




Complete Genome Sequences of Four Bovine Coronavirus Isolates from Pennsylvania

Maurice Byukusenge,^a Ruth Helmus Nissly,^a Sunitha Manjari Kasibhatla,^{c,d} Lingling Li,^a Rebekah Russell,^b Hayley Springer,^b Rhiannon Barry,^a Robert Van Saun,^b David Wolfgang,^{b*} Ernest Hovingh,^b Urmila Kulkarni-Kale,^c  Suresh V. Kuchipudi^a

^aAnimal Diagnostic Laboratory, Department of Veterinary and Biomedical Sciences, The Pennsylvania State University, University Park, Pennsylvania, USA

^bDepartment of Veterinary and Biomedical Sciences, The Pennsylvania State University, University Park, Pennsylvania, USA

^cBioinformatics Centre, INDI, Pune, India

^dHPC-Medical & Bioinformatics Group, Centre for Development of Advanced Computing, Savitribai Phule Pune University, Pune, India

ABSTRACT We report four full-genome sequences of bovine coronavirus (BCoV) isolates from dairy calves in Pennsylvania obtained in 2016 and 2017. BCoV is a pathogen of great importance to cattle health, and this is the first report of full-genome sequences of BCoV from PA cattle.

Bovine coronavirus (BCoV), a member of the *Betacoronavirus* genus of the *Coronaviridae* family, is an enveloped virus with an approximately 31-kb single-stranded positive-sense RNA genome. Infection of cattle with bovine coronavirus is a major contributor to diarrhea in calves, is involved in the etiology of winter dysentery in adult cattle, and serves as a contributing pathogen in bovine respiratory disease complex (1, 2).

Coronaviruses are difficult to cultivate in the laboratory, and only 17 full-genome sequences of unique BCoV isolates are publicly available in GenBank (3). We generated full-genome sequences of four BCoV isolates, 7-16-23, 4-17-03, 4-17-08, and 4-17-25, recovered from fecal samples of 3- to 14-day-old dairy calves in July 2016 and April 2017. Fecal samples were screened for presence of BCoV genetic material by real-time reverse transcription-PCR (qRT-PCR), as described previously (4). The study has been approved by the Pennsylvania State University Institutional Animal Care and Use Committee (IACUC protocol no. 46948). Fecal extracts from positive samples were cultured on HRT-18G cells, and after 3 to 5 days, culture medium samples were rescreened by qRT-PCR. Libraries were prepared from viral RNA extracted from positive-culture media using the TruSeq stranded mRNA kit without the poly(A) selection step. Genetic material was subjected to whole-genome sequencing using a 150-nucleotide (nt) read-length single-read approach on the Illumina MiSeq platform. Sequence files were assembled with the BCoV Mebus strain (GenBank accession number U00735) as a reference using SeqMan NGen build 15.0.1.1.

The four sequences generated contained 31,016 to 31,032 nucleotides, with 99.36% nucleotide identity between isolates. Following alignment of full-genome nucleotide sequences by the MUSCLE algorithm implemented in the MEGA7 package (5, 6), phylogenetic analysis indicated that the four isolates were closely related to one another and shared 99% coverage and 99% identity with their closest relative, sable antelope coronavirus US/OH1/2003 (GenBank accession number EF424621). Other closely related sequences were those of isolates from diverse cattle and captive

Received 23 April 2018 Accepted 29 April 2018 Published 31 May 2018

Citation Byukusenge M, Nissly RH, Kasibhatla SM, Li L, Russell R, Springer H, Barry R, Van Saun R, Wolfgang D, Hovingh E, Kulkarni-Kale U, Kuchipudi SV. 2018. Complete genome sequences of four bovine coronavirus isolates from Pennsylvania. *Genome Announc* 6:e00467-18. <https://doi.org/10.1128/genomeA.00467-18>.

Copyright © 2018 Byukusenge et al. This is an open-access article distributed under the terms of the [Creative Commons Attribution 4.0 International license](https://creativecommons.org/licenses/by/4.0/).

