Molecular typing of group A bovine rotavirus of calves in the provinces of Tehran, Alborz and Qazvin

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Key words:
A bovine rotavirus (BRV-A), molecular typing, calf gastro-enteritis, Iran.

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Received: 5 September 2012
Accepted: 17 October 2012

Abstract:

BACKGROUND: Group A bovine rotavirus (BRV-A) is one of the most important causes of gastroenteritis and diarrhea in newborn calves. **OBJECTIVES:** Major types of BRV-A in Tehran, Alborz and Qazvin were detected in this study. **METHODS:** A total 125 fecal samples of calves showing clinical signs of diarrhea were collected from 26 industrial dairy farms located in the provinces of Tehran, Alborz and Qazvin, during two years. **RESULTS:** BRV-A was detected in 39.2 % (49/125) of total samples using a commercial ELISA kit. Thirty five positive samples were analyzed by seminested multiplex RT-PCR for P and G genotyping. G10 was the most prevalent genotype, accounting for 57.1% of samples, G6 accounted for 22.9% of samples and in 5.7% of samples (2/35), mixed infection of both genotypes G6 and G10 were detected. Also, the detected P types were P[11] and P[5], accounting for 71.4% and 14.2%, respectively. In our study, none of the genotypes G8 and P[1] were detected. The incidence of genotype combinations corresponded to the B223-like strains (G10P[11]), UK-like strains (G6P[5]) and KN4-like strains (G6P[11]) were 51.4%,14.3% and 8.6%, respectively. Mixed infections G6/G10P[11] were detected in 2.85% of all samples analyzed with RT-PCR. **CONCLUSIONS:** To the best of our knowledge, this is the first report about the determination of P genotypes of BRV-A and distribution of the most common BRV-A strains circulating in Iran. Our study also indicated that the incidence of the G genotypes of BRV-A in the provinces of Tehran, Alborz and Qazvin, which is one of the greatest husbandry centers in Iran, has changed in the past years. Furthermore, this finding could be valuable in rotavirus vaccine design.

Introduction

Rotavirus group A (RV-A) is one of the most important causes of diarrhea in newborn of human and many animal species including bovine (Estes and Kapikian, 2007; Saif, 2011). The disease in bovine (caused by bovine group A rotavirus) commonly occurs in calves, 1-8 weeks of age and causes notable economic loss due to increased morbidity and mortality, treatment costs and particularly, reduced growth rate in thess animals as a result of rotavirus infection (Cashman et al., 2010). Bovine group A rotavirus (BoRV-A) like other viruses classified as RV-A, belongs to genus Rotavirus in the family Reoviridae and contains a genome 11 segments of

double stranded RNA which are encircled within a non-enveloped triple capsid protein (Cashman et al., 2010; Estes and Kapikian, 2007).

Rotaviruses could be classified based on genotypic and serologic analysis. Variation in the group-specific antigen on VP6 (a structural protein forming the middle layer of the virion particle) is used to define seven major groups which are alpha-betically named A-G (Estes and Kapikian, 2007; Saif, 2011). The VP7 and VP4 proteins are two in-dependent neutralizing antigens which are structural components of the outer layer of the viral capsid and used to define the G (glycoprotein; VP7) and P (protease sensitive protein; VP4) serotypes and genotypes using monoclonal antibodies and serotypes specific primers, respectively (Estes and Kapikian, 2007).

Because of their specific neutralizing antibody response, both the VP7 and VP4 proteins are considered important for development of a successful rotavirus vaccine and so identification of their genotypes are the most important subject in epidemiology of RV-A (Alfieri et al., 2004; Cashman et al., 2010; Estes and Kapikian, 2007; Garaicoechea et al., 2006; Rodriguez-Limas et al., 2009). To date, 23 G and 31 P genotypes are recognized (Cashman et al., 2010). Regarding bovine rotavirus, G6, G8 and G10 together with P[1], P[5] and P[11] are the most common G and P genotypes, respectively,although G8 and P[1] have been detected less than the other two (Alkan et al., 2010; Cashman et al., 2010; Fukai et al., 2002; Garaicoechea et al., 2006; Monini et al., 2008; Reidy et al., 2006; Rodriguez-Limas et al., 2009; Swiatek et al., 2010).

