



Investigation of the Prevalence of Rotavirus Infection in Calves using Polyacrylamide Gel Electrophoresis

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ABSTRACT

Bovine rotaviruses cause loss of calves and cause great financial losses to breeders. Bovine rotaviruses, which are classified in the *Reovirales* order, *Sedoreoviridae* family and *Rotavirus* genus, are mostly classified as G and P genotypes according to VP7 and VP4 gene regions. In addition, 10 different species (group A-J) have been identified according to genetic and antigenic properties of another major antigen, VP6. Group A rotaviruses are the most common cause of diarrhea in calves, while group B and C infections are also known. For the protection of calves, rotavirus screening should be performed on a herd basis and the infection status of cattle should be revealed. For this purpose, stool samples of 100 calves with diarrhea symptoms in the inventory of Atatürk University, Faculty of Veterinary Medicine, Department of Virology were used. Polyacrylamide gel electrophoresis (PAGE), which allows the examination of segments of the genome, was used to check for the presence of the virus. Nucleic acid extraction was performed on the stool samples before electrophoresis and then extracts were loaded into the prepared polyacrylamide gel and run. The samples were stained with silver nitrate stain, segment patterns were determined, and the presence of rotavirus was analyzed. While 27 of the analyzed samples were positive, 5 samples were suspicious, and 68 samples were negative. The segment pattern of the positive samples was compatible with group A and all of them were classified in this group. Although they were in the same group, it was determined that the positive samples had 3 different electrophoretotypes. As a result, it was determined that rotaviruses still have an important role in the etiology of calf diarrhea. Besides, the detected rotaviruses showed variation, although they were in group A, and breeders in the region should pay attention to control and hygiene measures.

Keywords: Cattle, Diagnosis, Polyacrylamide gel electrophoresis, Rotavirus infections.

ÖZ

Buzağların Rotavirus Enfeksiyonunun Prevalansının Poliakrilamid Jel Elektroferez Yöntemi ile Araştırılması

Sığır rotavirus enfeksiyonları yenidoğanların ölümlerine neden olduğundan yetiştiriciler için önemli ekonomik kayıplara sebep olmaktadır. *Reovirales* takımı, *Sedoreoviridae* ailesi ve *Rotavirus* genusu içerisinde sınıflandırılan sığır rotavirusları en çok VP7 ve VP4 gen bölgelerine göre G ve P genotipleri olarak sınıflandırılmaktadır. Bunun haricinde diğer bir major antijen olan VP6 genetik ve antijenik özellikleri temel alınarak 10 farklı tür (grup A-J) belirlenmiştir. Grup A rotaviruslar sığırlarda en fazla ishale neden olurken grup B ve C enfeksiyonları da bilinmektedir. Buzağların korunması için sürü bazında rotavirus taraması yapılmalı ve sığırların enfeksiyon durumu ortaya konulmalıdır. Bu amaçla Atatürk Üniversitesi Veteriner Fakültesi Viroloji Anabilim Dalı envanterinde bulunan ishal semptomu olan 100 adet buzağıya ait gaita örneği kullanıldı. Virus varlığını tespit etmek için grup ayırımına imkân veren poliakrilamid jel elektroferezi (PAGE) kullanıldı. Elektroferez öncesi gaita örneklerine nükleik asit ekstraksiyonu işlemi yapıldı ve sonrasında hazırlanan poliakrilamid jele yüklenerek yürütüldü. Yürütülen örnekler gümüş nitrat boyanarak segment paternleri belirlenerek rotavirus varlığına bakıldı. İncelenen örneklerin 27'sinde pozitiflik saptanırken 5 örnek şüpheli ve 68 örnek negatif olarak belirlendi. Pozitif bulunan örneklerin segment paterni grup A ile uyumlu bulundu ve tümünün bu grup içerisinde sınıflandığı görüldü. Her ne kadar aynı grup içerisinde yer alsada tespit edilen pozitif örneklerin 3 farklı elektroforetipe sahip olduğu tespit edildi. Sonuç olarak rotavirusların buzağı ishalleri etiyolojisinde halen önemli rolü olduğu, tespit edilen rotavirusların grup A içerisinde yer almakla beraber varyasyon gösterdiği ve bölgede yetiştiricilerin kontrol ve hijyen önlemlerine dikkat etmesi gerektiği tespit edilmiştir.

Anahtar Kelimeler: Poliakrilamid jel elektroferezi, Rotavirüs enfeksiyonları, Sığır, Tanı.



INTRODUCTION

Neonatal calf diarrhea is one of the main problems in cattle breeding because of the serious economic losses. One of the main viral agents contributing to early-stage calf loss by diarrhea is bovine rotaviruses. Bovine rotaviruses are classified under the family *Sedoviridae*, genus *Rotavirus* (Karayel-Hacıoğlu et al. 2022; Aksoy and Azkur 2023). Genetic material of the bovine rotavirus is a double-stranded RNA that has 11 segments and encodes 6 structural and 6 non-structural proteins (Aksoy and Azkur 2023; Ates and Yesilbag 2023). Besides having a zoonotic potential, rotaviruses have a high degree of reassortment by exchanging the segment with other species in the same genus. This leads to the widening of the host spectrum and interspecies transmission of rotaviruses (Karayel-Hacıoğlu et al. 2022).

Rotaviruses are mostly classified with a binary nomination namely G and P depending on VP7 and VP4 respectively. There is an extended genotype classification Gx-P[x]-Ix-Rx-Cx-Mx-Ax-Nx-Tx-Ex-Hx based on genes VP7-VP4-VP6-VP1-VP2-VP3-NSP1-NSP2-NSP3-NSP4-NSP5/6 to compare full genomes by rotavirus classification working group (RCWG. 2024). According to this classification, 42 G, 58 P, 32 I, 28 R, 24 C, 24 M, 39 A, 28 N, 28 T, 32 E, and 28 H genotypes have been described (RCWG. 2024). Moreover, based on the genetic and antigenic of the full genome and VP6 rotaviruses are classified into ten groups (or species, designated from A to J) (Banyai et al. 2017). Calf diarrhea is mostly caused by bovine rotaviruses which belong to group A, but groups B and C are also reported (Karayel-Hacıoğlu et al. 2022; Ates and Yesilbag 2023).

Bovine rotaviruses are common causative agents of calf diarrhea which have been previously serologically and molecularly reported from our country and region from herds with diarrhea (Alkan et al. 2010; Alkan et al. 2015; Karayel et al. 2017; Bulut et al. 2020; Karayel-Hacıoğlu et al. 2022; Aksoy and Azkur 2023; Ates and Yesilbag 2023).

As the disease can lead to a significant potential loss to cattle husbandry, diagnosis of the virus bears quite importance. Virus can be diagnosed by molecular methods, ELISA kits and rapid diagnostic tests. Diagnosis with reverse transcription polymerase chain reaction (RT-PCR) is the preferred method of detailed studies because subsequent phylogenetical analyses provide a larger perspective in examining mutations and segment changes (WHO 2009; Bulut et al. 2020). However, this method can be challenging because of the need to analyse multiple gene regions, mutations and the presence of novel strains, PCR inhibitors present in stool or other technical issues. An initial diagnosis is beneficial to make sure virus is present in the sample to work on RT-PCR optimizations. Polyacrylamide gel electrophoresis (PAGE) is an optimal method to visualize the segments of the virus. Thus, after PAGE is performed presence of the virus nucleic acid is known in the sample, so further molecular studies can be performed (WHO 2009). Also, molecular study of single segment can be an option when segments are separately migrated removing the segment and using gel extraction.

This study aims to investigate rotavirus presence, determine rotavirus groups according to segment patterns, and observe different variations in segment migration patterns of rotaviruses in calves with diarrhea.

MATERIAL AND METHODS

Material

Material of the study consisted of 100 stool samples of calves of different ages. Material is collected within Erzurum province. This study has been approved by Ataturk University Veterinary Faculty Local Ethics Committee with number 2024/04.

Polyacrylamide Gel Electrophoresis

Stools were first processed for dsRNA nucleic acid extraction. Gels were cast and run using Mini-Protean (Bio-Rad, California ABD) system. Gels were run vertically using the vertical tank. Gel staining was done using silver nitrate to visualize dsRNA segments. All processes were conducted as instructed by WHO Rotavirus Manual (WHO 2009).