Address correspondence to Suresh V. Kuchipudi, skuchipudi@psu.edu.

* Present address: David Wolfgang, Pennsylvania Department of Agriculture, Bureau of Animal Health and Diagnostic Services, Harrisburg, Pennsylvania, USA. M.B. and R.H.N. contributed equally to this work.

ruminants obtained in Ohio, a neighboring state with a border approximately 270 km away from the collection sites of these samples.

The ORF1ab, HE, S, NS5, E, M, and N genes of the four sequences were subjected to BLASTN analysis (7, 8). According to these analyses, the ORF1ab and HE genes of the four isolates were closely related to the strain calf-giraffe coronavirus US/OH3/2006 (GenBank accession number EF424624), with >99% nucleotide identity. Similarly, the S, E, and M genes from all four isolates had >99% nucleotide identity with sequences with GenBank accession numbers HE616738 (strain VB 7/09/MAYABEQUE/2009), KX982264 (strain BCoV_2014_13), and EF424615 (strain E-AH65), respectively. Of interest, the first codon position of the E gene of 4-17-25 contained a point mutation shifting ATG to ACG. The N gene sequence was similar to that of BCoV strain E-AH65 in most of the isolates, but that of 4-17-08 shared >99% nucleotide identity with that of the giraffe coronavirus US/OH3/2003 strain (GenBank accession number EF424623).

Accession number(s). The complete genome sequences of the BCoV isolates 4-17-03, 4-17-25, 4-17-08, and 7-16-23 have been deposited in GenBank under the accession numbers [MH043952](#) to [MH043955](#).

ACKNOWLEDGMENTS

This work was funded by the Pennsylvania Soybean Board under grants R2016-P02 and R2017-P03.

We thank Craig Praul, Kerry Hair, and the staff of the Penn State Genomics Core Facility for library preparation and whole-genome sequencing services.

REFERENCES

1. Hasoksuz M, Hoet AE, Loerch SC, Wittum TE, Nielsen PR, Saif LJ. 2002. Detection of respiratory and enteric shedding of bovine coronaviruses in cattle in an Ohio feedlot. *J Vet Diagn Invest* 14:308–313. <https://doi.org/10.1177/104063870201400406>.
2. Park SJ, Kim GY, Choy HE, Hong YJ, Saif LJ, Jeong JH, Park SI, Kim HH, Kim SK, Shin SS, Kang MI, Cho KO. 2007. Dual enteric and respiratory tropisms of winter dysentery bovine coronavirus in calves. *Arch Virol* 152: 1885–1900. <https://doi.org/10.1007/s00705-007-1005-2>.
3. Benson DA, Cavanaugh M, Clark K, Karsch-Mizrachi I, Lipman DJ, Ostell J, Sayers EW. 2013. GenBank. *Nucleic Acids Res* 41:D36–D42. <https://doi.org/10.1093/nar/gks1195>.
4. Cho Y-I, Kim W-I, Liu S, Kinyon JM, Yoon KJ. 2010. Development of a panel of multiplex real-time polymerase chain reaction assays for simultaneous detection of major agents causing calf diarrhea in feces. *J Vet Diagn Invest* 22:509–517. <https://doi.org/10.1177/104063871002200403>.
5. Kumar S, Stecher G, Tamura K. 2016. MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. *Mol Biol Evol* 33: 1870–1874. <https://doi.org/10.1093/molbev/msw054>.
6. Edgar RC. 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Res* 32:1792–1797. <https://doi.org/10.1093/nar/gkh340>.
7. Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. 1990. Basic local alignment search tool. *J Mol Biol* 215:403–410. [https://doi.org/10.1016/S0022-2836\(05\)80360-2](https://doi.org/10.1016/S0022-2836(05)80360-2).
8. Altschul SF, Madden TL, Schäffer AA, Zhang J, Zhang Z, Miller W, Lipman DJ. 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res* 25:3389–3402. <https://doi.org/10.1093/nar/25.17.3389>.