



Genomic Sequence of a *Megrivirus* Strain Identified in Laying Hens in Brazil

Priscilla F. Gerber,^a Huigang Shen,^b Ying Zheng,^b Ganwu Li,^b Zélia I. P. Lobato,^c Tanja Opriessnig^{b,d}

^aAnimal Science, School of Environmental and Rural Science, University of New England, Armidale, Australia

^bDepartment of Veterinary Diagnostic and Production Animal Medicine, College of Veterinary Medicine, Iowa State University, Ames, Iowa, USA

^cLaboratório de Pesquisa em Virologia Animal, Departamento de Medicina Veterinária Preventiva, Escola de Veterinária, Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais, Brazil

^dThe Roslin Institute and The Royal (Dick) School of Veterinary Studies, University of Edinburgh, Midlothian, United Kingdom

ABSTRACT A new strain of chicken megrivirus was identified in fecal samples of layer chickens in a commercial flock in Minas Gerais, Brazil. It is most closely related to the family *Picornaviridae*, genus *Megrivirus*, species *Melegrivirus A*, and has an overall nucleotide identity of up to 85.1% with other megrivirus strains.

The *Picornaviridae* family currently consists of 40 genera (1), with at least 13 of these genera identified from avian sources (2). Avian picornaviruses of the genera *Megrivirus*, *Gallivirus*, and *Avisivirus* have been frequently identified in healthy and diseased poultry (3–5). Recent metagenomics studies focusing on the poultry gut virome have identified an ever-growing number of enteric picornaviruses in fecal samples from broiler chickens (3–8). In this study, two pooled fecal samples (2 g of fresh fecal material from five sites each in two sheds) were collected from a 102-week-old and a 57-week-old commercial laying flock in Minas Gerais State, Southeast Brazil, in August 2012. No health problems had been reported in the examined flocks.

Total nucleic acid was extracted from a 1:5 dilution of fecal samples using the MagMAX pathogen RNA/DNA kit (Thermo Fisher Scientific, MA) with KingFisher (Thermo Fisher Scientific) (9). Double-stranded cDNA was synthesized using the NEXTflex rapid transcriptome sequencing (RNA-seq) kit (Bioo Scientific Corp., TX). The sequencing library was prepared using the Nextera XT DNA library preparation kit (Illumina, CA) with dual indexing. The pooled libraries were sequenced on an Illumina MiSeq platform at the next-generation sequencing (NGS) lab located in the Veterinary Diagnostic Laboratory at Iowa State University, using the 300-cycle v2 reagent kit (Illumina) to generate 150 base-pair paired-end reads by following standard Illumina protocols. Raw reads of each sample were demultiplexed automatically on the MiSeq platform with the default settings.

Raw sequencing reads were preprocessed using Trimmomatic v0.36 to remove adapters and trim low-quality ends (10). Raw reads and preprocessed reads were subjected to sequencing quality analysis with FastQC (<https://www.bioinformatics.babraham.ac.uk/projects/fastqc/>) to ensure the efficiency of cleaning. Cleaned reads were fed to a comprehensive reference-assisted virus genome assembly pipeline (9, 11) with modifications. Briefly, the cleaned reads were aligned to the host reference genome using the Burrows-Wheeler Aligner MEM algorithm (BWA-MEM) (12); the nonhost reads were classified using Kraken v1.0 (13), and the unclassified reads were classified using Kaiju v1.6.2 (14); KronaTools v2.7 (15) was used to generate hierarchical classification results in which chicken picornavirus was identified; additional (supplementary) reads were collected before *de novo* assembly (9) by mapping the quality-trimmed reads to publicly available chicken picornavirus

Citation Gerber PF, Shen H, Zheng Y, Li G, Lobato ZIP, Opriessnig T. 2019. Genomic sequence of a *Megrivirus* strain identified in laying hens in Brazil. *Microbiol Resour Announc* 8:e01438-18. <https://doi.org/10.1128/MRA.01438-18>.

Editor Jelle Matthijnsens, KU Leuven

Copyright © 2019 Gerber 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 Tanja Opriessnig, tanja.opriessnig@roslin.ed.ac.uk.