RESULTS

Rotavirus genome was detected in 27 samples, no segment was observed in 68 of the samples and result of 5 samples could not be determined due to heavy smear in corresponding well of the samples. Thus, 5 samples were regarded as suspicious. Sample gels including positive and negative samples are presented in Figure 1 and Figure 2. All positive samples had a pattern compatible to rotavirus group A pattern. Although all positive samples were of group A, there were minor differences in migration pattern. Three different patterns were distinguishable among positive samples. Thus, these electropherotypes were designated type 1, type 2, type 3 as shown in Figure 3.

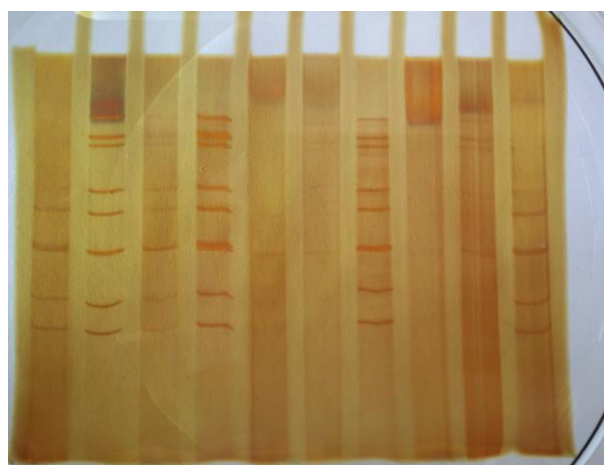


Figure 1: Sample PAGE gel image from various positive and negative cases. Image is visualised with normal camera in direct light. Positive samples have varying degree of visible segment patterns. Only well 8 (counted from left) does not have visible segments and considered negative. Some segments are not as apparent due to lack of contrast (Wells 5, 6 and 9). This is caused by decreased amount of nucleic acid in samples.

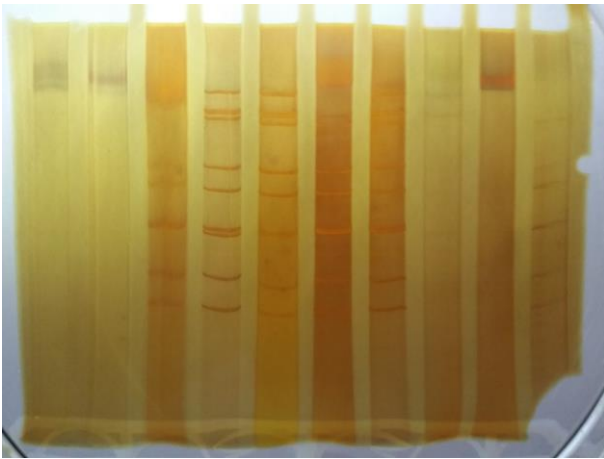


Figure 2: Sample PAGE gel image from various positive and negative cases. Image is visualised with normal camera in direct light. Positive samples have varying degree of visible segment patterns. Wells 1, 2, 8, 9 (counted from left) does not have visible segments and considered negative. Some segments are not as apparent due to lack of contrast (Wells 3 and 10).