The objective of this study was to determine the distribution of the three common G and P genotypes of BoRV-A in fecal samples from calves with diarrhea collected from the provinces of Tehran, Alborz and Qazvin, Iran.

Material and method

Fecal samples: During the period from November 2010 to march 2012, a total of 125 stool samples from calves with diarrhea were collected from 26 industrial dairy farms located in three provinces of Iran (Tehran, Alborz and Qazvin). These provinces are located in a geographical area which is one of the greatest husbandry centers in Iran. All samples were collected from calves up to 8 weeks old and were stored at -20°C until analysis.

Sampling method was performed randomly and the sample size was estimated based on confidence level of 95% and precision level of 10%, assuming that the proportion of the presence of rotaviral infection in population is about 35% according to previous information (Badaracco et al., 2012; Dhama et al., 2009; Fukai et al., 2002).

Primers: The specificities of the selected primers used in the present study for G- and P-typing procedures have been evaluated previously (Gouvea et al., 1994a; Isegawa et al., 1993; Iturriza-Gomara et al., 2004). Two distinct sets of primers were used for G- and P-typing. For the first round amplification, primers VP7-F and VP7-R (Iturriza-Gomara et al., 2004) were used to amplify 881bp within the VP7 gene and primers Con2 and Con3 (Gentsch et al., 1992) were used to amplify 876 bp within the VP4 gene (Table 1)

The upstream consensus primer on the VP7 gene and three specific G-typing primers were used in a second round of PCR amplification for the characterization of the G6, G8, and G10 serotypes. Similarly, the upstream generic primer on the VP4 gene and three specific P-typing primers were used in a second amplification for the characterization of the P[1], P[5], and P[11] serotypes (Table 1).

Enzyme immunoassay: The presence of RV-A in fecal samples was confirmed before genotyping by a commercial ELISA kit (Pourquier ELISA Trikit, Institut Pourquier, France). The test was performed and interpreted according to the manufacturer's instructions.

RT-PCR (RNA extraction): Genomic viral RNA was extracted using VETEK viral DNA/RNA extraction kit (Intron, South Korea) according to the manufacturer's protocol. Briefly, 200 microliters of stool samples was suspended in 800 microliters PBS. 300 microliters of stool suspension was added to 500 microliters lysis buffer. After 10 minutes the process was followed by adding 700 microliters of loading buffer at room temperature. The mixture was applied to a VETEK spin column followed by centrifugation at 15000 ×g for 1 minute. Loaded RNA was washed two times using solution A and B before elution. The extracted RNA was used directly for synthesis of cDNA or stored at -70°C.

Reverse transcription-polymerase chain reaction (RT-PCR): Determination of each of the genotypes, G and P was carried out in three steps as described previously (Gentsch et al., 1992; Gouvea et al., 1990; Iturriza-Gomara et al., 2004) with some modifications. Briefly, at the first step, cDNA was generated in the presence of random hexamers primer using vivantis 2-step RT-PCR kit (Vivantis, Malaysia) according to manufacturer's recommendation. In summary, eight microliters of extracted RNA was mixed with one microliter dNTP and one microliter random hexamers primer and then denatured by incubation at 95°C for five minutes followed by immediately chilling on ice for two minutes. After that, 10 microliters of cDNA mixture composed of 2 microliters 10× M-MuLV buffer (Vivantis, Malaysia), 100 units M-MuLV reverse transcriptase (Vivantis, Malaysia), and 7.5 microliters nuclease free water was added to denatured RNA mixture and followed by 10 minutes incubation at room temperature. Finally, the total mixture was incubated at 42°C for 60 minutes and then at 85°C for 5 minutes to deactivated reverse transcriptase enzyme. The synthesized cDNA was directly used as template for the first round of PCR.