Received 17 October 2018

Accepted 18 December 2018

Published 24 January 2019

genomes using BWA-MEM (12), SAMtools (16), and seqtk (<https://github.com/lh3/seqtk>); contigs were assembled using Assembly by Short Sequences (ABYSS) v1.3.9 (17); and the resulting contigs were manually curated to remove contaminated (nonviral) contigs and to trim chimeric (misassembled) contigs in the SeqMan Pro DNASTAR Lasergene 11 core suite.

Eight contigs of chicken picornaviruses were assembled from the raw data (SRA accession no. [SRR8290010](https://www.ncbi.nlm.nih.gov/sra/SRR8290010)) with an N_{50} value of 1,268 bp, and specific primers (provided upon request) were then designed to perform reverse transcriptase PCR (RT-PCR) and close the gaps by sequencing of the Nextera XT DNA library of the amplicon on a MiSeq platform (SRA accession no. [SRR8290011](https://www.ncbi.nlm.nih.gov/sra/SRR8290011)). Finally, a near-complete sequence of a megrivirus, chicken picornavirus MG/9567, was identified with a genome length of 9,567 nucleotides. The genome had 78.3% nucleotide similarity with the reference *Megrivirus C* isolate BL 21 (GenBank accession no. [KF961186](https://www.ncbi.nlm.nih.gov/GenBank/ accession=KF961186)).

The chicken picornavirus MG/9567 genome shared the highest deduced polyprotein amino acid similarity (94.9%) with chicken picornavirus 5 isolate 27 (GenBank accession no. [KF979336](https://www.ncbi.nlm.nih.gov/GenBank/ accession=KF979336)), which originated from a chicken sample collected in Hong Kong in 2008, and shared 87.7% amino acid (aa) identity with chicken megrivirus strain 3R, collected from a healthy chicken in Brazil in 2018 (GenBank accession no. [MG846465](https://www.ncbi.nlm.nih.gov/GenBank/ accession=MG846465)). The genome nucleotide similarities were 85.2% and 78.3%, respectively.

Data availability. The genome sequence reported here has been deposited in GenBank under the accession no. [MH806866](https://www.ncbi.nlm.nih.gov/GenBank/ accession=MH806866). The sequence data are available under SRA accession no. [SRR8290010](https://www.ncbi.nlm.nih.gov/sra/SRR8290010) and [SRR8290011](https://www.ncbi.nlm.nih.gov/sra/SRR8290011).

ACKNOWLEDGMENTS

We thank Maria Isabel Guedes, Isabela Rehfeld, and Leonardo Lara for providing chicken samples.

Z. I. P. Lobato received a fellowship from Conselho Nacional de Desenvolvimento Científico e Tecnológico, Brazil (CNPq). T. Opriessnig was funded by Biotechnology and Biological Sciences Research Council (BBSRC) Institute Strategic Programme Grants BB/J004324/1 and BBS/E/D/20241864, awarded to the Roslin Institute. This study received no funding from outside sources.