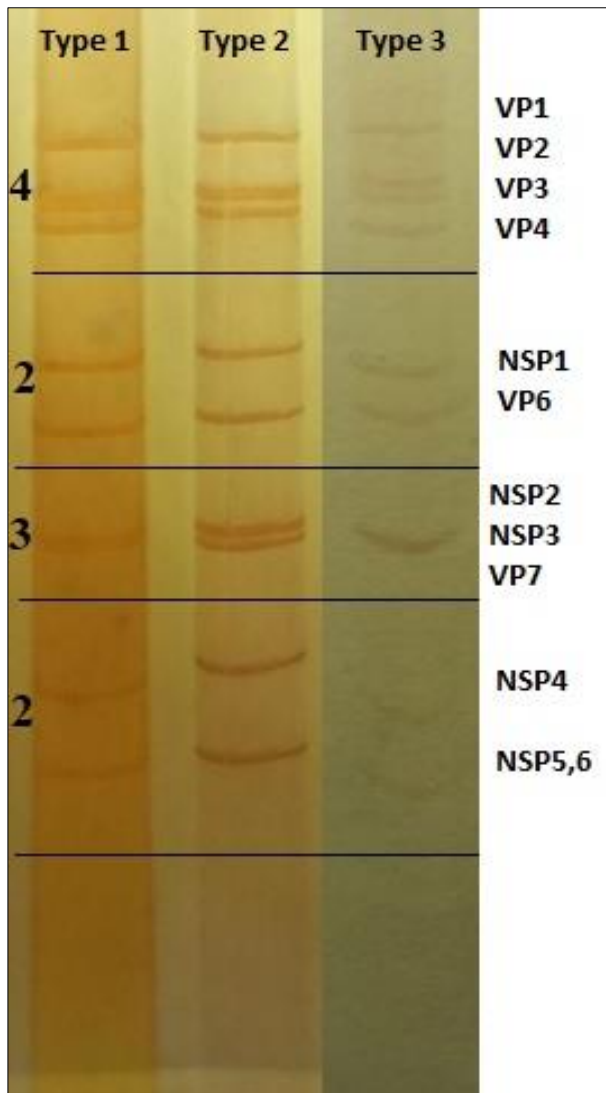


Figure 3: Comparison of three different electropheretotypes. Type names given are located on top. Genome segment names are shown on the right. All types show 4/2/3/2 pattern indicating rotavirus group A.

DISCUSSION AND CONCLUSION

Bovine rotaviruses classified in group A are common agents in outbreaks of neonatal calf diarrhea and cross-species infections are reported from humans (da Costa Mendes et al 1993; Cabalar et al. 2001; Matthijnsens et al. 2009; Alkan et al. 2010; Martella et al. 2010; Chen et al. 2023; Carossino et al. 2024; Sadiq and Khan 2024). The most common genotype in Türkiye between 1997-2008 was reported as G6P[11] and G10P[11] (Alkan et al. 2010).

Only partial information is known at genomic level of how these combinations occur; this situation is the cause of the limited understanding of rotavirus infection between ruminants, humans, and other mammals (Karayel-Hacıoglu et al. 2022). Genetic reassortment seems to be the leading cause of interspecies transmission of rotaviruses, segment changes enable rotaviruses to infect different species. Transmission from bovine to other species transmission include rabbits and horses (Ghosh et al. 2013; Schoondermark-van et al. 2013).

In this study, rotaviruses were found to be an important causative agent of diarrhea in calves under six months of age in Erzurum (27/100 for positivity rate, 27%). It was found that 27% of animals with diarrhea symptoms belonged to Group A rotavirus. The rate of detection of rotavirus in 27% of diarrhea cases was higher than the 6.1% previously reported in Erzurum (Aydın and Timurkan 2018). This difference observed in our region suggests that there are ongoing problems with sanitation and lack of care for calf diarrhea in livestock holdings and may also be due to environmental or social factors. Additionally, the prevalence observed in this study was similar to previous studies conducted outside Erzurum in other regions of our country, where the prevalence reported in other studies ranged from 1.6% to 30%. Beside Erzurum, prevalence was reported as 1.6% (1/59, calves) in Van (Cabalar et al. 2001), 30% (9/30, calves) in Elazığ (Al and Balıkcı, 2012), and 18.75% (18/96, calves) in Konya-Afyon (Uyunmaz Saklı et al. 2019).

Reports from different parts of the world have shown electrophoretotyping as a potential tool for studying the molecular epidemiology of rotavirus infections. Different electropheretotypes can be examined differently with two perspectives. Firstly we can determine and cluster the group (A, B, C etc) of the rotaviruses, which allows us evaluate the origin of the virus. Secondly, within the same group, there may be minor differences in patterns belonging to same group such as our study. This information can be considered as an indication of genetic changes including virulence, but the concept has not been studied extensively and regarded only preliminary (Özkul et al. 2002). In this context, electropheretotyping studies in humans are abundant. In one study (Ayolabi et al. 2013), 12 different electropheretotypes (7 long E-types and 5 short E-types) were found and L5 was found to be dominant. Regardless of the country and region, the predominance of the long pattern over the short pattern is in accordance with the findings of other researchers and they considered this situation normal. The same study also reported that more electrophoretic patterns were observed in Nigeria than previously reported. In another study, in 1998, the African rotavirus study group reported 2 and 7 patterns from Jos and Zaria cities, respectively. However, 22 electrophoretic patterns were reported in Bangladesh and 7 patterns were reported in Kenya by the same study group (Ayub et al. 1993). Bukrinskaia et al. (1990) identified several mobility patterns of both long and short-migration types of rotavirus RNA during outbreaks in

children in Russia in the winter of 1988-1989. This reports the presence of antigenic variants of rotaviruses in an outbreak.

