AMERICAN SOCIETY FOR MICROBIOLOGY | Microbiology Resource Announcements

GENOME SEQUENCES

Genome of Bovine Viral Diarrhea Virus (BVDV) Contaminating a Continuous LFBK-$\alpha_V\beta_6$ Cell Line

Lauren G. Holinka-Patterson,[a] Ian H. Fish,[a,b] Miranda R. Bertram,[a] Ethan J. Hartwig,[a] George R. Smoliga,[a] Carolina Stenfeldt,[a] Luis L. Rodriguez,[a] Jonathan Arzt[a]

[a]Foreign Animal Disease Research Unit, Plum Island Animal Disease Center, ARS, USDA, Orient Point, New York, USA
[b]Oak Ridge Institute for Science and Education, PIADC Research Participation Program, Oak Ridge, Tennessee, USA

ABSTRACT Here, we report the genome of bovine viral diarrhea virus 1 (BVDV-1) contaminating a continuous fetal bovine kidney cell line. The cell line (LFBK-$\alpha_V\beta_6$) is used for the rapid isolation and serotyping of foot-and-mouth disease virus (FMDV). The sequence contains the full polyprotein-coding sequence and partial untranslated regions (UTRs).

An immortalized line of fetal bovine kidney cells (LFBK-$\alpha_V\beta_6$) (1, 2) which was engineered to express the bovine integrins that serve as receptors for foot-and-mouth disease virus (FMDV) has been utilized extensively because of its enhanced sensitivity for virus isolation (VI) of FMDV. Bovine viral diarrhea virus 1 (BVDV-1) was inadvertently found as a contaminant of the LFBK-$\alpha_V\beta_6$ cell line (3) during virus isolation and next-generation sequencing of FMDV samples. BVDV-1 (*Pestivirus*, *Flaviviridae*) is an enveloped, positive-sense, single-stranded RNA virus with a 12.3-kb genome. BVDV-1 causes a highly infectious disease with major economic losses to the cattle industry worldwide. The virus exists in cytopathic (cp) and noncytopathic (ncp) biotypes, designated by its biological activity in cultured cells (4). Several cell lines have been reported to be persistently infected with ncpBVDV (5–8).

A total of 152 individual clinical samples collected from field FMDV-infected animals in Vietnam and India as well as from experimentally FMDV-infected cattle in our laboratory were subjected to virus isolation (VI) utilizing LFBK-$\alpha_V\beta_6$ cells. All 152 VI-supernatant RNAs were deep-sequenced as previously described, with uninfected LFBK-$\alpha_V\beta_6$ cells used for RNA extraction control (9, 10). Briefly, RNA was extracted using the MagMAX total RNA isolation kit, and host DNA was depleted using the DNA-free DNase kit (Ambion). RNA underwent first-strand synthesis using the Superscript II first-strand synthesis system (Invitrogen) coupled with random primers and two FMDV-specific primers (11). Double-stranded cDNA was generated using the NEBNext Ultra nondirectional RNA second-strand synthesis module and sequenced as previously described (10) using the Nextera XT kit on a NextSeq 500 platform with 150-cycle paired-end reads. There were 4,978,000 total reads and an average read length of 138.2 nt. All analyses were performed in CLC Genomics Workbench v21.0. Paired reads were quality trimmed using default parameters. Trimmed reads were *de novo* assembled, and a BLASTn search of the resulting contigs revealed the presence of BVDV in all 152 samples as well as in uninfected cells. Reads were then mapped to the nearest full-length genome, BVDV strain Oregon C24V (GenBank accession number AF091605) to obtain the near-full-length coding sequence of BVDV-1. Nucleotide consensus sequences were extracted using default parameters and a threshold of 50. All nucleotide sequences were >99% identical, and sequences were most similar (93.2% pairwise identity) to the published BVDV strain Oregon C24V (GenBank number AF091605). One representative sequence, designated ncpBVDV LFBK-$\alpha_V\beta_6$ [PI], was

Editor Simon Roux, DOE Joint Genome Institute

Address correspondence to Jonathan Arzt, Jonathan.Arzt@usda.gov.

The authors declare no conflict of interest.

Received 9 December 2021
Accepted 13 January 2022
Published 3 February 2022

chosen based on highest overall read coverage with 28,433 total mapped reads and a minimum coverage of 166 reads and maximum coverage of 575 reads per site.

The 12,166-nucleotide (nt) nearly complete genome contains an 11,697-nt open reading frame (ORF) (nt 362 to nt 12055) flanked by a 361-nt 5′ untranslated region (UTR) and a 108-nt 3′ UTR. The ncpBVDV LFBK-$\alpha_V\beta_6$ [PI] strain had 93.4% to 93.0% identity with five strains of BVDV-1a (GenBank accession numbers HQ174292.1, JN380080.1, HQ174293.1, MK509773.1, and MH490942.1) and 84.1% to 80.4% identity with 28 strains of BVDV-1b at the whole-genome level. The closest homology was with an isolate from a persistently infected calf in Alabama (HQ174292) (12). Partial sequences of BVDV with homology to this isolate have previously been identified in 5 experiments in our laboratory using the LFBK-$\alpha_V\beta_6$ cell line.