The first round VP7 PCR reactions were carried out in 25 µL reaction volume consisting of 2.5 µL of 10 × buffer A (Vivantis, Malaysia), 1 µL of 50 mM MgCl2, 0.5 µL of 10 mM dNTPs mix (Vivantis, Malaysia), 0.25 µL of 5 U/µL Taq DNA polymerase (Vivantis, Malaysia) and 0.75 µL of 50 pM solution of each reverse and forward primer VP7-F and VP7-R. The PCR reaction was run under the following thermal program using a thermal cycler (Techne, UK); 94°C for 5 min and then 35 cycles of 94°C for 1 min, 52°C for 1 min, 72°C for 1 min and a final extension of 72°C for 7 min.

The first round of VP4 PCRs was performed under identical PCR condition except that 1.25 mM of 50 mM MgCl2 and 1 mM of 50 mM solution of each forward and reverse primer (Con2 and Con3) were used.

Nested multiplex PCR: For G type determination, 2 µL of the first round product were used as template for second round of genotyping PCR using typing primers specific to G6, G8 and G10 and consensus antisense primer VP7-F (Gouvea et al., 1994a; Iturriza-Gomara et al., 2004). The PCR reaction mixture was prepared up to 25µL total volume containing 2.5 µL 10× buffer A (Vivantis, Malaysia), 0.75 µL 0f 50 mM MgCl2, 0.5 µL of 10 mM dNTPs mix (Vivantis, Malaysia), 0.25 µL of 5 U/µL Taq polymerase (Vivantis, Malaysia) and 1 µL of 50 pM solution of each type specific and antisense primer which was described above. Amplification was carried out using a thermal cycler (Techne, UK), through an initial denaturation at 94°C for 5 min followed by 30 cycles of 94°C for 1 min, 42°C for 2 min, 72°C for 1 min and a final extension of 72°C for 7 min.

Characterization of P types was performed with primer Con 2 and primers specific for genotypes P[1], P[5] and P[11] (Gentsch et al., 1992; Gouvea et al., 1994b). The PCR reaction mixture was prepared similar to that as described above but the volume of 50 mM MgCl2 was increased to 1µL and the annealing temperature was modified to 45°C.

All of the PCR products were examined using 1.5% agarose gel electrophoresis at 100V for 30 min and visualized under ultraviolet light followed by staining by ethidium bromide.

Because the standard strains were not available for us to use for setting up the RT-PCR reactions, after set up, some of each of the PCR amplicon of expected sizes which were specific for each G and P types were submitted directly to sequencing using ABI 3730XL DNA Analyzer automated sequencer (Applied Biosystems). Sequences were compared against the GenBank database using the BLAST program.

Results

Results of ELISA: Bovine rotavirus group A (BRV-A) was detected in 49 (39.2%) of 125 fecal samples examined by ELISA method and rotavirus was present in 76.9% of the herds that were sampled (20/26) (Table 2). 35 positive samples were randomly selected for the following typing processes.

Results of RT-PCR, G and P genotyping and sequencing: Table 3 shows the results of BRV-A G- and P-genotypes and genetic combination carried out by the multiplex seminested RT-PCR technique (Figure1). The G types were identified in 85.7% (30/35) of tested positive field samples and P types were identified 85.7% (30/35), and complete genotyping of both G and P types was feasible in

Table 1. Primers used for G- and P-typing in this study. (*) Primer locations are indicated as originally reported in the corresponding reports.

Assay	Primer	Sequence 5' to 3'	Sense	Position*	Reference
G-typing	VP7-F	ATG TAT GGT ATT GAA TAT ACC AC	+	51-71	(Iturriza-Gomara et al., 2004)
	VP7-R	AAC TTG CCA CCA TTT TTT CC	-	932-914	(Iturriza-Gomara et al., 2004)
	DT6	CTA GTT CCT GTG TAG AAT C	-	499-481	(Gouvea et al., 1994a)
	HT8	CGG TTC CGG ATT AGA CAC	-	273-256	(Gouvea et al., 1994a)
	ET10	TTC AGC CGT TGC GAC TTC	-	714-697	(Gouvea et al., 1994a)
P-typing	Con 3	TGG CTT CGC TCA TTT ATA GAC A	+	11-32	(Gentsch et al., 1992)
	Con 2	ATT TCG GAC CAT TTA TAA CC	-	887-868	(Gentsch et al., 1992)
	pNCDV	CGA ACG CGG GGG TGG TAG TTG	+	269-289	(Gouvea et al., 1994b)
	P5 K	GCC AGG TGT CGC ATC AGA G	+	336-354	(Gouvea et al., 1994b)
	pB223	GGA ACG TAT TCT AAT CCG GTG	+	574-594	(Gouvea et al., 1994b)

