Veterinary Microbiology 172 (2014) 51–62

Contents lists available at ScienceDirect

Veterinary Microbiology

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

Comparison of pathogenicities and nucleotide changes between porcine and bovine reassortant rotavirus strains possessing the same genotype constellation in piglets and calves

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ARTICLE INFO

Article history:
Received 28 October 2013
Received in revised form 11 April 2014
Accepted 15 April 2014

Keywords:
RVA
Reassortant
Pathogenicity
Calves
Piglets

ABSTRACT

Although reassortment is one of the most important characteristics of group A rotavirus (RVA) evolution, the host range restriction and/or virulence of reassortant RVAs remain largely unknown. The porcine 174-1 strain isolated from a diarrheic piglet was identified as a reassortant strain, harboring the same genotype constellation as the previously characterized bovine strain KJ56-1. Owing to its same genotype constellation, the pathogenicity of the porcine strain 174-1 in piglets and calves was examined for comparison with that of the bovine reassortant KJ56-1 strain, whose pathogenicity has already been demonstrated in piglets and calves. The porcine 174-1 strain induced diarrhea and histopathological changes in the small intestine of piglets and calves, whereas KJ56-1 had been reported to be virulent only in piglets, but not in calves. Therefore, full genomic sequences of 174-1 and KJ56-1 strains were analyzed to determine whether specific mutations might be associated with clinical and pathological phenotypes. Sequence alignment between the 174-1 and KJ56-1 strains detected one nucleotide substitution at the 3′ untranslated region of the NSP3 gene and 16 amino acid substitutions at the VP7, VP4, VP1, VP3, NSP1 and NSP4 genes. These mutations may be critical molecular determinants for different virulence and/or pathogenicity of each strain. This study presents new insights into the host range restriction and/or virulence of RVAs.

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http://dx.doi.org/10.1016/j.vetmic.2014.04.010
0378-1135/

1. Introduction

Group A rotavirus (RVA), a member of the *Reoviridae* family, is one of the most important causes of severe dehydrating diarrhea in young children, as well as in a wide variety of young animals (Estes and Kapikian, 2007). The RVA is a non-enveloped virus containing eleven segments of double-stranded (ds) RNA which encode for six structural proteins (VP1-4, VP6-7) and six nonstructural proteins (NSP1-6) (Estes and Kapikian, 2007; Gentsch et al., 2005; Parashar et al., 2006). RVAs are classified into eight distinct groups (A to H), based on serology and sequence comparisons (Matthijnssens et al., 2012). Genetic reassortment can occur within each group, but not among viruses of different groups (Estes and Kapikian, 2007). As a result, reassortant progeny viruses may have novel or atypical phenotypes (Estes and Kapikian, 2007; Gentsch et al., 2005; Parashar et al., 2006).

Each genomic segment of RVA is classified into independent genotypes by the Rotavirus Classification Working Group (RCWG) based on nucleotide percentage identity cut-off values (Matthijnssens et al., 2008a, 2008b). Among the 11 genomic segments, the G genotype for the glycoprotein encoded by VP7 and the P genotype for the protease-sensitive encoded by VP4 are frequently used for RVA classifications, because of their high relevance to immune protection and vaccine development (Estes and Kapikian, 2007; Matthijnssens et al., 2010). Currently, 27 G and 37 P genotypes have been found from various countries with various combinations of G and P genotypes in humans and animals (Collins et al., 2010; Matthijnssens et al., 2011; Trojnar et al., 2013). In pigs, G3, G4, G5 and G11 genotypes in combination with two dominant P[6] and P[7] genotypes are the most common representative genotype combinations which have been identified to date (Gouvea et al., 1994; Papp et al., 2013; Winiarczyk et al., 2002). In calves, G6, G8, and G10 genotypes, together with P[1], P[5] and P[11] genotypes, are the major genotypes (Alfieri et al., 2004; Cashman et al., 2010; Chang et al., 1996; Papp et al., 2013).

