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ORIGINAL ARTICLE

Characterization of bovine rotavirus isolates from diarrheic calves in Türkiye

Ozer Ates[1,2] · **Kadir Yesilbag**[1]

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Abstract

Background Neonatal calf diarrhea, which is the most common cause in calf deaths, leads to significant economic losses in dairy farming around the world. Diarrhea develops due to infectious and non-infectious reasons. Group A Rotaviruses (RVA) are the leading and predisposing factor for acute neonatal gastroenteritis.

Methods and results In this study, 20 diarrheic fecal samples were collected from one farm in Balıkesir province of Turkey. During virus isolation, a total of 2 stool samples were detected to produce cytopathogenic effects in MA-104 cell line. The two samples (RV-36, RV-38) were tested positive with antigen ELISA kits detecting RVA antigens. In order to detect the presence of rotavirus viral nucleic acid in cell supernatants, VP6 gene region-specific RT-PCR test was performed and the samples RV-36 and RV-38 were positive for RVA viral nucleic acid. By RT-PCR using genotype specific primers, both the isolates RV-36 and RV-38 formed amplicons compatible with G10 and P[11] genotypes of RVA. RVA nucleic acids segments were also visualized by poliacrilamide gel electrophoresis (PAGE) method. The phylogenetic tree constructed according to the VP6 gene region showed that these isolates were in the Rotavirus A group and in the I2 cluster same as other bovine and some human RVA isolates.

Conclusion Succesful isolation of RVA G10P[11] was echieved in the cattle farm. As rotaviruses play the most important role in the etiology of diarrhea in newborn calves respected genotype G10P[11] should be considered in selection of the vaccines applied to the dams. Those isolates can be further evaluated as vaccine candidate.

Keywords Cattle · Bovine rotavirus · Virus isolation · RT-PCR · Genotyping PAGE

Introduction

Neonatal calf diarrhea, which has the first place in calf deaths, causes significant economic losses in dairy farming around the world, due to treatment costs, labor, poor performance, growth retardation and deaths [1]. Despite scientific studies and chemotherapeutic advances on neonatal calf diarrhea, the disease still leads to high morbidity and mortality [1, 2]. Diarrhea of especially with infectious origin (bacterial, viral and parasitic) occurs in the neonatal period, is commonly caused by *E. coli*, cryptosporidium, rotavirus and coronaviruses. Diarrhea may also develop due to non-infectious causes including alimentary, predisposing and environmental factors [3]. Among the infectious factors, Group A rotaviruses (RVA) are the leading cause of acute viral gastroenteritis in young animals as well as in children worldwide [4, 5].

Rotaviruses, first identified by electron microscopy in 1974, are classified in the *Rotavirus* genus of the *Reoviridae* family; have a non-enveloped virion with double-stranded, positive polarity, and a 11 segmented RNA genome. The viral genome encodes a total of 12 proteins composed of 6 structural (VP1-4, VP6, VP7) and 6 nonstructural (NSP1-6) [6]. There are 3 well known major antigenic structures in a rotavirus particle including VP4, VP6 and VP7 [7]. According to the analysis of the gene region encoding VP6, rotaviruses that cause infection in different species are classified in 10 different groups (A–J) [6]. While rotavirus group A is

✉ Kadir Yesilbag
kyesilbag@uludag.edu.tr

Ozer Ates
ozerates@gmail.com

[1] Department of Virology, Faculty of Veterinary Medicine, Bursa Uludag University, 16059 Bursa, Turkey

[2] Department of Laboratory Animals Science, Faculty of Veterinary Medicine, Afyon Kocatepe University, 03204 Afyonkarahisar, Turkey

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prominent in calf diarrhea, group B and C rotaviruses also cause spontaneous infections in cattle [5, 8]. Historically, rotavirus strains have been typed using the outer capsid proteins VP7 (glycoprotein, G) and VP4 (protease-sensitive protein, P) [9]. In general, rotaviruses have reported to share 42 different G genotypes (G1–G42) and 58 different P genotypes (P[1]–P[58]) [10]. The majority of bovine rotavirus genotypes detected during field studies were identified as G6, G10, and less commonly G8, and these genotypes are generally associated with P[5], P[11], and less frequently with P[1] [11]. In addition, classification patterns for 11 RNA segments have been published by the Rotavirus Classification Working Group (RCWG), and the VP6 gene region represents the I genotype [12]. As a result of loss in mature enterocytes in gut where rotaviruses multiply, secretion of digestive enzymes is reduced, followed by carbohydrate malabsorption and diarrhea. In addition, the effect of NSP4 on cellular conduction and calcium mobilization also causes secretoric diarrhea [13]. RVA infection in adult cattle is usually subclinical and these animals play an important role in the transmission of the agent to newborn calves by the fecal-oral route. Death may occur as a result of fever, weakness, dehydration, and watery diarrhea, or an increase in mortality may be detected as a result of secondary factors [14].

Although diarrhea-related mortality rates in neonatal calf losses change according to farm conditions and regions, it has been reported that it is high even in farms where preventive veterinary medical services are applied [2]. RVA infection in cattle has been reported in different parts of the world. Due to the characteristics of the agent, isolation of the bovine rotaviruses in cell culture systems are rather problematic. Hence the diagnosis of the infection is commonly conducted either by antigen detection using ELISA and lateral flow systems or by genome detection using PCR and polyacrylamide gel electrophoresis (PAGE). Despite the common detection of RVA in Turkish cattle has been revealed, attempts to the isolation of the strains are quite limited in success. This study reports successful propagation and characterization of RVA strains from diarrheic stool samples of calves.

Material and methods

Samples

In 2019, 20 stool samples (n = 20) from calves with diarrhea were collected by the farm veterinarian into sterile stool containers sent under cold chain conditions to the Virology laboratory at Bursa Uludağ University Faculty of Veterinary Medicine for virological examination. The samples were used for diagnostic purposes, and the owner of the farm in the Balıkesir province of Turkey gave permission for their use in this study. The collected samples were diluted 1:10 in phosphate-buffered saline (PBS) containing 100 IU/ml penicillin, 100 µg/ml streptomycin, and 250 µl/ml amphotericin B solution. Stool samples diluted separately with PBS were subsequently vortexed and centrifuged at + 4 °C, 3000 rpm for 10 min. The supernatant separated after centrifugation was filtered through 0.45 µm pore diameter polyethersulfone filters and the filtrate was kept at – 80 °C to be used as inoculum in the tests.

Cell line and virus isolation

Cell lines were obtained from the culture collection of the department. Inoculum prepared from the collected samples were simultaneously inoculated onto MA-104, HRT-18, MDBK and Vero cell lines for virus isolation. HRT-18, MDBK and Vero cell lines were cultured in Dulbecco's Modified Eagle Medium (DMEM) while Glasgow Modified Essential Medium (GMEM) was used for MA-104 cell line. Culture media were enriched with 10% fetal calf serum (FCS) (Capricorn, FBS-11A, South America). In addition, 100 IU/ml penicillin, 100 µg/ml streptomycin, and 250 µl/ml Amphotericin B solution were added to prevent possible bacterial and fungal contamination in cell cultures. Both the cell cultures and FCS have been tested free from pestivirus contamination by RT-PCR and immunoperoxidase methods before use.

Virus isolation in MDBK, HRT-18 and Vero cell lines

For each of the cell lines (MDBK, HRT-18, Vero), 1 ml of cell suspension (1×10^5 cell/ml) was placed in each well of different 24-well cell culture plates. The next day, the culture media on the confluenting cell layer covering > 80% of the plate surface was removed and washed with FCS-free DMEM. For the isolation of possible enteric viruses other than rotavirus in cell culture, stool samples that were not treated with pancreatin were inoculated into each well by the adsorption-dependent virus inoculation method and left to incubate for one hour at 37 ºC, 5% CO_2 atmosphere. At the end of the period, the contents were removed, 1 ml of DMEM was added and it was taken back to the incubator under the same conditions. The plates were examined daily with the aid of an inverted microscope (Nikon, TS100) for six days to detect cytopathological changes in cell morphology (CPE), then were frozen at – 80 °C and thawed at 37 °C. The process was repeated three times by using the cell supernatants from the previous serial passage. Thus, a total of four blind passages were performed.

Virus isolation in MA-104 cell line

A unique protocol previously optimized for rotavirus propagation was employed [15]. For virus isolation attemps, 1 ml of MA-104 cell culture suspension in 1×10^5 cells/ml concentration was placed to each well of 24 well cell culture plates. The cells covering the plate surface were washed with GMEM. An amount of GMEM containing pancreatin (10 µg/ml) (Sigma, SLBL18810V) enough to cover the cell monolayers was added to the plate wells. Simultaneously inocula were individually treated with pancreatin (10 µg/ml) in tubes. The 24-well plates and the inocula were separately left to incubate for 30 minutes at 37 ºC in an incubators containing 5% CO_2. After the incubation, 200 µl of each inoculum was inoculated in a separate well of 24 well-plate and incubated for 1 h under the same conditions. At the end of the period, the content was removed, the monolayers washed out and 1 ml of GMEM containing pancreatin (10µg/ml) was added to each well and incubated under the same conditions for 6 days. All the wells were checked daily for CPE formation by an inverted-light microscope. During this period, pancreatin solution at 10 µg/ml of final concentration was added onto culture media at two days intervals. At the end of the 6 days, the plates were frozen at – 80 °C and then thawed at 37 °C. The process was repeated three times by using the culture supernatants from the previous serial passage thus a total of four blind passages were performed.