Table 2. The frequency of rotavirus group A, detected in fecal samples of calves with diarrhea collected from the industrial herds of the provinces of Tehran, Alborz and Qazvin using ELISA.

	No. of samples	No. of positive samples	Frequency	No. of herds	No. of positive herds	Frequency of positive herds
Tehran	51	22	43.1	13	10	76.9
Alborz	60	22	36.6	10	8	80
Qazvin	14	5	35.7	3	2	66.6
Total	125	49	39.2	26	20	76.9

Table 3. G and P genotypes of BRV-A field strains in the provinces of Tehran, Alborz and Qazvin, Iran.

Genotypes	No. (%) of each genotype and genetic combination				
	G10	G6	G6/G10	Non G-typable	Total
P[11]	18 (51.4)	3 (8.6)	1 (2.85)	3 (8.6)	25 (71.4)
P[5]	-	5 (14.3)	-	-	5 (14.3)
Non P-typable	2 (5.7)	-	1 (2.85)	2 (5.7)	5 (14.3)
Total	20 (57.1)	8 (22.9)	2 (5.7)	5 (14.3)	35 (100)

71.1% (27/35) of the samples and 5.7% (2/35) remained completely untypable. G typing revealed that the most predominant G type is G10 which was present in 57.1% (20/35) of samples, followed by G6 detected in 22.9% (8/35). The mixed infection of both G10 and G6 were detected in two samples (5.71%). G8 was not detected. G typing of three samples which were successfully P typed (8.6%), failed to identify. Regarding P-genotypes, P[11] was detected in 71.4% (25/35) and P[5] in 14.3% (5/35) of tested samples. P typing of three samples which were successfully G typed (8.6%), failed to identify (Table 3).

The combination of G and P types are summarized in Table 3. The most prevalent genetic combination was G10P[11] (B223-like strain) which was determined in 51.4%, followed by G6P[5] (UK-like strains) and G6P[11] (KN4-like strains) that accounted for 14.2% and 8.5% of tested samples, respectively. The other detected combination was G6/10P[5], which was detected in one sample (2.85%). None of the genotypes P[1] and G8 were identified. 5.7% (2/35) of the samples remained completely untypable (Table 3).

Sequencing data confirmed each PCR product submitted to sequencing was a sub genomic amplicon of related BRV-A genomic fragments (VP7 and VP4 genes).

Discussion

Group A bovine rotavirus is one of the causes of acute gastroenteritis in calves. Rotavirus infection causes great economic losses to the cattle industry (Cashman et al., 2010; Estes and Kapikian, 2007; Saif, 2011). Studies worldwide show that the prevalence of infection in different regions of the world is very high and this virus is present in 30-40%

