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Putative vaccine breakthrough event associated with heterotypic rotavirus infection in newborn calves, Turkey, 2015

Ilke Karayel[a], Enikő Fehér[b], Szilvia Marton[b], Nüvit Coskun[c], Krisztián Bányai[b], Feray Alkan[a,*]

[a] Ankara University, Faculty of Veterinary Medicine, Department of Virology, Diskapi, 06110 Ankara, Turkey
[b] Institute for Veterinary Medical Research, Centre for Agricultural Research, Hungarian Academy of Sciences, P.O. Box 18, H-1581 Budapest, Hungary
[c] Kafkas University, Faculty of Veterinary Medicine, Department of Virology, Kars, Turkey

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ABSTRACT

Group A rotaviruses (RVA) are regarded as major enteric pathogens of large ruminants, including cattle. Rotavirus vaccines administered to pregnant cows are commonly used to provide passive immunity that protects newborn calves from the clinical disease. In this study we report the detection of RVA from calves with severe diarrhea in a herd regularly vaccinated to prevent enteric infections including RVA. Diarrheic disease was observed in newborn calves aged 4–15 days, with high morbidity and mortality rates, but no diarrhea was seen in adult animals. Rotavirus antigen was detected by enzyme-immunoassay in the intestinal content or the fecal samples of all examined animals. Besides RVA, bovine coronavirus and bovine enteric calicivirus were detected in some samples. Selected RVA strains were characterized by whole genome sequencing. Two strains, RVA/Cow-wt/TUR/Amasya-1/2015/G8P[5] and RVA/Cow-wt/TUR/Amasya-2/2015/G8P[5] were genotyped as G8-P[5]-I2-R2-C2-M2-A3-N2-T6-E2-H3 and showed >99% nucleotide sequence identity among themselves. This genomic constellation is fairly common among bovine RVA strains; however, phylogenetic analysis of the G8 VP7 gene showed close genetic relationship to some European human RVA strains (up to 98.4% nt identity). Our findings is the first indication regarding the circulation of G8 RVA strains in Turkey. Given that the administered RVA vaccines contained type G6 and G10 VP7 antigens some concerns raised with regard to the level of heterotypic protection elicited by the vaccine strains against circulating bovine G8 RVA strains. Enhancement of surveillance of circulating RVA strains in calves across Turkey is needed to support ongoing vaccination programs.

1. Introduction

Neonatal diarrhea in calves is an important disease, which causes economic loss in the cattle industry due to high mortality and morbidity (Cho et al., 2013). There are various infectious agents which cause diarrhea and various factors including herd management, animal nutrition and environmental conditions affect the severity of the disease (Cho et al., 2013; Kaplon et al., 2013). Rotaviruses (RV) are one of the major causative agents of neonatal diarrhea in calves worldwide (Okada and Matsumoto, 2002; Alkan et al., 2010; Martella et al., 2010; da Silva Medeiros et al., 2015). Vaccines containing RV antigens are commercially available and commonly used to prevent RV diarrhea in young calves.

With a 11-segmented dsRNA genome that is enclosed in a triple-layered protein capsid RVs are members of the *Reoviridae* family (Lee et al., 2000; Reidy et al., 2006). Based on the genetic and antigenic characteristics of the inner capsid protein, VP6, RVs are divided into eight groups (or species; designated from A to H) (Matthijnssens et al., 2009) and new data suggest that a candidate ninth group can also be distinguished (Mihalov-Kovács et al., 2015). Group A rotaviruses (RVA) are the most commonly detected RV species in the gastrointestinal infection in cattle, although Group B and C rotaviruses are also regularly identified. RVAs are classified into G (glycoprotein) and P (protease sensitive) types according to the sequence diversity of the outer capsid proteins VP7 and VP4, respectively, which independently generate sero-type-specific neutralizing antibodies in vivo (Monini et al., 2008; Matthijnssens et al., 2011; Papp et al., 2013). Increased diversity in

* Corresponding author.
E-mail addresses: falkan@ankara.edu.tr, alkanferay@gmail.com (F. Alkan).

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RVA strains is generated principally by accumulation of point mutations and by reassortment of cognate genes. In addition, interspecies transmission is another important way to increase viral diversity in a host species (Papp et al., 2013). To date, at least 27 G and 37 P genotypes have been defined in mammals and avians (da Silva Medeiros et al., 2015). Among these, the most common G and P genotypes in cattle are G6, G8, G10 (for VP7) and P[1], P[5], P[11] P[15], and P[21] (for VP4) (Martella et al., 2010; Papp et al., 2013). More recently, the classification system of RVAs was extended to include the remaining capsid and non-structural protein genes; this new system denotes the VP7-VP4-VP6-VP1-VP2-VP3-NSP1-NSP2-NSP3-NSP4-NSP5/6 genes of a rotavirus strain by the descriptor Gx-P[x]-Ix-Rx-Cx-Mx-Ax-Nx-Tx-Ex-Hx, where x indicates the genotype number (Matthijnssens et al., 2011).

Determination of the genotype(s) of bovine RVA strains that causes the infection is amongst the most important factors for the implementation of effective vaccination to protect newborn calves from RVA infection and severe dehydrating diarrhea (Saif and Fernandez, 1996; Dulgheroff et al., 2012). Calves could be protected from diarrhea by appropriate management techniques and vaccination strategies. However, the potential of gene reassortment among various strains, the antigenic and genetic diversity between vaccine and field strains, the insufficient protection raised by vaccine strains against heterotypic strains and inappropriate vaccination strategy might be reasons for vaccine failure and thus remain a concern (Papp et al., 2013; da Silva Medeiros et al., 2015).