Characterization of the adventitious BVDV-1 in LFBK-$\alpha_V\beta_6$ cells is important for continued use and contaminant cleanup of this cell line. The genome reported here will enable distinguishing the adventitious BVDV strain from field strains in VI samples.

Data availability. The genome nucleotide sequence has been deposited in GenBank under accession number MZ442301. The raw sequence data are available in the NCBI Sequence Read Archive under SRA accession number SRX11379699.

ACKNOWLEDGMENTS

This research was funded in part by ARS-CRIS project 1940-32000-061-00D. I.H.F. was the recipient of a Plum Island Animal Disease Center Research Participation Program fellowship, administered by the Oak Ridge Institute for Science and Education (ORISE) through an interagency agreement between the U.S. Department of Energy (DOE) and the U.S. Department of Agriculture (USDA). All opinions expressed in this paper are the authors' and do not necessarily reflect the policies and views of the USDA, DOE, or ORISE.

REFERENCES

1. LaRocco M, Krug PW, Kramer E, Ahmed Z, Pacheco JM, Duque H, Baxt B, Rodriguez LL. 2013. A continuous bovine kidney cell line constitutively expressing bovine $\alpha V\beta 6$ integrin has increased susceptibility to foot-and-mouth disease virus. J Clin Microbiol 51:1714–1720. https://doi.org/10.1128/JCM.03370-12.
2. LaRocco M, Krug PW, Kramer E, Ahmed Z, Pacheco JM, Duque H, Baxt B, Rodriguez LL. 2015. Correction for LaRocco et al.: a continuous bovine kidney cell line constitutively expressing bovine $\alpha V\beta 6$ integrin has increased susceptibility to foot-and-mouth disease virus. J Clin Microbiol 53:755. https://doi.org/10.1128/JCM.03220-14.
3. LaRocco M, Ahmed Z, Rodriguez-Calzada M, Azzinaro PA, Barrette R, Krug P, Rodriguez LL, de los Santos T, Medina GN. 2021. An adventitious agent-free clonal cell line that is highly susceptible to foot -and-mouth disease virus. Biologicals 72:33–41. https://doi.org/10.1016/j.biologicals.2021.05.003.
4. Fulton RW, Saliki JT, Confer AW, Burge LJ, d'Offay JM, Helman RG, Bolin SR, Ridpath JF, Payton ME. 2000. Bovine viral diarrhea virus cytopathic and noncytopathic biotypes and type 1 and 2 genotypes in diagnostic laboratory accessions: clinical and necropsy samples from cattle. J Vet Diagn Invest 12:33–38. https://doi.org/10.1177/104063870001200106.
5. Nuttall PA, Luther PD, Stott EJ. 1977. Viral contamination of bovine foetal serum and cell cultures. Nature 266:835–837. https://doi.org/10.1038/266835a0.
6. Bolin SR, Matthews PJ, Ridpath JF. 1991. Methods for detection and frequency of contamination of fetal calf serum with bovine viral diarrhea virus and antibodies against bovine viral diarrhea virus. J Vet Diagn Invest 3:199–203. https://doi.org/10.1177/104063879100300302.
7. Levings RL, Wessman SJ. 1991. Bovine viral diarrhea virus contamination of nutrient serum, cell cultures and viral vaccines. Dev Biol Stand 75:177–181.
8. Dezengrini R, Weiblen R, Flores EF. 2006. Selection and characterization of canine, swine and rabbit cell lines resistant to bovine viral diarrhea virus. J Virol Methods 137:51–57. https://doi.org/10.1016/j.jviromet.2006.05.032.
9. Palinski RM, Bertram MR, Vu LT, Pauszek SJ, Hartwig EJ, Smoliga GR, Stenfeldt C, Fish IH, Hoang BH, Phuong NT, Hung VV, Vu PP, Dung NK, Dong PV, Tien NN, Tho ND, Dung DH, Arzt J. 2019. First genome sequence of foot-and-mouth disease virus serotype O sublineage Ind2001e from southern Vietnam. Microbiol Resour Announc 8:e01424-18. https://doi.org/10.1128/MRA.01424-18.
10. Bertram MR, Palinski RM, Ranjan R, Biswal JK, Pauszek SJ, Hartwig EJ, Smoliga GR, Fish IH, Vierra D, Subramaniam S, Mohapatra JK, Das B, Pattnaik B, Arzt J. 2019. Genome sequences of 18 foot-and-mouth disease virus outbreak strains of serotype O sublineage Ind2001d from India, 2013–2014. Microbiol Resour Announc 8:e00776-19. https://doi.org/10.1128/MRA.00776-19.
11. Xu L, Hurtle W, Rowland JM, Casteran KA, Bucko SM, Grau FR, Valdazo-González B, Knowles NJ, King DP, Beckham TR, McIntosh MT. 2013. Development of a universal RT-PCR for amplifying and sequencing the leader and capsid-coding region of foot-and-mouth disease virus. J Virol Methods 189:70–76. https://doi.org/10.1016/j.jviromet.2013.01.009.
12. Neill JD, Newcomer BW, Marley SD, Ridpath JF, Givens DM. 2011. Genetic change in the open reading frame of bovine viral diarrhea virus is introduced more rapidly during the establishment of a single persistent infection than from multiple acute infections. Virus Res 158:140–145. https://doi.org/10.1016/j.virusres.2011.03.024.