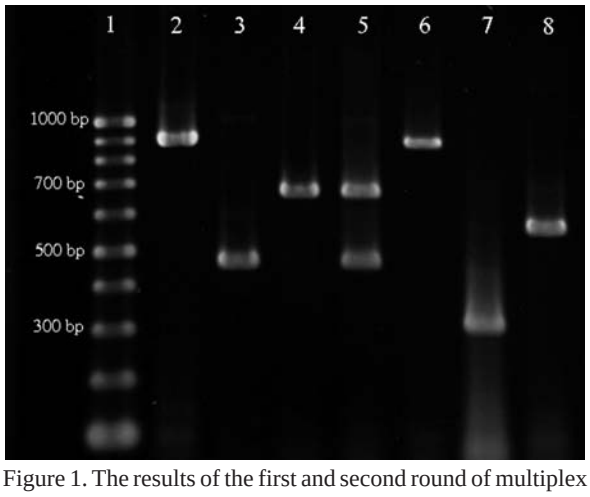

Figure 1. The results of the first and second round of multiplex RT-PCR of both G and P genotypes of BRV-A field strains in fecal samples. Line 1; 100 bp DNA ladder (Cinagen, Iran); lines 2 to 5 represent products of first and second round of G-typing; line 2, product of first round PCR of VP7 gene; line 3, PCR product of G6 genotypes; line 4, PCR product of G10 genotypes; line 5, PCR product of mixed infection of G6/G10 genotypes, lines 6 to 8 represent products of first and second round P-typing; line 6, product of first round PCR of VP7 gene; line 7, PCR product of P[11] genotypes and line 8, PCR product of P[5] genotypes.

of fecal samples of calves with diarrhea (Badaracco et al., 2012; Dhama et al., 2009; Fukai et al., 2002). Several studies have been done in Iran to determine the prevalence of rotavirus infection in calves. In these studies the prevalence of infection in stool samples collected from calves with diarrhea was estimated from 29 to 57.4%, of which the infection rate in Tehran and its surrounding area was 57.4% from 1998 to 1999 (Badiei et al., 2010; Ghorbanpour et al., 2004; Mayameei et al., 2010). In the present study, rotavirus antigen was found in 49 of 125 samples (39.2%), which means the prevalence of rotavirus infection in these provinces has reduced. One reason for this reduction could be the improved quality of hygiene management in dairy farms in recent years.

According to the reports of several epidemiological studies worldwide G6 and G10, along with P[5] and P[11] are the most common genotypes among bovine rotavirus group A strains, while two other genotypes, G8 and P[1] are less common (Alfieri et al., 2004; de Verdier Klingenberg et al., 1999; Falcone et al., 1999; Fukai et al., 2002; Reidy et al., 2006; Swiatek et al., 2010). G10 genotype was the most frequently identified in this study, occurring in 57.1% (20 out of 35) of rotavirus infections. The G6 genotype was the other detected G genotype in this study, identified in 22.9% (8 out of 35) of samples. However, regarding a previous study in Iran, the G6 type was the most frequently detected genotype (48%) in Tehran and its surrounding area (Mayameii et al., 2007). These observations confirm the fact that the incidence of G and P types may change significantly with time (Fukai et al., 2002; Monini et al., 2008). A similar predominance of G10 type in calves was observed in Sweden, México and India (de Verdier Klingenberg et al., 1999; Rodriguez-Limas et al., 2009; Varshney et al., 2002), whereas in most countries such as Australia, Brazil, Canada, Germany, Ireland, Italy, Japan, New Zealand, Turkey and the United States, G6 is the most common genotype (Alfieri et al., 2004; Alkan et al., 2010; Cashman et al., 2010; Falcone et al., 1999; Howe et al., 2008; Hussein et al., 1995; Ishizaki et al., 1996; Monini et al., 2008; Reidy et al., 2006; Suzuki et al., 1993). No G8 has been found either in our study or the previous studies in Iran (Mayameii et al., 2007) (Table 3).

One outstanding feature of our study is determination of the P genotypes of BRV-A circulating in Iran, for the first time. In the present study, P[11] genotype was the most frequent and was identified in 71.4% of samples followed by P[5] detected in 14.2% of samples. These results are contrary to the results obtained in many countries such as Japan, Latin America, the United States and Australia as well as most of the countries of Europe, as in these countries P[5] is more common compared to P[11](Alfieri et al., 2004; Badaracco et al., 2012; Brussow et al., 1994; Cashman et al., 2010; Chang et al., 1996; Garaicoechea et al., 2006; Ishizaki et al., 1996; Redmond et al., 1992; Reidy et al., 2006; Suzuki et al., 1993; Swiatek et al., 2010). However, in some countries like India, Italy, México and Turkey, similar to Iran, P[11] has been more frequently detected (Alkan et al., 2010; Falcone et al., 1999; Monini et al., 2008; Varshney et al., 2002) (Table 3).