Accumulating epidemiological data suggest that RVA can be transmitted from animal to human, as well as from animal to another animal, by the contribution of one or several genes to reassortants (Griffin et al., 2002; Martella et al., 2006; Palombo et al., 2000; Park et al., 2011; Pongsuwanna et al., 1996; Steyer et al., 2008). The G8 genotype, which is usually found predominantly in bovine species, was first isolated in children of Jakarta, Medan and Indonesia, and the occurrence of the G8 strain is continuously increasing, as depicted in a recent study (Santos and Hoshino, 2005). With various combinations of P types, G8 strains have emerged as important human RVA infection strains in parts of Africa (Estes and Kapikian, 2007; Santos and Hoshino, 2005; Gentsch et al., 2005), and are emerging strains in Iran (Kargar et al., 2012). However, it is still unclear which segments or specific domains (molecular basis of viral proteins) play key roles to determine host range restriction of RVAs or virulence. A few articles have proposed that six rotaviral genome segments (VP4, VP7, VP3, NSP1, NSP2 and NSP4) may affect host range restriction and virulence (Bridger et al., 1998; Broome et al., 1993; Burke and Desselberger, 1996; El-Attar et al., 2001; Hoshino et al., 1995; Kim et al., 2012; Kojima et al., 1996; Mori et al., 2003). Ample evidence has been provided which regardless of symptomatic or asymptomatic shedding of RVAs, some RVAs that were first isolated from specific hosts were also frequently isolated from other non-specific hosts (Ha et al., 2009; Matthijnssens et al., 2006; Tsugawa and Hoshino, 2008). These reports suggested that reassortant RVA strains can transmit naturally to another hosts (Ha et al., 2009; Matthijnssens et al., 2006). Nevertheless, there is only limited evidence that these reassortant RVA strains can replicate efficiently to induce diarrhea and cause pathology in the small intestine, which might occur through specific mutations in the genomic segments or changes in different domains.

Porcine and bovine RVAs are influential pathogens due to their significant economic impact on the livestock industry, as well as their high probability of transmission to humans (Kim et al., 2010; Matthijnssens et al., 2008a; Okitsu et al., 2011; Park et al., 2011). Molecular analyses have been performed on RVAs which were isolated from porcine and bovine diarrheic fecal samples collected across South Korea (Ha et al., 2009; Kim et al., 2010; Park et al., 2011). Among the isolated Korean RVAs strains, the G8P[7] genotype has been ranked as the most predominant genotype in Korean calves (Park et al., 2011), and as the second most important genotype in piglets (Kim et al., 2010). Among them, the bovine KJ56-1 strain isolated from diarrheic calves was characterized as a porcine and bovine reassortant G8P[7] strain (Park et al., 2011). A pathogenicity study of KJ56-1 showed that it can only induce diarrhea in piglets, but not in calves (Kim et al., 2012). This point prompted us to investigate the pathogenicity and full-genomic characteristics of the reassortant porcine G8P[7] 174-1 strain (Kim et al., 2010).

Previous studies have demonstrated that many RVAs can not only cause gastrointestinal tract infections, but were also detected in extra-intestinal organs (Blutt and Conner, 2007; Blutt et al., 2003; Kim et al., 2011, 2012). These data suggest that RVAs infect the gastrointestinal tract, and consequently cause viremia, in which infection can reach the extra-intestinal organs via the blood (Azevedo et al., 2005; Blutt and Conner, 2007; Blutt et al., 2003; Ciarlet et al., 2002; Crawford et al., 2006; Fenaux et al., 2006; Kim et al., 2011, 2012). This study determined whether the reassortant porcine strain 174-1 can efficiently infect, and induce diarrhea and pathology in the intestinal tracts and extra-intestinal organs and tissues of piglets and calves. For this experiment, major organs, feces and blood were sampled from experimental animals in order to analyze morphological and antigen distribution changes using an immunofluorescence assay, and viral RNA presence by RT-PCR and real-time RT-PCR. To obtain a better understanding of the potential consequences of viral genetic variations, infection characteristics between the porcine 174-1 and bovine