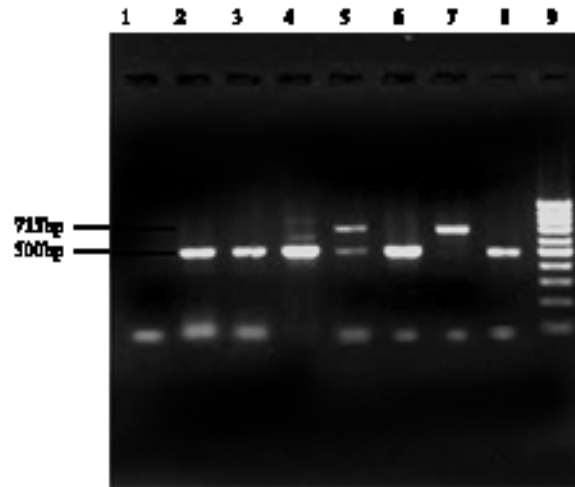


Fig. 2: The results of Multiplex semi-nested PCR with G6, G8 and G10 specific primers. Lane 1: negative control Lanes 2, 3, 4, 6 and 8 were positive for the type G6 (500 bp). Lane 7 represents a G10 (715 bp) positive sample and lane 5 shows the pattern of a mix infection (G6 and G10). Lane 9: 100 bp DNA Ladder

Table 1: The Frequency of G6, G8 and G10 types in fecal samples

Type	No. of positive samples	% of positive samples
G6	24	48
G8	0	0
G10	13	26
G6+G10	12	24
Unspecific	1	2
Total	50	100

amplification of DNA fragments of 500 bp (G6), 715 bp (G10) or both in 49 samples (Fig. 2). As shown in Table 1, the most frequently found G serotype was G6, being present in 48% of all the specimens.

DISCUSSION

Global surveys of rotavirus serotypes are essential to obtain epidemiological data on rotavirus infection and to provide basic knowledge necessary to develop and evaluate candidate vaccines (Taniguchi *et al.*, 1992; Alfieri *et al.*, 2004).

Surveillance of rotavirus has been performed worldwide by ELISA with monoclonal antibodies to various serotypes and has yielded useful information on rotavirus epidemiology (Taniguchi *et al.*, 1992). However, molecular typing has been preferred, due to its good correlation with serotypes, high sensitivity and using universal synthetic reagents (Gourvea *et al.*, 1990; Parwani *et al.*, 1993; Hussein *et al.*, 1996). The other advantages of molecular methods are stability of genomic

RNA compared to outer capsid proteins and using the PCR products for further characterization by direct sequencing, cloning, etc (Gouvea *et al.*, 1990; Taniguchi *et al.*, 1992). In Iran, bovine rotavirus is one of the major causes of diarrhea of calves (Kargar *et al.*, 1981), but there is few information about types of circulating rotaviruses. In fact, this study is the first molecular G typing of bovine rotaviruses in Iran.

In the present study, among 50 ELISA-positive samples analyzed by RT-PCR, G6 was the predominant G type (48%), followed by the type G10 (26%). Twenty four percent of the samples showed a mixed infection by G6/G10 types and one sample was negative by PCR assay. Negative reaction of this samples in PCR may be due to presence of unknown PCR inhibitor(s) in the samples.

Bovine G8 strains have been reported to be rare in some area, while they are detected more frequently in other reports (Reidy *et al.*, 2006). In this study, no sample was found to be positive for the type G8.

A higher prevalence of G6 serotype we found in our study is in agreement with reports from many other parts of the world. Fukai *et al.* (1998) showed by RT-PCR, that G6 was the most common bovine group A rotavirus in Japan. Analysis of the VP7 gene of 21 rotavirus positive samples collected from diarrheic buffalo calves in southern Italy, revealed that 57% of the isolates were G6, 23.8% were G8 and 19% were G10 (Pisanelli *et al.*, 2005). Alfieri *et al.* (2004) showed that in Brazil the type G6 was also the most frequent type, occurring in 66% (33/50) of rotavirus infections and G10 was identified in 16% of the samples. In a study in Ireland by using nested RT-PCR, G6 was again the most prevalent type (80.6%) and G10 accounted only for 9.7% of the samples (Reidy *et al.*, 2006).

Molecular typing of group A rotavirus, as performed in this study, is an essential tool for epidemiological studies of rotaviruses and is also important for vaccine consideration (Gentsch *et al.*, 1996), because, it has been shown that the most predominant G and P types could change periodically. There is, therefore, a need to reinforce continuous surveillance of rotavirus types in communities to identify circulating strains (Asmah *et al.*, 2001; Fukai *et al.*, 2002).

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