Veterinary Microbiology 161 (2013) 239–246

Contents lists available at SciVerse ScienceDirect

Veterinary Microbiology

journal homepage: www.elsevier.com/locate/vetmic

Equine G3P[3] rotavirus strain E3198 related to simian RRV and feline/canine-like rotaviruses based on complete genome analyses

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ARTICLE INFO

Article history:
Received 1 June 2012
Received in revised form 10 July 2012
Accepted 19 July 2012

Keywords:
Equine rotavirus
Feline rotavirus
Canine rotavirus
G3
P[3]
Interspecies transmission

ABSTRACT

Equine group A rotavirus (RVA) strains are the most important cause of gastroenteritis in equine neonates and foals worldwide, and G3P[12] and G14P[12] are epidemiologically the most important genotypes. The genotype constellation of an unusual Argentinean G3P[3] RVA strain (RVA/Horse-wt/E3198/2008/G3P[3]) detected in fecal samples of a diarrheic foal in 2008 was shown to be G3–P[3]–I3–R3–C3–M3–A9–N3–T3–E3–H6. Each of these genotypes has been found typically in feline and canine RVA strains, and the genotype constellation is reminiscent to those of Cat97-like RVA strains. However, the phylogenetic analyses revealed only a distant relationship between E3198 and known feline, canine and feline/canine-like human RVA strains. Surprisingly, a rather close relationship was found between E3198 and simian RVA strains RVA/Simian-tc/USA/RRV/1975/G3P[3] for at least 5 gene segments. RRV is believed to be a reassortant between a bovine-like RVA strain and a RVA strains distantly related to feline/canine RVA strains. These analyses indicate that E3198 is unlikely to be of equine origin, and most likely represents a RVA interspecies transmitted virus, possibly in combination with one or more reassortments, from a feline, canine or related host species to a horse. Further studies are in progress to evaluate if this strain was a single interspecies transmission event, or if this strain started to circulate in the equine population.

1. Introduction

The genus *rotavirus* belongs to the family of *Reoviridae* (Ball, 2005), and is further divided into 8 groups (A–H) based on serological characterization of the inner capsid protein VP6 and recently also based on amino acid sequence comparisons of VP6 (Matthijnssens et al., 2012). The infectious group A rotavirus (RVA) virion is a non-enveloped, triple layered particle with an eleven-segmented, double-stranded RNA genome. Cumulatively, the viral genome encodes five or six nonstructural proteins (NSP1–NSP5, and sometimes, NSP6) and six structural proteins (VP1–VP4, VP6 and VP7) (Estes and Kapikian, 2007). The two outer capsid proteins, VP7 and VP4 are the basis for a widely used classification system defining G-types (glycosylated) and P-types (protease sensitive), respectively. To date, at least 27 G-genotypes and 35 P-genotypes have been described in the literature (Matthijnssens et al., 2011a). Currently, a uniform nomenclature and an extended classification system based on

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http://dx.doi.org/10.1016/j.vetmic.2012.07.033

nucleotide sequence identity for each of the 11 genome segments is used worldwide, and the acronym Gx–P[x]–Ix–Rx–Cx–Mx–Ax–Nx–Tx–Ex–Hx defines the genotypes of the VP7–VP4–VP6–VP1–VP2–VP3–NSP1–NSP2–NSP3–NSP4–NSP5/6 encoding gene segments (Matthijnssens et al., 2008a,b, 2011a). This new classification system has been proven very useful to study evolution, interspecies transmission and reassortments among a wide range of human and animal RVA strains (Bányai et al., 2009, 2010; Ciarlet et al., 2008; Martella et al., 2010, 2011; Matthijnssens et al., 2006, 2009, 2010a; McDonald et al., 2009; Mukherjee et al., 2011; Park et al., 2011).