Detection of viral antigens by ELISA

For detection of RVA viral antigens, all the collected stool samples and the two culture supernatants (RV-36, RV-38) producing cytopathogenic effect in MA-104 cell culture were tested with a commercially available RVA antigen ELISA kit (BioX Diagnostic BIO K 343/2). The test protocol was applied as recommended by the manufacturer company. The test plate was scanned in ELISA automatic microplate reader (Thermo-Multiskan EX, Finland) at 450 nm wavelength and the results were evaluated using suggested calculations. In addition, the culture supernatants from two samples (RV-36, RV-38) were also tested with the BCoV antigen ELISA kit (BioX Diagnostic BIO K 392/2).

Hemaglutination assay

The obtained rotavirus isolates (RV-36, RV-38) were tested for hemagglutination (HA) activity using red blood cells from chicken, cow and horse. For that purpose, 50 µl of cell culture supernatant at the 4th passage level and 50 µl of erythrocyte suspension (0.1%) were mixed on the glass lamina. The mixtures were incubated for 30 min at room temperature and evaluated [16].

RNA extraction and reverse transcriptase polymerase chain reaction (RT-PCR)

Viral RNA was extracted using a commercial kit (NucleoSpin Virus, Macharey-Nagel, Germany) from cell culture supernatants at the 4th passage level. cDNA synthesis was performed using the iScript cDNA synthesis kit (Biorad, 170-8891). The kit protocols were applied as recommended by the manufacturers. Gene-specific primers were used for identification of VP6 gene region of RVA by RT-PCR (Table 1.) The 25 µl of reaction mixture was consisted of 9.5 µl of nuclease-free water, 12.5 µl Maxima Hot Start Dream Taq (2X) PCR Master Mix, 1 µl each of forward and reverse primers (10pmol) and 1 µL each of cDNA. Applied thermal profile was as follows: 95ºC for 4 min; 35 cycles of 95ºC for 1 min, 58ºC for 1 min, 72 ºC for 2 min and final extension at 72 ºC for 10 min. A 1.5% agarose gel (peQLab Biotechnologic GmbH, 35-1010, Germany) including Safe View Classic (Applied Biological Materials, Canada) prepared in 1x TAE buffer (Fermantas, B49, Lithuania) was used to visualize the obtained 379 bp products.

Table 1 The information of oligonucleotide primer pairs and target region for RT-PCR

Target region		Primer	Primer sequence (5′-3′)	Location	Size of product (bp)	Reference
VP6		VP6-F	GACGGVGCRACTACATGGT	747–766	379	
		VP6-R	GTCCAATTCATNCCTGGTGG	1126–1106		[26]
VP7	G genotyping	Beg9-F	GGCTTTAAAAGAGAGAATTTCCGTCTGG	1–28		[17]
		G(6)-R	CTAGTTCCTGTGTAGAATC	499–481	500	
		G(8)-R	CGGTTCCGGATTAGACAC	274–256	274	
		G(10)-R	TTCAGCCGTTGCGACTTC	715–697	715	
VP4	P genotyping	P-GenF	TTCATTATTGGGACGATTCACA	1064–1085		
		P[1]-R	TTAAATTCATCTCTTAGTTCTC	1526–1505	463	
		P[5]-R	GGCCGCATCGGATAAAGAGTCC	1725–1704	662	
		P[11]-R	TGCCTCATAATATTGTTGGTCT	1398–1377	335	