The mechanisms for generating wide genomic diversity among rotavirus strains (since they have segmented genomes) are mostly due to genetic reassortment. This genetic reassortment can occur either naturally or possibly due to suppression by the host immune system (Biryahwaho et al. 1987; Ward et al. 1988). This means that there are several strains circulating in the region in our study and this also emphasizes the need for regular rotavirus surveillance to detect these new/unusual and emerging strains that may have an impact on current vaccines. As important epidemiological data, it can be emphasized that the emergence of genomic variations and their carry-over to antigenic properties of circulating rotaviruses can be frequently encountered between herds at different intervals. In this study, 3 different rotavirus patterns were detected in the limited sample size. With more comprehensive studies, the relationship of these patterns with genotypes, the approach to the pathogenesis of the disease, and the similarity/difference with virus strains can be revealed.

PAGE allows distinguishing rotavirus groups without performing RT-PCR and sequencing. Group A rotaviruses are identified according to migration of the pattern the 11 segments of the genome as 4/2/3/2, while Group B has a migration pattern of 4/2/2/3, and Group C has 4/3/2/2 (WHO 2009; Ates and Yesilbag 2023). Even within the same group, like in our study all positive samples are group A, we can have different electropherotypes to interpret segment migration pattern changes to predict antigenic differences. A fast interpretation in this regard is advantageous when molecular diagnostic equipment/sequencing is not available. Three electropherotypes in our study are examined are mostly constant in segment patterns. Among positive samples type 1 was the dominant type and 22 of 27 positives were of this type. Whereas 4 of 27 were type 2 and only one sample was classified as type 3. The main difference is with type 3 is with segment 3 (VP3), it has migrated faster thus VP2 and VP3 is distinguishable. Type 2 has differed in segment 4 (VP4) has slower migration and segment 9 (VP7) is faster migration. Since VP7 is responsible for G genotype and VP4 is for P genotype is expected to have a different G type and P genotype than other strains if related genes were sequenced. Three main antigenic sites are defined by genes VP7, VP6 and VP4. Especially outer capsid proteins, VP7 and VP4, that independently generate serotype-specific neutralizing antibodies (Özkul et al. 2002; Ates and Yesilbag 2023). These segments can be examined with PAGE, although results can only be preliminary because there is not enough data which compare segment patterns/serological response.

There are two previous studies on rotaviruses that reported rotavirus presence detected with PAGE (Özkul et al. 2002; Ates and Yesilbag 2023). Özkul et al. (2002) determined 5 different electrophoretic types according to migration patterns in 83 stool samples in which rotavirus infection was detected. All of the rotaviruses examined in the study were found in group A and exhibited short migration patterns. They included 7 enterprises in their study and all of them showed a unique pattern, but in one enterprise, they detected rotaviruses with a pattern that changed over the years. They observed the greatest differences in electrophoretic migration in segments 2-4 and 6-9, but found that segments 1, 10 and 11 exhibited the most constant migration. Ates and Yesilbag (2023)

worked with 20 stool samples and were able to isolate 2 strains in cell culture. These two isolates were examined with PAGE and researchers reported they belonged to rotavirus group A. Our study had similar results to these studies; all strains in this study also belonged to group A.

Bovine rotaviruses can impact herds by loss of calves when prevention measures are not taken carefully. The most important way of protection is vaccination, but since passive immunization is mostly acquired by colostrum vaccination of pregnant cows is the most effective form of prophylaxis (Alkan et al. 2010; Bulut et al. 2020).

In conclusion, the following data has been gathered in light of our results: Firstly, bovine rotavirus group A has been shown to still cause problems to breeders in the Erzurum region. Secondly when vaccine strains are taken into consideration current vaccine has been found suitable for field strains. Thirdly there is need for further research regarding antibody response for different electropherotypes since different types were found in our results.

CONFLICTS OF INTEREST

The authors report no conflicts of interest.

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AUTHOR CONTRIBUTIONS

Idea / Concept: NC, MÖT

Supervision / Consultancy: MÖT

Data Collection and / or Processing: HA, MÖT, NC

Analysis and / or Interpretation: HA, MÖT, NC, VY

Writing the Article: NC, MÖT

Critical Review: NC, MÖT

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