11-15-2018

# First Complete Genome Sequence of a Genotype A2, Subgroup 4 Small Ruminant Lentivirus

Aspen M. Workman
*USDA, ARS, U.S. Meat Animal Research Center*, aspen.workman@ars.usda.gov

Michael L. Clawson
*USDA, ARS, U.S. Meat Animal Research Center*, Mike.Clawson@usda.gov

Michael P. Heaton
*USDA, ARS, U.S. Meat Animal Research Center*, mike.heaton@usda.gov

Aaron M. Dickey
*USDA, ARS, U.S. Meat Animal Research Center*, aaron.dickey@usda.gov

AMERICAN SOCIETY FOR MICROBIOLOGY | Microbiology Resource Announcements

GENOME SEQUENCES

# First Complete Genome Sequence of a Genotype A2, Subgroup 4 Small Ruminant Lentivirus

Aspen M. Workman,[a] Michael L. Clawson,[a] Michael P. Heaton,[a] Aaron M. Dickey[a]

[a]USDA, ARS, U.S. Meat Animal Research Center, Clay Center, Nebraska, USA

**ABSTRACT** Genetic variation in the ovine *TMEM154* gene associates with susceptibility to small ruminant lentivirus (SRLV) infection. We report here the first complete genome sequence for a genotype A2, subgroup 4 SRLV isolated from a Hampshire ewe with two copies of a *TMEM154* frameshift mutation predicted to abolish protein function.

Small ruminant lentiviruses (SRLVs) are a heterogeneous group of viruses that infect sheep and goats, causing incurable, progressive diseases that impact animal welfare and productivity (1). SRLVs in the United States have been classified into two main phylogenetic groups, genotype A2, which includes visna/maedi virus-like strains (called ovine progressive pneumonia virus in the United States), and genotype B1, which includes caprine arthritis encephalitis virus-like strains (2). U.S. genotype A2 isolates have been further classified into 4 subtypes based on variation in the *gag* gene sequence (3). Viral tropism and host susceptibility to these strains are determined by host and virus genetics. Genetic variation in the ovine transmembrane 154 (*TMEM154*) gene associates with infection susceptibility in sheep (4–6), and SRLV A2 subgroups 1 and 2 infect sheep in association with their *TMEM154* diplotypes (7). While the biological function of TMEM154 and the role it plays in SRLV infection remain unknown, apparent coevolution between *TMEM154* and SRLVs suggests that TMEM154 plays an important role in the virus life cycle. Thus, it was hypothesized that sheep lacking a functional copy of TMEM154 may be resistant to SRLV infection. However, a novel SRLV A2 subgroup (subgroup 4) was recently identified that naturally infected two sheep lacking functional TMEM154 and did not appear to be restricted by host *TMEM154* diplotypes (3). Thus, it was of interest to obtain a full-length genomic sequence from this strain to further our understanding of SRLV evolution.

One of the naturally infected ewes harboring two copies of the defective (knockout) *TMEM154* variant died unexpectedly in 2017 (Hampshire ewe, animal number 1150 [3]). Lung tissue was collected at necropsy and homogenized using a gentleMACS dissociator (Miltenyi Biotec, San Diego, CA) and clarified by centrifugation followed by filtration through a 0.2-$\mu$m syringe filter. Encapsidated viral RNA was enriched by treating the sample with 20 units of RNase ONE (Promega, Madison, WI) and 30 U of Turbo DNase (Thermo Fisher Scientific, Waltham, MA) in 1$\times$ DNase buffer at 37°C for 90 min. The remaining nucleic acids were isolated using TRIzol LS (Life Technologies, Inc., Carlsbad, CA) and used to generate an RNA TruSeq library (Illumina, San Diego, CA) without the initial step of poly(A) selection on oligo(dT) beads (8). The library was sequenced on an Illumina MiSeq instrument with a 600-cycle kit (v3) or a 500-cycle kit (v2), which generated 18,392,804 paired-end reads (2 $\times$ 300 bp and 2 $\times$ 200 bp, respectively).

Raw sequence reads were processed using Geneious software (v11.1.4; Biomatters, Auckland, New Zealand). Index adapters and low-quality reads were removed using BBDuk (v37.64) as implemented in Geneious. Assembly of the viral genomic sequence was accomplished using template-assisted assembly, where trimmed reads were

**Received** 2 October 2018 **Accepted** 26 October 2018 **Published** 15 November 2018

**Citation** Workman AM, Clawson ML, Heaton MP, Dickey AM. 2018. First complete genome sequence of a genotype A2, subgroup 4 small ruminant lentivirus. Microbiol Resour Announc 7:e01337-18. https://doi.org/10.1128/MRA.01337-18.