Regarding genetic combination, in Iran, similar to India and Mexico (Rodriguez-Limas et al., 2009; Varshney et al., 2002) G10P[11] (B223-like strains) was identified as the predominant combination (51.4 %). It should be noted that according to reports,

G10P[11] strains were associated with symptomatic and asymptomatic infections in children in India (Abe et al., 2009). G6P[5] was detected as the second most common strain in this study (14.3%). This genetic combination seemed to be the most prevalent combination in several countries worldwide such as Australia, Argentina, Brazil, Britannia, Ireland, Japan and the United States (Alfieri et al., 2004; Alkan et al., 2010; Badaracco et al., 2012; Cashman et al., 2010; Chang et al., 1996; Ishizaki et al., 1996; Reidy et al., 2006; Suzuki et al., 1993; Swiatek et al., 2010). Another BRV genotype combination is G6P[11],which was detected in a low rate in the present study (8.6%). G6P[11] has been detected in a high rate in Argentina (21%), Japan (9.7 - 17.5%) and in lower rates in Ireland (7.4%) (Badaracco et al., 2012; Falcone et al., 1999; Reidy et al., 2006; Rodriguez-Limas et al., 2009; Suzuki et al., 1993) and according to the latest reports was the most prevalent BRV genotype in Italy, during 2003 - 2005, and Turkey (Alkan et al., 2010; Monini et al., 2008). Not any G6P[1] (NCDV-like strains) was detected in our study. Other combinations of G6/10P[11] were detected in the lowest rate (2.85%) (Table 3).

The RT-PCR-based genotyping method used was further confirmed to be a useful epidemiological tool for examining strain diversity, although it has gaps in encompassing novel or uncommon rotavirus genotypes and mixed infections (Desselberger et al., 2001; Iturriza-Gomara et al., 1999; Monini et al., 2008). In this study only 5.7% of the analyzed samples failed to be completely genotyped. This value is comparable with results of other studies, (de Verdier Klingenberg et al., 1999; Monini et al., 2008; Reidy et al., 2006), indicating that the use of random hexamers primers for synthesis of cDNA are sensitive enough to be used in epidemiologic studies of rotavirus in Iran.

Preventing the spread of viral infection in herds is based on good hygiene and sanitation measures as well as vaccine prophylaxis. The vaccination strategy is based on immunizing pregnant cows with inactivated vaccines in order to induce passive immunity in new borne calves following the ingestion of colostrum (Saif and Fernandez, 1996). According to studies, cross immunization among different serotypes is poor and induces antibodies protected against infection with homologous, but not heterologous sero- or genotypes (Hoshino and Kapikian, 2011; Saif and Fernandez, 1996; Snodgrass et al., 1984). In a study in Iran, Mohammadi and coworkers evaluated the effectiveness of a commercial inactivated rotavirus vaccine in vaccinated pregnant cows compared with unvaccinated control pregnant cows. Their results indicated that vaccination of cows during pregnancy was not helpful in reducing the incidence of neonatal diarrhea in newborn calves in this herd (Mohammadi et al., 2004). One reason for these results may be due to heterology between vaccine strain and field strains. Another reason could be that the commercial vaccine did not include all field strains circulating in the region. This fact emphasizes the important role of epidemiologic studies in the development of targeted vaccines. Based on data presented in our study, any future vaccines in Iran should contain all G6, G10, P[11] and P[5] to be able to develop a protective immunity. In addition, monitoring the distribution and occurrence of BRV-A that circulate in herds is important for the control of outbreaks in both human and animals. Based on data presented in this study, the preponderant genetic combination in Iran, G10P[11], has the potential to be zoonotic (Abe et al., 2009; Varshney et al., 2002).