Determination of G and P genotypes

For genotyping the isolates, the cDNAs obtained from the cell culture supernatants from the 4th passage level were tested by RT-PCR. G and P genotyping protocols were applied as including minor modifications from previous study [17]. The Beg9-F forward primer sequence specific for the VP7 gene region was coupled with genotype specific reverse primers (G6-R, G8-R, G10-R) specific for the genotypes G6, G8 and G10 as shown in Table 1. The 25 µl of reaction mixture was consisted of the content described above. For G8 and G10 genotyping, applied thermal profile was as follows: 95 ºC for 5 min; 40 cycles of 94 ºC for 1 min, 55 ºC for 1 min, 72 ºC for 2 min, and final extension at 72 ºC for 7 min. Unlike this protocol, the annealing temperature for G6 genotyping was 43.5 °C that is optimized in this study. P genotyping was performed as similar to G genotyping. However, RT-PCR was applied using the P-GenF primer sequence specific to the VP4 gene region and reverse primer sequences (P[1]–R, P[5]–R, P[11]–R) specific for the P[1], P[5] and P[11] genotypes were subjected as shown Table 1. The 25 µl of reaction mixture was as described above. The concentration for forward and reverse primers was 25 pmol. Thermal profile applied for P genotyping was as follows: 95 ºC for 5 min; 40 cycles of 94 ºC for 1 min, 50 ºC for 1 min, 72 ºC for 2 min, and final extension at 72 ºC for 7 min. Visualization of the PCR products was as described above. The targeted product sizes for the G and P genotypes are shown in Table 1. In addition, the two samples (RV-36, RV-38) were tested by RT-PCR for the simultaneous presence of pestiviruses, bovine coronavirus, and bovine torovirus (data not shown).

Sequencing and phylogenetic analysis

The samples positive in RVA-RT-PCR were selected for Sanger sequencing (BM Lab, Turkey). Sequence data were aligned by the ClustalW multiple alignment tool using the BioEdit software. Maximum likelihood analysis was performed with 1000 bootstrap replication using MEGA11. For designing the phylogenetic trees, the reference sequences were obtained from GenBank.

SDS-PAGE

RNA extraction was performed as described [18] to show 11 segments of rotavirus from original fecal samples of the isolates (RV-36, RV-38) in sodium dodecyl sulfate-polyacrilamide gel electrophoresis. The obtained RNA was loaded onto a commercially available gel system containing 5–14% acrylamide (Biorad, 4561083) and run for 6 h (30 mA and 100 V). Silver-nitrate staining was performed as described and the results were evaluated macroscopically [18].

Results

Virus isolation, HA activity and antigen ELISA results

All the 20 stool samples were entirely subjected to blind passages in MA-104, MDBK, HRT-18, and Vero cell lines for four times and 2 stool samples (RV-36, RV-38) were detected to produce CPE at every passage levels in MA-104 cells (Fig.1). For detection of possible enteric viruses other than rotavirus, inoculum was inoculated onto MDBK, HRT-18 and Vero cells without treatment with pancreatin and no changes in cell morphology were detected in these cell lines after four blind passages.

Using the two cell culture supernatants (RV-36, RV-38) at 4th passage level resulted negative in the hemagglutination test performed with chicken, bovine and horse erythrocytes. Five out of 20 stool samples were positive for rotavirus antigens by RVA Ag ELISA test. The two samples (RV-36, RV-38) producing cytopathogenic effect in MA-104 cell line were detected positive for rotavirus antigens by ELISA at the 4th passage level. In addition, all the collected stool samples and the two samples (RV-36, RV-38) producing cytopathogenic effect in MA-104 cell line were detected as negative by BCoV Ag ELISA.

RT-PCR, genotyping and phylogenetic analyses

RT-PCR was used for nucleic acid detection of the suspected virus in the cell supernatants of two samples producing cytopathologic effects at the 4th passage level. VP6 gene region-specific RT-PCR produced a 379 bp amplicon for both the samples RV-36 and RV-38 which confirmed the presence of rotavirus (Fig. 2). In addition the two samples (RV-36, RV-38) that produced a cytopathogenic effect in MA-104 cell line were found negative for pestivirus, BCoV and BToV with RT-PCR.

By genotype-specific RT-PCR, both of the isolates (RV-36, RV-38) produced expected amplicons for G10 (715 bp) and P[11] (335 bp) (Fig. 3), despite being negative for all the other genotypes tested (G6, G8, P[1], P[5]).

Phylogenetic analysis was performed with previously reported VP6 gene sequences of the RVA genome. According to the phylogenetic tree constructed for the partial (379bp) VP6 gene region, RV-36 sequence (RVA/bovine/TR/RV-36/2019/G10P[11], accession number: OP432877) and RV-38 sequence (RVA/bovine/TR/RV-38/2019/G10P[11], accession number: OP432878) dropped into the cluster of I2 in the Rotavirus group A (Fig.5).