**Editor** Christina A. Cuomo, Broad Institute of MIT and Harvard



Address correspondence to Aspen M. Workman, aspen.workman@ars.usda.gov.

mapped to a reference U.S. SRLV A2 genome (GenBank accession number KY358787 [9]). A total of 25,099 reads mapped to the reference genome, resulting in an average coverage depth of 389×. The complete genome sequence of strain 1150 is 9,195 nucleotides long and shares 88.7% and 87.6% sequence identities with the SRLV A2 subgroup 1 and subgroup 2 reference genomes, respectively. This strain represents the third complete genome sequence belonging to SRLV genotype A2 and the first belonging to subgroup 4.

**Data availability.** The genome sequence of strain 1150 was deposited in DDBJ/EMBL/GenBank under the accession number MH916859. The partial *gag* sequence of this strain was previously deposited in GenBank (accession number KP120550 [3]). The raw sequence data were deposited in the NCBI Sequence Read Archive under accession number PRJNA497454.

## ACKNOWLEDGMENTS

This work was supported by the Agricultural Research Service (CRIS 3040-32000-034-00D).

We thank Steven Anderson, Neil Dyer, and Teresa Newell for the lung sample; Sue Hauver for technical support; and Stephanie Schmidt for secretarial support.

The use of product and company names is necessary to accurately report the methods and results; however, the United States Department of Agriculture (USDA) neither guarantees nor warrants the standard of the products, and the use of names by the USDA implies no approval of the product to the exclusion of others that may also be suitable.

## REFERENCES

1. Blacklaws BA. 2012. Small ruminant lentiviruses: immunopathogenesis of visna-maedi and caprine arthritis and encephalitis virus. Comp Immunol Microbiol Infect Dis 35:259–269. https://doi.org/10.1016/j.cimid.2011.12.003.
2. Ramírez H, Reina R, Amorena B, de Andrés D, Martínez HA. 2013. Small ruminant lentiviruses: genetic variability, tropism and diagnosis. Viruses 5:1175–1207. https://doi.org/10.3390/v5041175.
3. Clawson ML, Redden R, Schuller G, Heaton MP, Workman A, Chitko-McKown CG, Smith TP, Leymaster KA. 2015. Genetic subgroup of small ruminant lentiviruses that infects sheep homozygous for TMEM154 frameshift deletion mutation A4Delta53. Vet Res 46:22. https://doi.org/10.1186/s13567-015-0162-7.
4. Heaton MP, Clawson ML, Chitko-Mckown CG, Leymaster KA, Smith TP, Harhay GP, White SN, Herrmann-Hoesing LM, Mousel MR, Lewis GS, Kalbfleisch TS, Keen JE, Laegreid WW. 2012. Reduced lentivirus susceptibility in sheep with TMEM154 mutations. PLoS Genet 8:e1002467. https://doi.org/10.1371/journal.pgen.1002467.
5. Leymaster KA, Chitko-McKown CG, Clawson ML, Harhay GP, Heaton MP. 2013. Effects of TMEM154 haplotypes 1 and 3 on susceptibility to ovine progressive pneumonia virus following natural exposure in sheep. J Anim Sci 91:5114–5121. https://doi.org/10.2527/jas.2013-6663.
6. Leymaster KA, Chitko-McKown CG, Heaton MP. 2015. Incidence of infection in 39-month-old ewes with TMEM154 diplotypes "1 1," "1 3," and "3 3" after natural exposure to ovine progressive pneumonia virus. J Anim Sci 93:41–45. https://doi.org/10.2527/jas.2014-8553.
7. Sider LH, Heaton MP, Chitko-McKown CG, Harhay GP, Smith TP, Leymaster KA, Laegreid WW, Clawson ML. 2013. Small ruminant lentivirus genetic subgroups associate with sheep TMEM154 genotypes. Vet Res 44:64. https://doi.org/10.1186/1297-9716-44-64.
8. Workman AM, Harhay GP, Heaton MP, Grotelueschen DM, Sjeklocha D, Smith TP. 2015. Full-length coding sequences for 12 bovine viral diarrhea virus isolates from persistently infected cattle in a feedyard in kansas. Genome Announc 3:e00487-15. https://doi.org/10.1128/genomeA.00487-15.
9. Workman AM, Dickey AM, Heaton MP, Clawson ML, Smith TP. 2017. Complete genome sequences of two genotype A2 small ruminant lentiviruses isolated from infected U.S. sheep. Genome Announc 5:e00109-17. https://doi.org/10.1128/genomeA.00109-17.