In Turkey, RVAs have been associated repeatedly with diarrhea in calves (Alkan et al., 2004, 2010; Can-Sahna and Alkan., 2003; Ozkul et al., 2002) and RVA-specific antibodies have been demonstrated in the sera of adult bovines (Alkan et al., 2004; Can-Sahna and Alkan., 2003). In a study on VP4 and VP7 diversity of the RVAs circulating in Turkey, RVAs isolated from diarrheic calves from several Turkish geographical areas between 1997 and 2008 (Alkan et al., 2010). In this study, the majority (40/53) of the RVAs were characterized as G6, followed by G10 (15/53), while G8 viruses were not detected. The most common VP4 type was P[11] followed by P[5]. Additionally, a large outbreak of enteric disease with high morbidity and mortality rates in young kids was reported as associated with the RVA strain (RVA/goat-tc/TUR/Kirklareli/2007/G8P[1]) characterized as G8P[1], with E2 NSP4 and VP6 I2 genotype (Alkan et al., 2012).

In this study, we detected and characterized RVA strains from a herd regularly vaccinated against RVA and found differences in the virion components between the vaccine strains and the field strains. This finding may have implications for specific prevention of RVA disease in cattle herds.

2. Material and methods

2.1. History of the herd and the outbreak

During January and February 2015, an outbreak of diarrhea occurred in newborn calves at age 4–15 days in a large Turkish cattle herd (no. of cows, 917). The outbreak was associated with high mortality among newborn calves born in January and February 2015 (14 of 73 calves died). In this herd a systematic RV vaccination program was carried on in the past 3 years. Due to economic reasons the management decided to change the vaccine type in 2014. 'Vaccine A' was used in 2012–2013 and contained two RVA genotypes, G6 and G10, (in addition to coronavirus, *Escherichia coli* having the K99 pili adherence factor and *Clostridium perfringens* type C), whereas 'vaccine B' contained a single RVA genotype, G6P[5], (in addition to coronavirus, *Escherichia coli* with K99 pilus type),

2.2. Diagnosis of the infection

In February, specimens from the diarrhea outbreak were obtained for diagnostic investigation. The intestinal content from a dead calf and four fecal samples from calves with diarrhea were tested for rotavirus, coronavirus, *E. coli* K99 antigens by using a commercial ELISA kit (IDEXX Rota-Corona-K99, Ag Test) according to manufacturer's instructions. For this reason, microplates coated with a mix of antibodies against 3 mentioned antigens were used. The faeces diluted (1/10) in dilution buffer were plated triplicate into the wells and incubated on the microplate for 30 min at room temperature (approximately 25 °C). After this first incubation step, the plate was washed by manually using the washing solution, and then each pathogen specific conjugates, peroxidase-labelled anti-pathogen monoclonal antibodies, were added to the related wells. The plate was then incubated for 30 min at room temperature again. Following the final wash, the chromogen (tetramethylbenzidine) was added to each well and the plate was held at room temperature away from light for 10 min. A stop solution was added and the optical densities were measured at 450 nm using an ELISA Reader. The ELISA reader optical density data was calculated according to the manufacturer's instruction. The samples were also tested for bovine enteric caliciviruses (BEC) by RT-PCR (Park et al., 2007, 2008).

2.3. Genotyping and genome sequencing

All ELISA positive samples were tested for possible RVA G genotypes by RT-PCRs using oligonucleotides specific for RVA G6 and G10 genotypes. (Iturizza-Gomara et al., 1999; Gouvea et al., 1990, 1994; Isegawa et al., 1993). Briefly, synthesis of cDNA was achieved following denaturation of RNA at 70 °C for 5 min. The cDNA was synthesized using Moloney Murine Leukemia Virus (MMLV) reverse transcriptase (RT) (Fermentas, Lithuania) and random hexamers (Fermentas, Lithuania), by incubating at 25 °C for 10 min, and thereafter at 37 °C for 1 h. MMLV-RT was therefore inactivated at 70 °C for 10 min (Iturizza-Gomara et al., 1999). Detection and characterization of BRV was performed as described elsewhere (Gouvea et al., 1990, 1994; Isegawa et al., 1993) with minor modifications using oligonucleotide primers designed by same researchers. The resulting amplicons were analyzed on 1.5% agarose gel after electrophoresis at 80 V for 30 min and visualized under ultraviolet light.

Following these laboratory diagnosis, two RVA strains were selected for whole-genome sequencing using the protocol from published studies (Dóró et al., 2014; Mihalov-Kovács et al., 2015). In brief, after nuclease treatment, viral RNA was extracted by using Direct-zol RNA Mini Prep Kit (Zymo Research) according to manufacturer's instructions. Random RT-PCR was carried out and then gel extraction of products was processed to start barcoded library preparation for Ion Torrent New Generation sequencing. Sequencing was carried out on a 316 chip using the 200 bp sequencing protocol. Raw sequencing data were evaluated by the CLC Genomics Workbench version 7 (CLC Bio-Qiagen, Aarhus, Denmark). By using a combination of de novo assembly and reference sequence mapping a single consensus sequence was obtained for all 11 viral gene segments of both bovine RVA strains, RVA/Cow-wt/TUR/Amasya-1/2015/G8[5] and RVA/Cow-wt/TUR/Amasya-2/2015/G8[5].

For each genome segment, multiple sequence alignments were prepared by the Muscle algorithm as implemented in Aliview Software (Edgar, 2004; Larsson, 2014). Cognate sequences of reference RVAs representing different genotypes for all genes were retrieved from GenBank through the Blast engine. Phylogenetic analyses of the full-length nucleotide sequences of the VP1-4, VP6, VP7 and NSP1-5 genes of the two field RVA strains, RVA/Cow-wt/