



Complete Genome Sequence of *Campylobacter fetus* Isolated from a Sheep

Daniela Costa,^{a,b} Virginia Aráoz,^c Maila Barcellos,^a Rubén Darío Caffarena,^c Martín Fraga,^c Federico Giannitti,^c Cecilia Monesiglio,^c Ruben Pérez,^a Caroline da Silva Silveira,^c  Lucía Calleros^a

^aSección Genética Evolutiva, Instituto de Biología, Facultad de Ciencias, Universidad de la República, Montevideo, Uruguay

^bLaboratorio de Genómica Microbiana, Institut Pasteur de Montevideo, Montevideo, