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Identification of the novel lapine rotavirus genotype P[22] from an outbreak of enteritis in a Hungarian rabbitry

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Abstract

Application of improved molecular techniques in the detection and characterization of rotavirus strains has led to the recent description of several new combinations, specificities, and genetic variants of the outer capsid genes, VP7 and VP4. In spite of the enormous diversity of mammalian rotavirus strains, the few lapine rotaviruses characterized to date, appear to carry a narrow range of such antigen combinations; only P[14], G3 and, based on a more recent study, P[22], G3 rotaviruses have proved to be epidemiologically important in rabbits. In the present study, we characterized a lapine group A rotavirus with a super-short electropherotype detected in an outbreak of fatal enteritis in a Hungarian commercial rabbitry. Based on sequence and phylogenetic analysis of the VP7, VP4, and NSP4 genes, our lapine strain is a P[22], G3 rotavirus that carries the NSP4 genotype shared by most lapine rotaviruses. Although the P[22] VP4 specificity has been newly identified, the relatively high sequence variation between our strain and those identified in Italy (89.1–90.4% nucleotide identity; region VP8*) implies that these strains diversified far before they were described for the first time, strongly suggesting that this genotype may have circulated in rabbitries or in nature without prior detection. We conclude that genotype P[22] lapine rotaviruses show a wider geographical dispersal than previously thought, although understanding their true epidemiological significance needs further investigation.

Keywords: Sequencing; Phylogenetic analysis; Molecular characterization; Diarrhea

1. Introduction

Group A rotaviruses, members of the genus *Rotavirus* within the family *Reoviridae*, are a major cause of gastroenteritis in young birds and mammals (Estes, 2001). The rotavirus genome consists of 11 segments of double-stranded RNA, encased in a triple-layered, round capsid carrying lollipop-like projections on the surface. The genome segments encode six structural (VP1–VP4, VP6 and VP7) and six non-structural (NSP1–NSP6) proteins (Estes, 2001). The antigenic/genetic diversity of group A rotavirus is accounted for by a broad variety of gene alleles. The dsRNA genome segments may be resolved by electrophoresis on polyacrylamide gel and distinct electropherotypes (E-types) may be distinguished by the relative gel migration profile of genome segments 10 and 11. Two main E-types – 'long' and 'short' – and a third, uncommon E-type, termed 'super-short' have been described (Estes, 2001). On the basis of VP6 antigenic specificity, four subgroups (sg), designated sg I, sg II, sg I + II and sg non I-non II, have been identified (Estes, 2001). Based on their antigenic/genetic variability, the outer capsid genes,

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VP7 and VP4, have been classified into 15 G-types and 23 P-types, respectively (Santos and Hoshino, 2005). In addition, sequence comparison and phylogenetic analyses have demonstrated the existence of at least six NSP4 genotypes, designated as A–F (Mori et al., 2002). Epidemiological studies frequently utilize a combination of these features and typing methods to characterize individual rotavirus strains and to analyze unusual rotaviruses that may have arisen through reassortment.

Although rotaviruses seem to display a weak host range restriction, certain specificities appear to be associated only with a single, or narrow, well-defined spectrum of host species. For example, the G14 VP7 serotype has been described so far only in horses; the P[13] VP4 serotype is thought to occur primarily in pigs; the NSP4 genotype D has been observed only in murine strains. The serotype G3 VP7 gene, instead, is common in a broad range of mammals, including primates, rodents, rabbits, ruminants, and carnivores (Estes, 2001). As more and more epidemiologic and sequence data become available for rotavirus strains of yet under-represented host species, many of the above mentioned single-host specificities will most likely be found to occur in other species as well.

Group A rotaviruses cause severe loss in herds of domesticated animals, including livestock and poultry. In addition to their economic impact, several animal rotaviruses are thought to be a potential source of human infection; this likelihood has led to intensify epidemiological surveillance and detailed molecular characterization of certain strains. In spite of the potential economic impact of rabbit rotaviruses, only a few strains have been selected for characterization in Canada, the United States, Japan, and Italy (Tanaka et al., 1988; Sato et al., 1982; Thouless et al., 1986; Ciarlet et al., 1997; Martella et al., 2003). The first reports on the occurrence of lapine rotaviruses in Hungary were published in the 1980s (Kudron et al., 1982; Vetési et al., 1982; Nagy et al., 1988). Some of those strains were characterized by genome profiling and all were categorized as ‘long’ E-type strains (Szücs and Nagy, unpublished data).

Our research group is currently setting up a surveillance system to screen and analyze animal rotavirus strains in Hungary. In February 2004, a number of lethal enteritis cases were reported at a Hungarian rabbitry. Molecular characterization of the rotavirus strain detected in the intestinal content of a dead rabbit is described in the present study.

2. Materials and methods

2.1. Cases and laboratory diagnosis

In February 2004, 60 young rabbits died of acute enteritis in a flock of 5400. Three 7-week-old rabbits that had died of enteritis were sent to the Central Veterinary Institute for post-mortem examination, where routine bacterial examination of the intestinal content was also done. *Klebsiella* spp. and *Escherichia coli* with unassigned serotype were grown from all three samples. Virological examination included detection of rotaviruses by polyacrylamide gel electrophoresis of the extracted RNA, using methods and algorithm described earlier (Bányai et al., 2003, 2004). Briefly, total RNA of the intestinal content was extracted with the TRIZOL reagent (Invitrogen) according to the manufacturer’s recommendation. The extracted RNAs were subsequently run in 10% polyacrylamide gel using Laemmli’s discontinuous buffer system (Laemmli, 1970) without sodium-dodecylsulfate and then stained with silver-nitrate, as described by Dolan et al. (1985). The rotavirus genome was identified in one of the three samples.

2.2. Sequencing and phylogenetic analysis

The genes encoding outer capsid antigens, VP7 and VP4, and the non-structural enterotoxigenic protein, NSP4, were sequenced with the BigDye Cycle Termination kit v1.1 (Applied Biosystems) following reverse transcription-polymerase chain reaction and gel extraction (QIAquick; QIAGEN) of the resulting amplicons. The amplicons were generated with primers, described earlier for the VP7 and VP4 genes (Gouvea et al., 1990; Gentsch et al., 1992; Das et al., 1994), and with NSP4-specific primers designed on the basis of an alignment made from a subset of available NSP4 nucleotide sequences of mammalian rotavirus strains (nt 8–27 (+ sense), 5′-AAA AGT TCT GTT CCG AGA GA-3′; nt 719–738 (– sense), 5′-CCR TTC CTT CCA TTA ACG TC-3′). The same primers were used in the cycle sequencing reaction. All three genes were amplified and directly sequenced in duplicates. Overlapping sequences were constructed with the GeneDoc software (Nicholas et al., 1997), aligned with reference sequences, using the Multalin program (Corpet, 1988), and then subjected to phylogenetic analysis using the neighbor-joining algorithm and bootstrap analysis with the MEGA2 software (Kumar et al., 2001).

2.3. GenBank accession numbers

Partial sequences of the VP7, VP4 and NSP4 sequences of the rotavirus strain, designated 3489/3, were deposited in GenBank under the accession numbers AJ871481–AJ871483.

3. Results

3.1. Determination of the electropherotype

The rotavirus strain displayed a genome pattern that was similar to that of conventional group A rotavirus strains (4–2–3–2) but with some slight difference. The genome segments migrated in a 4–2–4–1 pattern (data not shown) that virtually corresponds to a ‘super-short’ group A rotavirus RNA profile.