In conclusion, details of our study show that the prevalence of G genotypes in Tehran and its surroundings have changed in recent years. Also, in this study the most common BRV-A P genotypes were determined for the first time in Iran. This region is one of the greatest husbandry centers in Iran, and cattle is distributed from this region and provinces to other regions. For this reason, our results indicate the most prevalent strains of BRV-A in Iran are different from strains that are common in many countries in the world and this fact should be considered for any decision about development of effective vaccines in Iran. Therefore, it is recommended that the epidemiological studies be continued in more geographic regions of Iran to monitor the distribution pattern of existing strains as well as the onset of unusual strains, and baseline information be gathered for better understanding of rotavirus ecology. It also recommended that further sequencing and phylogenetic studies on existing genotypes in Iran be done to compare with strains worldwide.

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مجله طب دامی ایران، ۱۳۹۱، دوره ۶، شماره ۴، ۲۲۶-۲۱۹

تعیین تیپ مولکولی روتاویروس‌های گروه A گاوی در سه استان تهران، البرز و قزوین

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(دریافت مقاله: ۱۵ شهریور ماه ۱۳۹۱ ، پذیرش نهایی: ۲۷ مهر ماه ۱۳۹۱)

چکیده

زمینه مطالعه: روتاویروس های گروه A گاوی، یکی از مهمترین عوامل مسبب گاستروانتریت و اسهال در گوساله های تازه متولد شده هستند. **هدف:** در این مطالعه تیپ های اصلی این ویروس ها با روش های مولکولی تعیین تیپ شدند. **روش کار:** در طی دو سال ۱۲۵ نمونه مدفوع از گوساله های زیر دو ماه مبتلا به اسهال از ۲۶ گاوداری صنعتی استان های تهران، البرز و قزوین که از بزرگترین مراکز پرورش گاو در ایران هستند، جمع آوری شدند. با استفاده از روش ELISA حضور روتاویروسهای گروه A در ۳۹/۲٪ (۴۹ از ۱۲۵) کل نمونه‌ها مورد تایید قرار گرفت. به منظور تعیین ژنوتیپ های G و P ۳۵ نمونه مثبت در آزمون ELISA با استفاده از seminested multiplex RT-PCR مورد بررسی قرار گرفتند. **نتایج:** از میان ژنوتیپ های G حضور ژنوتیپ های G10(۵۷/۱٪) و G6(۲۲/۹٪) مورد تایید قرار گرفت. عفونت همزمان با دو ژنوتیپ G10 و G6 در ۵/۷٪ (۲ از ۳۵) نمونه ها دیده شد. همچنین در مورد ژنوتیپ های P، P[11] شایع ترین ژنوتیپ شناخته شد (۷۱/۴٪) و بعد از آن ژنوتیپ P[5](۱۴/۳٪) قرار داشت. در این مطالعه ژنوتیپ های G8 و P[1] دیده نشد. ترکیب ژنوتیپ های موجود در این مطالعه شامل G10P[11] (B223-like strains)، G6P[5] (UK-like strains) و G6P[11] (KN4-like strains) بود که درصد وقوع هرکدام به ترتیب ۵۱/۴، ۱۴/۳و ۸/۶ بود. در ۲/۸۵٪ نمونه‌ها ترکیب ژنتیکی G6/G10P[11] دیده شد. **نتیجه‌گیری نهایی:** بنا بر اطلاعات موجود، در این مطالعه برای اولین بار ژنوتیپ های P روتاویروس های گروه A گاو در ایران انجام شده است. همچنین در این مطالعه برای اولین بار در ایران ترکیب کامل ژنوتیپی رایج ترین سویه های روتاویروس گروه A گاوی، گزارش می شود. نتیجه این تحقیق همچنین حاکی از آن است که در طی سالهای اخیر میزان وقوع ژنوتیپ های G در سه استان تهران، البرز و قزوین نسبت به مطالعات قبلی تغییر یافته است. علاوه بر این، نتایج این مطالعه برای طراحی و ساخت واکسن در ایران می تواند بسیار مفید باشد.

واژه‌های کلیدی: روتاویروس گاوی گروه A، تعیین هویت مولکولی، گاستروآنتریت گوساله ها، ایران.

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