Full-Length Genomic Sequence of Bovine Coronavirus (31kb)

Completion of the open reading frame 1a/1b sequences

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1. INTRODUCTION

Bovine coronavirus (BCV) is an important veterinary pathogen which causes neonatal diarrhea in newborn calves and winter dysentery in adult cattle. Recent studies indicate that BCV also infects the respiratory tract of cattle producing severe respiratory problems, especially in feedlot cattle. BCV belongs to the antigenic group II of coronaviruses and shares the antigenic and genetic similarities with mouse hepatitis virus (MHV), human coronavirus strain OC43, turkey coronavirus, and hemagglutinating encephalomyelitis virus of pigs. Approximately 10 kb of the 3' most BCV genome has been sequenced, and this region is known to encode all the structural proteins plus the 32k nonstructural protein which resides immediately upstream of the hemagglutinin-esterase (HE) glycoprotein gene. The comparative studies indicate that the genome organization of BCV is similar to but distinct from MHV. In BCV, the HE gene is functional coding for a major envelop protein while it is an optional gene in MHV. Similarly, the 32k protein gene is absent in some of the MHV variants. The region between the spike (S) protein gene and the small membrane (E) protein gene also shows a significant sequence divergence among coronaviruses. Two thirds of the BCV genome remain largely undetermined.

To date, coronaviruses of which the full length genomic sequences are available include mouse hepatitis virus (MHV) (Bredenbeek et al, 1990; Lee et al, 1991; Bonilla et al, 1994), human coronavirus 229E (Herold et al, 1992; The Nidoviruses (Coronaviruses and Arteriviruses). Edited by Ehud Lavi et al. Kluwer Academic/Plenum Publishers. 2001.)
1994), porcine transmissible gastroenteritis virus (TGEV) (Almazan et al., 2000), and avian infectious bronchitis virus (IBV) (Boursnell et al. 1987). Of these viruses, human coronavirus 229E and TGEV fall within the same antigenic group (group I), and IBV is a group III virus. In the antigenic group II which BCV belongs to, MHV is the only virus that the full-length sequence has been determined. My laboratory has recently completed sequencing of the entire genome of BCV, and in this report we present the structural organization of the BCV genome.

2. MATERIALS AND METHODS

2.1 cDNA Cloning

The Quebec strain of BCV was propagated in Mardin-Darby bovine kidney (MDBK) cells. Genomic RNA was prepared from the purified virions, and double stranded cDNA was synthesized by standard methods. After addition of Bam HI linkers, cDNAs were ligated into the Bam HI site of pTZ19R and used transform E. coli strain DH1. Specific clones were identified by colony hybridization using a $^{32}$P-labeled cDNA probe prepared by randomly primed reverse transcription of viral genomic RNA or by PCR. For RT-PCR, total cellular RNA was extracted from virus-infected cells using Trizol and used as a template for cDNA synthesis.

2.2 Reverse Transcription Polymerase Chain Reaction

For first-stranded cDNA synthesis, Superscript II RNase H Negative Reverse Transcriptase (GIBCO BRL) was used. The first strand cDNA was PCR-amplified using Vent DNA polymerase (New England Biolab) in GeneAmp Thermocycler PE2400 (Perkin Elmer).

2.3 DNA Sequencing

The nucleotide sequences were determined either by manual sequencing or BY using an automated DNA sequencer. For manual sequencing, a series of overlapping clones were generated and the overlapping clones were sequenced using universal primers. For automated sequencing, new primers were generated based on the determined sequence to walk along the unsequenced region. Sequences were assembled and analyzed using the GCG Wisconsin sequence analysis package. The sequence has been deposited to the GenBank database under accession number AF220295.