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Short Communication

Molecular characterization of equine rotaviruses isolated in Europe in 2013: Implications for vaccination

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ABSTRACT

Equine group A rotavirus (RVAs) mainly cause disease in foals under the age of 3 months. Only sporadic data are available on the circulation of RVAs in equine populations in Europe. In this study, 65 diarrheic samples from foals under 4 months of age were collected in Belgium ($n=32$), Germany ($n=17$), Slovenia ($n=5$), Sweden ($n=4$), Hungary ($n=3$), Italy ($n=2$), France ($n=1$) and The Netherlands ($n=1$). Forty percent of these samples ($n=26$) were found to be RVA positive by a quantitative RT-PCR assay. The viral load in 11 of these samples was sufficiently high to be (partially) genotyped. G3, G14 and P[12] were the main genotypes detected, and phylogenetic analyses revealed that they were closely related to contemporary equine RVA strains detected in Europe as well as in Brazil and South Africa. Regional variation was observed with only G14 and P[12] being detected in Germany, whereas mainly G3P[12] was encountered in Belgium. Surprisingly the only G14P[12] RVA strain detected in Belgium was also found to possess the very rare P[18] genotype, which has been described only once from equine RVA strain L338 detected in the UK in 1991. Despite the identification of this uncommon P[18] genotype, G3P[12] and G14P[12] RVA strains remained the most important genotypes in Europe during the study period. Based on this finding and the knowledge that G3P[12] and G14P[12] serotypes are partially cross-reactive it can be assumed that a vaccine based on an inactivated virus of the G3P[12] genotype is still relevant in the current European epidemiological situation, although the addition of a G14 strain would most likely be beneficial.

1. Introduction

Group A rotaviruses (RVAs) have been detected in horse populations around the world (Papp et al., 2013). Nowadays, classification of RVAs is performed almost exclusively by sequencing analyses of the gene segments encoding the VP7 and VP4 outer capsid proteins, and 27 G- and 37 P-genotypes have been recognized, respectively (Matthijnssens et al., 2011; Trojnar et al., 2013). To date, only genotypes G3P[12] and G14P[12] are considered to be of epidemiological importance in horses (Papp et al., 2013). Equine G3 RVA strains have been further subdivided into subtypes G3A and G3B based on their reactivity with mAbs (Browning et al., 1992). Interestingly, the subtype G3B seems to be restricted to Japan, whereas G3A seems to be prevalent in the rest of the world (Bailey et al., 2013).

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Equine RVAs mainly cause disease in animals below the age of 3 months, resulting in damage to the small intestine leading to severe watery diarrhea, which may lead to death (Imagawa et al., 1991; Sellon and Long, 2014). They cause significant economic losses because infected foals need to be quarantined from other foals and pregnant mares to avoid the further spread of the virus, which is a labor-intensive and costly event for horse-breeders (Ntafis et al., 2010; Papp et al., 2013). Besides good farm management, vaccination is currently the most widely used method to reduce the risk of disease caused by RVA in horse farms (Sellon and Long, 2014). Currently 3 inactivated vaccines are licensed globally and administered to pregnant mares. Vaccines based on the inactivated H-2 strain (G3AP[12]) are licensed in the US, New Zealand, Australia and several European countries (Zoetis Inc.). The vaccine licensed in Japan uses the HO-5 strain (G3BP[12]) (Nisseiken Co., Ltd). The third vaccine is licensed in Argentina and contains the equine RVA strain H-2, a simian G3P[2] RVA strain and the bovine G6P[1] RVA strain (Biogénesis Bagó).

The aim of this study was to investigate which equine RVAs genotypes circulated in 2013 in Europe and, hence, the relevance of the vaccine licensed in the European Union which contains an inactivated equine RVA strain of the G3AP[12] genotype.

