Complete Genome Sequence of *Campylobacter hepaticus* Strain UF2019SK1, Isolated from a Commercial Layer Flock in the United States

Ananta Arukha,a Thomas N. Denagamage,b Gary Butcher,b Subhashinie Kariyawasama

aDepartment of Comparative, Diagnostic, and Population Medicine, University of Florida, Gainesville, Florida, USA
bDepartment of Large Animal Clinical Sciences, College of Veterinary Medicine, University of Florida, Gainesville, Florida, USA

**ABSTRACT** The thermophilic *Campylobacter* species *Campylobacter hepaticus* is the causative agent of spotty liver disease (SLD) in chickens. This announcement describes the complete genome sequence of *C. hepaticus* strain UF2019SK1, isolated from the liver of a commercial layer chicken with SLD in the United States.

Spotty liver disease (SLD), or spotty liver syndrome, in chickens is an acute disease caused by the thermophilic *Campylobacter* species *Campylobacter hepaticus* (1–5). The disease is characterized by 1- to 2-mm grayish-white or cream spots in the liver, increased mortality, and a drop in egg production (3, 5, 6). Although cases of SLD have been reported most commonly in free-range and barn-housed commercial layer flocks around the time of peak production, occasional outbreaks have occurred in caged layers, as well as in broiler and layer breeders (1, 4, 5, 7). With the steady increase in free-range commercial layer management practices worldwide, *C. hepaticus* may remain as a major bacterial pathogen of table egg production. Although several genome sequences of *C. hepaticus* strains from other countries have been deposited in public databases (1, 2, 8, 9), to the best of our knowledge, only one *C. hepaticus* strain isolated in the United States has been sequenced to date (GenBank accession number CP063536.1). In the present study, the chromosome of *C. hepaticus* strain UF2019SK1, which was isolated from the liver of a 21-week-old chicken in a caged layer flock in Florida showing the typical signs and lesions of SLD, was sequenced and fully closed. For isolation of *C. hepaticus*, the liver specimen was collected aseptically, transported to the laboratory in Stuart’s transport medium (Oxoid) within 4 h of collection, and processed as described previously (1). Briefly, a section of the liver was macerated in 5 ml of modified Preston broth and incubated for 7 days at 37°C. After 7 days of incubation, subcultures were spread onto 5% sheep blood agar (Remel, Lenexa, KS) and incubated at 37°C for 3 days in a microaerophilic environment (85% N₂, 7.5% CO₂, 7.5% O₂) created with a MicroAero gas generator and a Mitsubishi AnaeroPack rectangular jar (Fisher Scientific, Portsmouth, NH). At this point, Gram staining and an oxidase test were performed to identify presumptive *Campylobacter* colonies (Gram negative and oxidase positive). The suspected *Campylobacter* colonies were grown one more time on 5% sheep blood agar at 37°C in a microaerophilic atmosphere for 3 days to ensure purity. The isolated *Campylobacter* colonies were confirmed as *C. hepaticus* by PCR, which targeted the glycerol kinase gene (*glk*), and 16S rRNA gene sequencing, as described previously (6, 10). Frozen stocks of *C. hepaticus* were made in 1% protease peptone water containing 15% glycerol and stored at −80°C for long-term storage. For DNA extraction, bacteria were grown from frozen stocks on 5% sheep blood agar under microaerophilic conditions at 37°C for 3 days. A single colony of bacteria was suspended in 100 µl phosphate-buffered saline (PBS) before spreading onto 5% sheep blood agar and then incubated as above. The entire bacterial growth was harvested into a tube containing 1 ml of

**Citation** Arukha A, Denagamage TN, Butcher G, Kariyawasam S. 2021. Complete genome sequence of *Campylobacter hepaticus* strain UF2019SK1, isolated from a commercial layer flock in the United States. Microbiol Resour Announc 10:e01446-20. https://doi.org/10.1128/MRA.01446-20.

**Editor** David A. Baltrus, University of Arizona

**Copyright** © 2021 Arukha et al. This is an open-access article distributed under the terms of the Creative Commons Attribution 4.0 International license.

**Address** correspondence to Subhashinie Kariyawasam, skariyawasam@ufl.edu.

**Received** 17 December 2020

**Accepted** 23 February 2021

**Published** 25 March 2021
0.1 M PBS (pH 7.2) solution and subjected to glk gene PCR to confirm the bacterial growth as *C. hepaticus* before proceeding to DNA extraction.