SDS-PAGE result

After silver nitrate staining on acrylamide gel, RVA nucleic acid sharing 11 RNA segments was detected as a result of

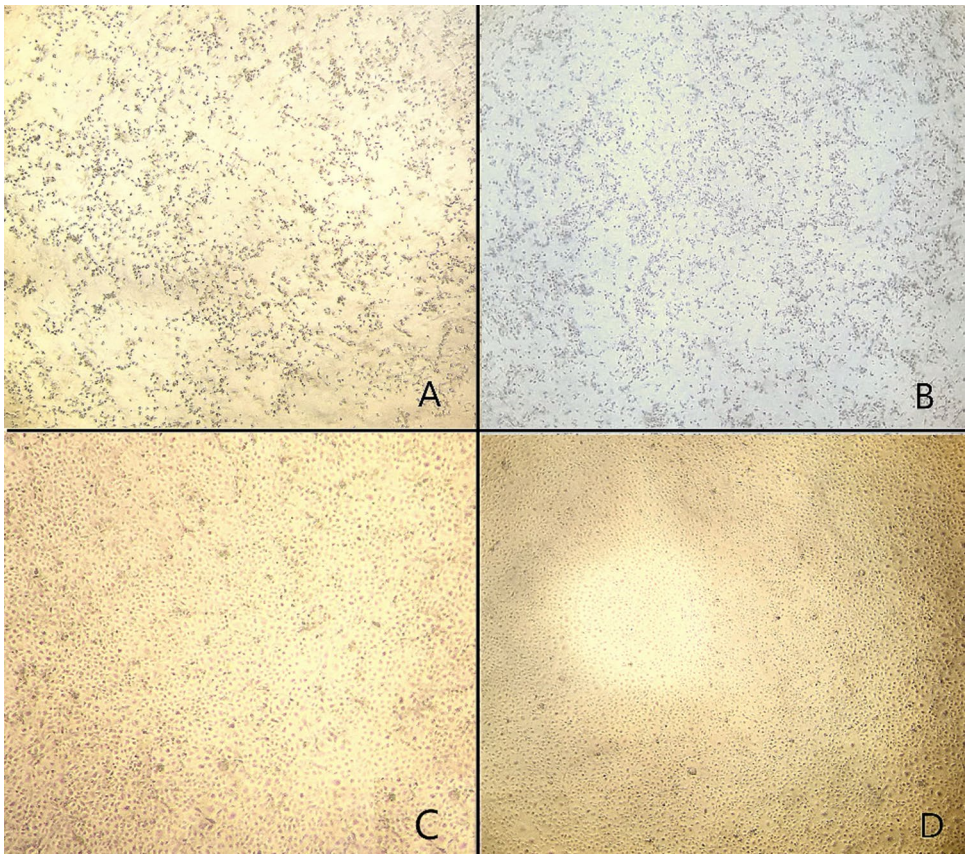

Fig. 1 Cytopathic effect caused by the isolates RV-36 and RV-38 on MA-104 cell monolayers (5th day, × 4). **(A)** MA-104 cell line infected with RV-36, **(B)** MA-104 cell line infected with RV-38. **(C)** Control well: non-infected, pancreatin-treated. **(D)** Cell control well: non-infected and pancreatin non-treated

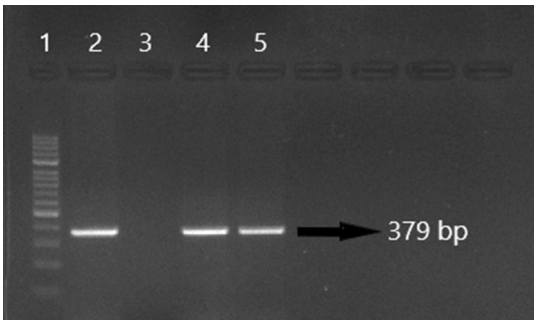

Fig. 2 RT-PCR amplification of VP6 gene region using 4th passage of the isolates in MA-104 cell culture. Line 1: 100 bp DNA ladder, Line 2: Positive control, Line 3: Negative control, Line 4: Isolate RV-36, Line 5: Isolate RV-38, (expected amplicon size 379 bp)

PAGE test applied on both RV-36 and RV-38. The detected segments migration pattern was as 4/2/3/2 (Fig.4) which indicates the two isolates are in the rotavirus group A.

Discussion

Group A rotaviruses are one of the most important etiological agents of diarrhea in newborns of many animal species around the world [4, 5]. Obtaining and characterization of rotavirus isolates from the field is not only important for following up on the epidemiological status of a defined region but also indispensable for confirmation of the protection by vaccines and immunsera in the field. It is also notchless for the development of vaccinal strains upon requirement. Detection of the disease factor in newborn calves in herds is an important situation in preventing economic losses and disease control. The detection of rotaviruses can be achieved by various methods such as virus isolation in cell culture, immunochromatographic test kits, polyacrylamide gel electrophoresis, fluorescent antibody assay, ELISA, RT-PCR and electron microscopy (EM) [7, 16], though sharing different sensitivities [3, 7, 16, 18]. Despite various results were reported, it is quite difficult to isolate and propagate bovine rotaviruses from the native specimens using cell culture systems [16, 19]. In this study, antigen ELISA and RT-PCR methods, which were successfully used in the identification of bovine rotavirus isolates, were used in accordance with previous studies, and the obtained isolates were typed by RT-PCR method.

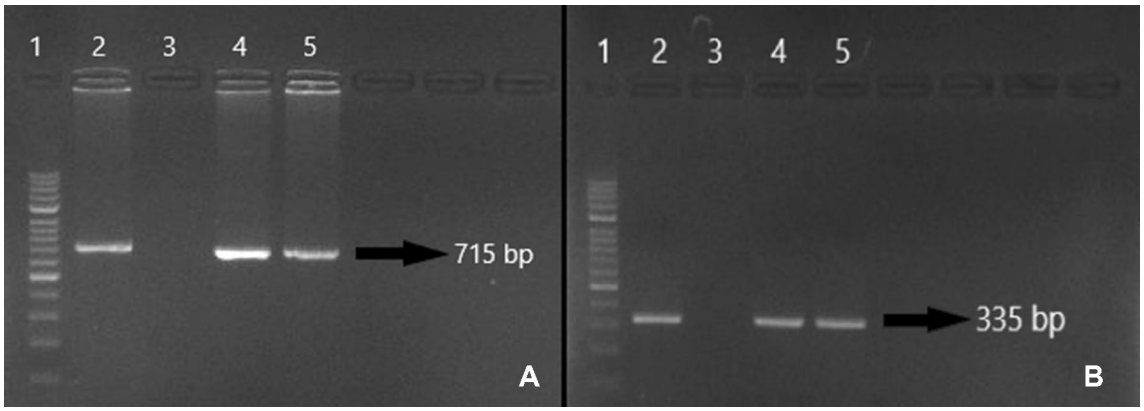

Fig. 3 The result of RT-PCR amplification for G10 (**A**), expected amplicon size of 715 bp) and P[11] (**B**), expected amplicon size of 335 bp) genotyping at 4th passage of isolates in MA-104 cell culture. Line 1: 100 bp DNA ladder, Line 2: Positive control, Line 3: Negative control, Line 4: Isolate RV-36, Line 5: Isolate RV-38

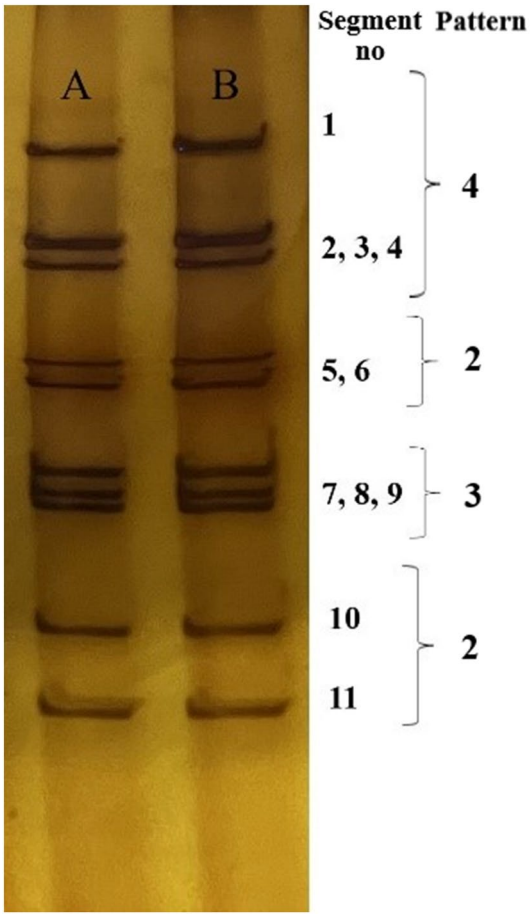

Fig. 4 Electrophoresis showing the characteristic 11 segmented genomic dsRNA in polyacrylamide gel. (**A**) Isolate RV-36 and (**B**) Isolate RV-38 represent the electrophoretic migration patterns of bovine rotavirus group A (pattern 4/2/3/2)

MA-104, MDBK and PK-15 cell lines were reported to be used for the isolation of RVA in cell culture by following different applications and successful results were obtained [16, 19–21]. There are many incomplete hypotheses regarding the cell entry of rotaviruses. In some studies, the entry of RVA into the host cell has been described as a complex event involving sialic acid-containing receptors and integrins [22]. Thus, numerous studies used proteolytic enzymes such as pancreatin, TPCK trypsin, and crystal trypsin in the isolation protocols of RVA. Successful results have also been reported in virus isolation according to the application method [16, 19–21]. One of which comprises pairing MA-104 cell line with the use of pancreatin enzyme [15]. Accordingly, MA-104 cells combined with pancreatin treatment were employed for virus isolation in this study. The pancreatin has a function to cleave VP4, which is one of the structural proteins of the rotavirus virion and sensitive to proteolytic enzymes, into VP8 and VP5. By this proteolysis, the adhesion and entry of the virus particle into the target cell is facilitated. In this study, inocula prepared from fecal samples were treated with 10% pancreatin to facilitate the adhesion of RVA to the receptors on the MA-104 cells and cytopathogenic effect was detected in two samples (Fig. 1). Isolation of enteric viruses in cell culture is mostly difficult. As well as the applied protocol and selected cell line, the sampling time is one of the important criteria in virus isolation from stool samples. The chance of virus isolation is high in the samples taken during the onset and peak period of clinical findings [3]. In the present study, samples were freshly collected from the clinical cases, quickly transferred to laboratory and inoculated into cell culture without a freeze-storage period. Togetger with the applied enzyme protocol, sampling and processing of the samples should have critical effect for succesful isolation of RVA from the stool samples. Other cell lines used in the study were

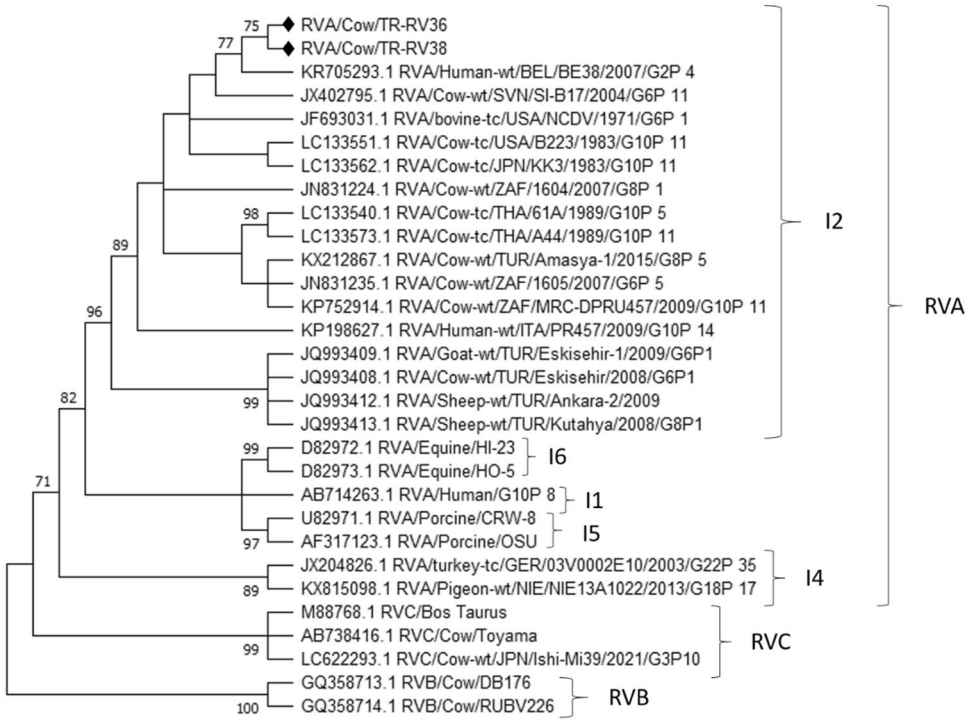

Fig. 5 Phylogenetic tree presenting the position of isolate RV-36 and RV-38 in I2 genotype of group A rotaviruses (RVA). The target sequences are on the VP6 genomic region of the RVA strains. The tree was obtained using the maximum likelihood method with 1000 bootstrap replicates. The Turkish RVA isolate sequences are shown as marked. GenBank Association number of the reference strains are submitted in the figure

inoculated without pancreatin treatement for possible isolation of enteric viruses other than rotavirus, but no CPE was recorded at the end of 4 blind passages. Those samples were also tested for the presence of bovine coronavirus, bovine pestivirus, and bovine torovirus with negative results (not submitted data). These results confirm that the detected isolates (RVA G10P[11]) is the causative viral agent of diarrhea in the study population.

