

TITLE

MOLECULAR CHARACTERIZATION OF ROTAVIRUS B AND ROTAVIRUS C STRAINS ISOLATED FROM NEONATAL DIARRHEA OUTBREAKS IN CATALONIA.

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CONTENT

Background and objectives

Rotaviruses B (RVB) and C (RVC) have been reported to cause neonatal diarrhea worldwide. Nevertheless, there are few reports concerning the molecular characterization of porcine isolates in Europe. The aim of this study was to deep-sequence and characterize seven positive samples to RVB and six to RVC in order to obtain their complete genome sequences and compare the genetic diversity with the published sequences available.

Materials and methods

Diarrheic and non-diarrheic samples from 31 farms suffering outbreaks of neonatal diarrhea were analyzed for a panel of enteric pathogens. Seven positive samples to RVB and six to RVC showing, by qRT-PCR, viral loads high enough to perform a next-generation sequencing (NGS) analysis (Ct values below 25), were selected. Total RNA was extracted using Trizol™ reagent and deep sequenced using the Illumina platform. Considering the segmented nature of the Rotavirus genome, the outputs were filtered gene by gene against a reference sequence, and a consensus sequence was generated for every genome fragment (VP1, VP2, VP3, VP4, VP6, VP7, NSP1, NSP2, NSP3, NSP4 and NSP5).

Results and Discussion

The NGS protocol was successfully applied to all samples. Complete genome sequences were obtained for seven RVB and six RVC strains. A salient feature was the great diversity observed among strains and, especially for RVB, the great differences reported with the genomes available at GenBank. For some genes, the highest nucleotide identities reported were below 80%. Actually, the strains characterized in this work might represent new RVB genotypes applying the nucleotide and aminoacidic thresholds published for several genome fragments.

Conclusion

Altogether, the results highlight the huge amount of hidden diversity not described for several species of Rotavirus.