Genomic DNA was extracted using a Genomic-tip 100/G kit (Qiagen, Inc., Germantown, MD, USA) according to the manufacturer’s instructions. The DNA sample was quantified using a Qubit version 2.0 fluorometer (Life Technologies, Carlsbad, CA, USA), and the sample purity and integrity were checked using NanoDrop and pulsed-field gel analysis, respectively. DNA library preparations, sequencing reactions, and initial bioinformatics analysis were conducted at Genewiz, LLC (South Plainfield, NJ, USA). An ~10-kb library for PacBio Sequel was constructed using the SMRTBell template prep kit version 1.0 (PacBio, Menlo Park, CA, USA). The library was bound to polymerase using the Sequel binding kit version 2.0 (PacBio) and loaded onto a PacBio Sequel instrument using the MagBead kit version 2 (PacBio). Sequencing was performed on a single PacBio Sequel single-molecule real-time (SMRT) cell, using Instrument Control Software version 5.0.0.6235, Primary analysis software version 5.0.0.6236, and SMRT Link version 5.0.0.6792. Reads were assembled using HGAP 4 (within the SMRT Link suite) and Canu. Sequencing yielded a total of 45,335 corrected PacBio reads with a read distribution (Nₜ₀) of 5,346 bp and a total of 1,600,012,565 bp. At this point, the corrected sequences were assembled with the CLC Genomics Workbench software version 20 (Qiagen, Inc.). The initial assembly was performed with reference-guided assembly using *C. hepaticus* strain HV10 (GenBank accession number NZ_CP031611) as the reference. *De novo* assembly was used to complete and verify the assembled genome sequence. After obtaining a draft circular genome sequence, the remaining gaps were filled by a primer-walking approach. At this point, the circularized genome sequence of *C. hepaticus* was annotated using NCBI Prokaryotic Genome Annotation Pipeline (PGAP; https://www.ncbi.nlm.nih.gov/genome/annotation_prok/) (11) and was independently analyzed on the Rapid Annotations using Subsystems Technology (RAST) server (http://rast.nmpdr.org/). (12). Since there were no inconsistencies observed, the PGAP annotation was used as the public-facing version. The SEED viewer (http://pubseed.theseed.org/) (13) was used for subsystem functional categorization of the predicted open reading frames (ORFs) obtained from annotation and initial subsystem assignments with RAST. Default parameters were used for all software, including those used for genome assembly and annotation, unless otherwise specified.

Analysis of the *C. hepaticus* UF2019SK1 chromosome revealed that it consists of a circular chromosome of 1.52 Mb. The sequence mapped to 1,525 open reading frames (ORFs), which included 1,470 coding DNA sequences (CDS), 9 rRNAs, 43 tRNAs, and 3 noncoding RNAs. Similar to other sequenced *C. hepaticus* strains, the average G+C content of the UF2019SK chromosome was approximately 28%. The SEED viewer assigned about 33% of genes from the predicted ORFs from RAST annotation to a particular biochemical pathway or a subsystem wherein protein metabolism and amino acid utilization pathways made up the two largest subsystems. The genomic data reported in this announcement will be useful in future studies of *C. hepaticus* pathogenesis and vaccine development.

**Data availability.** The complete annotated chromosome was deposited in NCBI GenBank under accession number CP065357.1, BioProject accession number PRJNA681575, and BioSample accession number SAMN16960974. The raw reads were deposited in the NCBI SRA under accession number SRX1000234.

**ACKNOWLEDGMENT**

This study was supported by the USDA National Institute of Food and Agriculture (Animal Health Project, 1023600).

**REFERENCES**

1. Crawshaw TR, Chanter JI, Young SCL, Cawthraw S, Whatmore AM, Koylass MS, Vidal AB, Salgueiro FJ, Irvine RM. 2015. Isolation of a novel thermophilic *Campylobacter* from cases of spotty liver disease in laying hens and experimental reproduction of infection and microscopic pathology. Vet Microbiol 179:315–321. https://doi.org/10.1016/j.vetmic.2015.06.008.