2. Materials and methods

2.1. Sample collection

Local veterinarians in Belgium, France, Germany, Hungary, Italy, Slovenia, Sweden and The Netherlands were offered the opportunity to send diarrheic samples from sick foals under three months of age to a Belgian laboratory (Dierengezondheidszorg, Torhout) for RVA testing in 2013. Samples tested positive were subsequently sent to a second lab (Laboratory of Clinical and Epidemiological Virology, Rega Institute for Medical Research, KULeuven) for genotyping.

2.2. RVA detection (qPCR)

Viral RNA was extracted from the fecal samples using the QIAamp Viral RNA mini kit (Qiagen/Westburg, Leusden, The Netherlands) according to the manufacturer's instructions. The extracted RNA was stored at −60 °C or below prior and after analysis. To confirm presence or absence of RVA, the commercial kit TaqVet™ Triplex Ruminant Rotavirus & Coronavirus (Laboratoire Service International, Lissieu, France) which is suitable to detect equine RVAs was used. Based on the manufacturer declaration, the test is able to detect all subtypes by detecting the conserved NSP5 gene on genome segment 11, encoding a non-structural protein of RVAs. It has a limit of detection of 7 RVA RNA copies and was shown to be more sensitive than an antigen ELISA in a comparative study (Ramos et al., 2009).

2.3. RVA genotyping

The extracted RNA was denatured at 95 °C for 2 min. Reverse transcriptase PCR (RT-PCR) was carried out using the Qiagen OneStep RT-PCR Kit (Qiagen/Westburg) using VP7 primers Beg9 (5′-GGCTTTAAAAGAGAGAATTTCCGTCTGG-3′) and End9 (5′-GGTCACATCATACAATTCTAATCTAAG-3′), and the VP4 primers GEN_VP4_P12_10F (5′-TGGCTTCTCTTATTTACAGACAG-3′) and GEN_VP4_P12_2360R (5′-TCACATCCTTCAGAAGCTACTC-3′). For sample EQ44, also primers GEN_P[18]_44F (5′-ATTCTTACGCAGTAGACTTGTCAG- 3′) and GEN_P[18]_2362R (5′-GGTCACATCCTGCATAAGCTAC-3′) were used. The reaction was carried out with an initial reverse transcription step at 50 °C for 30 min, followed by PCR activation at 95 °C for 15 min, 35 cycles of amplification (30 s at 94 °C, 30 s at 50 °C for VP7 and 1 min at 50 °C for VP4, 1 min at 72 °C), and a final extension of 10 min at 72 °C in a Thermocycler Biometra T3000 (Biometra, Westburg BV, The Netherlands). PCR products (1062 bp, 2351 bp and 2319 bp, respectively) were run on a polyacrylamide gel, stained with ethidium bromide and visualized under UV-light. The PCR amplicons were purified using ExoSAP-IT (Affymetrix USB, Santa Clara, USA), and sequenced using the dideoxynucleotide chain termination method with the ABI PRISM® BigDye Terminator Cycle Sequencing Reaction kit (Perkin-Elmer Applied Biosystems, Foster City, CA) on an automated sequencer (ABI PRISMTM 3130). The primers Beg9, VP4-P12-10F and GEN_P[18]_44F described above were used as sequencing primers. The chromatogram sequencing files were inspected using the computer application Chromas 2.3 (Technelysium, Helensvale, Australia), and samples were genotyped using BLAST.

Table 1
Number of equine stool samples collected in different European countries, positivity rate by qRT-PCR targeting the NSP5 gene, and number of samples that were genotyped.

Country	qRT-PCR					RT-PCR/Sequence Positive	
	Total Number	POS		NEG		VP7	VP4
		Number	%	Number	%		
Belgium	32	15	46.9	17	53.1	4	4
Germany	17	7	41.2	10	58.8	4	5
France	1	0	0.0	1	100.0	0	0
Hungary	3	0	0.0	3	100.0	0	0
Italy	2	1	50.0	1	50.0	1	1
The Netherlands	1	0	0.0	1	100.0	0	0
Sweden	4	2	50.0	2	50.0	0	0
Slovenia	5	1	20.0	4	80.0	1	0
Total	65	26	40.0	39	60.0	10	10