There are various studies showing the hemagglutination activity of some RVA strains with erythrocytes from many species [23]. The HA activity of the two isolates obtained in this study was evaluated with bovine, equine, and chicken erythrocytes, but failed to detect positive results. An association for the presence of a proteolytic enzyme in the culture media as well as the freezing-thawing process has been reported for hemagglutination activity of RVA isolates [16]. Inoba et al [24] reported that RVA field strains with no clear HA activity may show hemagglutination even at low titer concentrated after by ultracentrifugation. Thus, the hemagglutination activity of the isolated RVA strains (RV36, RV38) may be associated with the low number of the viral particles.

VP6 structural protein is considered to be a strongly conserved protein and showing little variability in defined groups of rotaviruses. On the other side, VP6 is the major structural component of the rotavirus capsid and the commonly targeted for RVA detection [25]. Thus, the faster and sensitive detection of rotavirus infections in humans and many animal species are to be made by RT-PCR targeting the VP6 gene region [26–28]. In this study, RT-PCR targeting the VP6 gene of the virus was applied to the supernatants of two samples in which CPE was detected at the 4th passage level. The presence of RVA viral nucleic acid was determined in both samples. Consistent with the results by RVA antigen ELISA, positive results confirmed the succesfull isolation of rotavirus from clinical specimens collected from calves with diarrhea. Bovine rotaviruses are frequently allocated in group A (RVA) while less frequent strains are classified in groups B and C [8]. VP6 gene region represents the I genotype and, in genotyping by VP6 gene sequences, bovine and human rotaviruses are clustered to be in the subgenotype I-2. [12, 27, 28]. With the phylogeny study, the RVA isolates (RV-36 and RV-38) obtained in the present study were in the Group A rotavirus, where bovine RVAs are frequently found, and in the I2 genotype according to the VP6 gene region (Fig. 5).

In various studies conducted around the world, G6 and G10 genotypes are commonly detected in cattle populations according to the VP7 encoding gene region of rotaviruses [25], while G1-G3, G5, G8, G11, G15, G17, G21 and G24 genotypes are also detected at a lesser extend [29]. For VP4 gene region, despite P[5] and P[11] genotypes are observed frequently [25], P[1], P[3], P[6], P[7], P[14], P[17], P[21], P[29] and P[38] are also reported more rarely [29]. Frequently observed dual genotype combination of Group A rotaviruses consists of G6P[1], G6P[5], G10P[11] and G8P[1] in cattle. G6P[5], G6P[11], G10P[5] and G10P[11] genotype combinations have been detected in Türkiye, with

the dominance of G6P[11] and G10P[11] combinations. The RVA combinations of the isolates obtained in this study were in G10P[11] compatible with the previous data [30]. These results show that the G10P[11] is one of the predominant genotype combination still causes diarrhea in calves despite the prevention and control studies.

PAGE is one of the gold standart methodology for RVA detection from native samples and culture fluids. In these method, A, B, and C groups of rotaviruses are defined according to migration patterns of the segments in polyacrylamide gel. In that approach, Group A rotaviruses are identified as grouping the 11 segment of the genome as 4/2/3/2 pattern, while Group B has a migration pattern of 4/2/2/3, and Group C has 4/3/2/2 [8]. In this study, RV-36 and RV-38 genome segments were ordered as 4/2/3/2 (Fig.4), clearly describing the isolates that put into Rotavirus Group A. The results obtained in the phylogeny tree are also compatible.

Consequently, detection of the circulating strains in the field and vaccinating the dams with the appropriate strains in calf diarrhea caused by rotavirus is of great importance for the control and eradication of the infection. Results of the present study highlight the presence and importance of RVA infection in field conditions. Two strains of RVA (G10P[11]) are successfully isolated and characterized in this study. Identification of these genotypes may require involvement of the G10 RVA strains in the neonatal calf diarrhea vaccines applied in Türkiye. These results may help to vaccine development studies in the future.

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Declarations

Conflict of interest The authors declare that they have no conflicts of interest.

Ethical approval This study was approved by the Bursa Uludag University Experimental Animals Local Ethics Committee in accordance with ethical guidelines for the Care and Use of Laboratory Animals (approval number: HADYEK; 2020–05/14).

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