REFERENCES

- King AMQ, Lefkowitz EJ, Mushegian AR, Adams MJ, Dutilh BE, Gorbalenya AE, Harrach B, Harrison RL, Junglen S, Knowles NJ, Kropinski AM, Krupovic M, Kuhn JH, Nibert ML, Rubino L, Sabanadzovic S, Sanfacon H, Siddell SG, Simmonds P, Varsani A, Zerbini FM, Davison AJ. 2018. Changes to taxonomy and the International Code of Virus Classification and Nomenclature ratified by the International Committee on Taxonomy of Viruses (2018). *Arch Virol* 163:2601–2631. <https://doi.org/10.1007/s00705-018-3847-1>.
- Boros A, Pankovics P, Reuter G. 2014. Avian picornaviruses: molecular evolution, genome diversity and unusual genome features of a rapidly expanding group of viruses in birds. *Infect Genet Evol* 28:151–166. <https://doi.org/10.1016/j.meegid.2014.09.027>.
- Lima DA, Cibulski SP, Finkler F, Teixeira TF, Varela APM, Cerva C, Loiko MR, Scheffer CM, Dos Santos HF, Mayer FQ, Roehe PM. 2017. Faecal virome of healthy chickens reveals a large diversity of the eukaryote viral community, including novel circular ssDNA viruses. *J Gen Virol* 98:690–703. <https://doi.org/10.1099/jgv.0.000711>.
- Day JM, Oakley BB, Seal BS, Zsak L. 2015. Comparative analysis of the intestinal bacterial and RNA viral communities from sentinel birds placed on selected broiler chicken farms. *PLoS One* 10:e0117210. <https://doi.org/10.1371/journal.pone.0117210>.
- Lau SK, Woo PC, Yip CC, Li KS, Fan RY, Bai R, Huang Y, Chan KH, Yuen KY. 2014. Chickens host diverse picornaviruses originated from potential interspecies transmission with recombination. *J Gen Virol* 95:1929–1944. <https://doi.org/10.1099/vir.0.066597-0>.
- Boros A, Pankovics P, Adonyi A, Fenyvesi H, Day JM, Phan TG, Delwart E, Reuter G. 2016. A diarrheic chicken simultaneously co-infected with multiple picornaviruses: complete genome analysis of avian picornaviruses representing up to six genera. *Virology* 489:63–74. <https://doi.org/10.1016/j.virol.2015.12.002>.
- Bullman S, Kearney K, O'Mahony M, Kelly L, Whyte P, Fanning S, Morgan JG. 2014. Identification and genetic characterization of a novel picornavirus from chickens. *J Gen Virol* 95:1094–1103. <https://doi.org/10.1099/vir.0.061085-0>.
- Castro CMO, Chagas EHN, Bezerra DAM, Ribeiro AF, da Silva SP, Cruz ACR, Júnior ECS, Silva RR, Mascarenhas JDAP. 2018. A proposed new strain of avian picornavirus in broiler chicken from Brazil. *Genome Announc* 6:e00012-18. <https://doi.org/10.1128/genomeA.00012-18>.
- Zhang J, Zheng Y, Xia XQ, Chen Q, Bade SA, Yoon KJ, Harmon KM, Gauger PC, Main RG, Li G. 2017. High-throughput whole genome sequencing of Porcine reproductive and respiratory syndrome virus from cell culture materials and clinical specimens using next-generation sequencing technology. *J Vet Diagn Invest* 29:41–50. <https://doi.org/10.1177/1040638716673404>.
- Bolger AM, Lohse M, Usadel B. 2014. Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics* 30:2114–2120. <https://doi.org/10.1093/bioinformatics/btu170>.
- Chen Q, Wang L, Zheng Y, Zhang J, Guo B, Yoon KJ, Gauger PC, Harmon KM, Main RG, Li G. 2018. Metagenomic analysis of the RNA fraction of the fecal virome indicates high diversity in pigs infected by porcine endemic diarrhea virus in the United States. *Virology* 515:95. <https://doi.org/10.1016/j.virol.2018.01.001-z>.
- Li H, Durbin R. 2009. Fast and accurate short read alignment with Burrows-Wheeler transform. *Bioinformatics* 25:1754–1760. <https://doi.org/10.1093/bioinformatics/btp324>.
- Wood DE, Salzberg SL. 2014. Kraken: ultrafast metagenomic sequence

- classification using exact alignments. *Genome Biol* 15:R46. <https://doi.org/10.1186/gb-2014-15-3-r46>.
14. Menzel P, Ng KL, Krogh A. 2016. Fast and sensitive taxonomic classification for metagenomics with Kaiju. *Nat Commun* 7:11257. <https://doi.org/10.1038/ncomms11257>.
 15. Ondov BD, Bergman NH, Phillippy AM. 2011. Interactive metagenomic visualization in a Web browser. *BMC Bioinformatics* 12:385. <https://doi.org/10.1186/1471-2105-12-385>.
 16. Li H, Handsaker B, Wysoker A, Fennell T, Ruan J, Homer N, Marth G, Abecasis G, Durbin R. 2009. The Sequence Alignment/Map format and SAMtools. *Bioinformatics* 25:2078–2079. <https://doi.org/10.1093/bioinformatics/btp352>.
 17. Simpson JT, Wong K, Jackman SD, Schein JE, Jones SJ, Birol I. 2009. ABySS: a parallel assembler for short read sequence data. *Genome Res* 19:1117–1123. <https://doi.org/10.1101/gr.089532.108>.