Diarrhea is one of the most common health problems of newborn foals, and equine RVA strains are the most common cause of foal enteritis in major horse breeding farms. Both single cases of RVA diarrhea and severe diarrhea outbreaks affecting all the susceptible foals in the farm occur. However, with the use of a commercially available vaccine, farm management practices, and hygiene measures, this disease can be controlled (Barrandeguy et al., 1998; Dwyer, 2007; Estes and Kapikian, 2007; Powell et al., 1997). Worldwide surveys of equine RVA field samples have revealed that the equine RVA population consists mainly of G3 and G14 genotypes associated with genotype P[12] (Ciarlet et al., 1994; Collins et al., 2008; Elschner et al., 2005; Garaicoechea et al., 2011; Monini et al., 2011; Nemoto et al., 2011; Tsunemitsu et al., 2001; van der Heide et al., 2005). We recently described for the first time the complete genomes of 6 equine RVA strains with these prevalent G3P[12] and G14P[12] genotype combinations in addition to the complete genome of the uncommon equine G13P[18] RVA strains L338 (Matthijnssens et al., 2011c). A limited number of RVA strains with unusual G/P combinations have been detected in horses on single occasions, such as RVA/Horse-tc/GBR/H1/1975/G5P[7] shown to be of porcine origin (Ghosh et al., 2012), and RVA/Horse-tc/JPN/R-22/1984/G10P[11] and RVA/Horse-tc/GBR/26-94/199X/G8P[1] believed to be of bovine origin (Imagawa et al., 1994; Iša et al., 1996).

In Argentina, Garaicoechea and colleagues reported that RVA circulating in horses between 1992 and 2008 were quite homogeneous in their VP7, VP8* and NSP4 encoding gene segments (Garaicoechea et al., 2011). An apparent shift was described from G3 to G14 over the time, showing that from 1992 to 1999 G3 was the predominant genotype, whereas G14 became the predominant genotype in 2008. In the same study, the equine RVA sequences of the viral enterotoxin (NSP4) were classified in the unusual E12 genotype, which has up to date only been described in RVA strains detected in guanaco and cattle from Argentina (Matthijnssens et al., 2009). Furthermore, a single G3P[3] RVA strain (RVA/Horse-wt/ARG/E3198/2008/G3P[3]) was detected in this study. The G3P[3] genotype combination is typically found in feline and canine RVA strain and has sporadically also been detected in other hosts such as a simian, a goat, cattle and humans (Agnello et al., 2006; Ghosh et al., 2007; Grant et al., 2011; Kang et al., 2007; Lee et al., 2003; Matthijnssens et al., 2010b, 2011b; Oka et al., 2001; Tsugawa and Hoshino, 2008). In this paper we describe the full genome characterization of this unusual equine G3P[3] RVA strain E3198 and its phylogenetic relationship with other human and animal RVA strains.

2. Materials and methods

2.1. Virus

Equine RVA strain RVA/Horse-wt/E3198/2008/G3P[3] was detected from feces of a 3 day-old diarrheic foal on August 12th 2008. The foal was born from a vaccinated mare at a thoroughbred breeding farm in Buenos Aires province, Argentina.

2.2. Rotavirus diagnosis

The fecal sample was initially screened for the presence of RVA antigen by a commercial enzyme-linked immunoassay (Pathfinder Rotavirus, Bio-Rad, Marnes-la-Coquette, France) following the manufacturer's instructions.

2.3. RNA extraction

Viral RNA was extracted using the QIAamp viral RNA kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions.

2.4. Reverse transcription-PCR

The RT-PCRs and used primers were described previously: VP1, VP2 and VP3 (Tsugawa and Hoshino, 2008), VP6, NSP1–NSP5 (Matthijnssens et al., 2006), VP7 (Garaicoechea et al., 2011), and VP4 (GEN_P[3]_13F: 5′-GCTTCGCTCATTTATAGACAATTGC-3′ and GEN_P[3]_2361R: 5′-GTCACATCCTCTAGAAATTGCTTAC-3′) (Matthijnssens et al., 2011b). Briefly, the extracted RNA was denatured at 95 °C for 2 min before the RT-PCR was carried out using the Qiagen OneStep RT-PCR kit (QIAGEN) on a IVEMA T18 Thermocycler.

2.5. Nucleotide sequencing

The PCR products were purified with the MinElute Gel Extraction kit (QIAGEN) and sent for sequencing to the Unidad de Genómica, Instituto de Biotecnología, CICVyA, INTA, Castelar. The sequencing was performed with the same forward and reverse primers used for the RT-PCR. Primer walking sequencing was performed to cover the complete sequence of the respective fragments on both strands. The 5′ and 3′ ends were determined as described previously (Matthijnssens et al., 2006).

2.6. Nucleotide and protein sequence analysis

The chromatogram sequencing files were analyzed using Bioedit 7.0.9.0 Sequence Alignment Editor (Hall, T.A. 1999). Multiple sequence alignments were constructed using Muscle in MEGA5.0 (Tamura et al., 2011).

2.7. Phylogenetic analysis

Phylogenetic and molecular evolutionary analyses were conducted using MEGA5.0 (Tamura et al., 2011). Genetic