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GENOME ANNOUNCEMENT

Complete Genome Sequence of a Novel Bovine Norovirus: Evidence for Slow Genetic Evolution in Genogroup III Genotype 2 Noroviruses

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A new genogroup III genotype 2 bovine norovirus, B309/2003/BE, was entirely sequenced and genetically compared to the original Newbury2/1976/UK strain and to Dumfries/1994/UK, detected in 1976 and 1994, respectively. Interestingly, except in well-defined coding regions (N-terminal protein, 3A-like protease, hypervariable region of the capsid protein, and C-terminal part of the minor structural protein), very low genetic differences were noted between the entire genomes of these three strains along a 30-year-long period. It allowed some hypotheses of hotspots of genetic evolution through a low genetic evolution background in genotype 2 genogroup III bovine noroviruses.

Noroviruses (NoVs), belonging to the family *Caliciviridae* and genus *Norovirus*, are major agents of acute nonbacterial gastroenteritis in humans, and genetically related viruses were also detected in stool samples from cattle. Five genogroups (G) are currently described in the genus *Norovirus*, and within genogroups, sequences are further distributed into genotypes following genetic homology and phylogenetic relationships. All bovine noroviruses (BoNoVs) clustered into genogroup III (GIII), and to date, only one genotype 1 (GIII.1) (Jena/1980/DE) and two genotype 2 (GIII.2) (Newbury2/1976/UK, Dumfries/1994/UK) BoNoV genomes were entirely sequenced. Three open reading frames (ORFs) were described in the NoV genome: ORF1 encodes a polyprotein further processed in 6 nonstructural proteins (N-terminal protein, NTPase, 3A-like protein, viral genome-linked protein [VPg], 3C-like proteinase, polymerase), ORF2 encodes the single capsid protein, and ORF3 encodes a minor structural protein.

During a diagnosis study of BoNoV infection by reverse transcription-PCR (RT-PCR), the third complete GIII.2 sequence was obtained from a calf stool specimen, allowing interesting comparisons with previously sequenced reference strains.

Viral RNA extraction was performed with the QIAamp viral RNA minikit (Qiagen) on centrifuged, 10% stool specimen suspensions in phosphate-buffered saline. Reverse transcription was conducted with the SuperScript III reverse transcriptase (Invitrogen). The entire sequencing of the strain was undertaken by generating long, high-fidelity PCR fragments covering the end of the polymerase gene until the poly(A) tail with the primer pair CBecUF (targeting the conserved motif of the polymerase gene [4]) and TVN-linker (3) by primer walking and by a 5′ rapid amplification of cDNA ends (RACE) system (Invitrogen) for the 5′ end of the genome. PCR products were purified with the QIAquick gel extraction kit (Qiagen) and cloned into the Topo Zero Blunt plasmid (Invitrogen). Sequencing reactions on at least three clones were performed at the GIGA facilities of the University of Liège. The entire genome of B309/2003/BE (7,317 bp) was obtained from assembled sequences. By BLAST, it was found to have high nucleotide homology with GIII.2 BoNoV (89.7% nucleotide identity with Newbury2/1976/UK), with the same genome organization. Divergence in nucleotide and amino acid sequences based on the capsid region (ORF2), the region most exposed to herd immunity, was 10.6% and 1.6%, respectively, with Newbury2/1976/UK during an interval longer than 20 years. Furthermore, a similarity plot (Simplot software version 3.5.1) showed very low genetic differences between B309/2003/BE, Newbury2/1976/UK, and Dumfries/1994/UK along their entire nucleotide sequences despite a 30-year-long evolution period (each strain was separated from the other one by 10 years), allowing hypotheses of lower genetic evolution than that already recorded for some human norovirus (HuNoV) strains (1, 2). However, four genomic regions showed higher genetic divergence: the N-terminal part of ORF1, the region of nucleotides 2250 to 2500, corresponding to the 3A-like protein-coding sequence, the C-terminal part of ORF2 (hypervariable capsid coding region), and the C-terminal part of the ORF3. These genomic regions may represent high spots for mutations and be particularly submitted to mechanisms that drive evolution in the BoNoV genomes, mutations under positive and negative selection due to the innate and adaptive immunities, fitness, and virulence modifications.

Nucleotide sequence accession number. The B309/2003/BE sequence was submitted to GenBank under accession number EU794907.

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