2. Van TTH, Elshagmani E, Gor MC, Scott PC, Moore RJ. 2016. *Campylobacter hepaticus* sp. nov., isolated from chickens with spotty liver disease. Int J Syst Evol Microbiol 66:4518–4524. https://doi.org/10.1099/ijs.0.001383.

3. Crawshaw T, Young S. 2003. Increased mortality on a free-range layer site. Vet Rec 153:664.

4. Crawshaw T. 2019. A review of the novel thermophilic *Campylobacter*. Transbound Emerg Dis 66:1481–1492. https://doi.org/10.1111/tbed.13229.
5. Grimes T, Reece R. 2011. “Spotty liver disease”—an emerging disease in free-range egg layers in Australia, p 53–56. In Proceedings of the 60th Western Poultry Disease Conference. American College of Poultry Veterinarians, Jacksonville, FL. https://aaap.memberclicks.net/assets/WPDC/wpdc_2011.pdf

6. Gregory M, Klein B, Sahin O, Girgis G. 2018. Isolation and characterization of Campylobacter hepaticus from layer chickens with spotty liver disease in the United States. Avian Dis 62:79–85. https://doi.org/10.1637/11752-092017-Reg.1

7. Groves PJ. 2010. A survey of the occurrence of the “spotty liver syndrome” in commercial layer chickens. In Proceedings of the Australian Veterinary Poultry Association Scientific Meeting. Australasian Veterinary Poultry Association, Sydney, Australia.

8. Petrovska L, Tang Y, Jansen van Rensburg MJ, Cawthraw S, Nunez J, Sheppard SK, Ellis RJ, Whatmore AM, Crawshaw TR, Irvine RM. 2017. Genome reduction for niche association in Campylobacter hepaticus, a cause of spotty liver disease in poultry. Front Cell Infect Microbiol 7:354. https://doi.org/10.3389/fcimb.2017.00354.

9. Van TTH, Lacey JA, Vezina B, Phung C, Anwar A, Scott PC, Moore RJ. 2019. Survival mechanisms of Campylobacter hepaticus identified by genomic analysis and comparative transcriptomic analysis of in vivo and in vitro derived bacteria. Front Microbiol 10:107. https://doi.org/10.3389/fmicb.2019.00107.

10. Van TTH, Gor M-C, Anwar A, Scott PC, Moore RJ. 2017. Campylobacter hepaticus, the cause of spotty liver disease in chickens, is present throughout the small intestine and caeca of infected birds. Vet Microbiol 207:226–230. https://doi.org/10.1016/j.vetmic.2017.06.022.

11. Tatusova T, DiCuccio M, Badretdin A, Chetvernin V, Zaslavsky L, Lomsadze A, Pruitt KD, Borodovsky M, Ostell J. 2016. NCBI Prokaryotic Genome Annotation Pipeline. Nucleic Acids Res 44:6614–6624. https://doi.org/10.1093/nar/gkw569.

12. Aziz RK, Bartels D, Best AA, DeLongh M, Disz T, Edwards RA, Formsma K, Gerdes S, Glass EM, Kubal M, Meyer F, Olsen GJ, Olson R, Osterman AL, Overbeek RA, McNiel LK, Paarmann D, Paczian T, Parrello B, Pusch GD, Reich C, Stevens R, Vassieva O, Vonstein V, Wilke A, Zagnitko O. 2008. The RAST server: Rapid Annotations using Subsystems Technology. BMC Genomics 9:7. https://doi.org/10.1186/1471-2164-9-75.

13. Overbeek R, Begley T, Butler RM, Choudhuri JV, Chuang H-Y, Cohoon M, de Crécy-Lagard V, Diaz N, Disz T, Edwards R, Fonstein M, Frank ED, Gerdes S, Glass EM, Goesmann A, Hanson A, Iwata-Reuyl D, Jensen R, Jamshidi N, Krause L, Kubal M, Larsen N, Linke B, McHardy AC, Meyer F, Neuweger H, Olsen G, Olson R, Osterman A, Portnoy V, Pusch GD, Rodionov DA, Rücker C, Steiner J, Stevens R, Thiele I, Vassieva O, Ye Y, Zagnitko O, Vonstein V. 2005. The subsystems approach to genome annotation and its use in the project to annotate 1000 genomes. Nucleic Acids Res 33:5691–5702. https://doi.org/10.1093/nar/gki866.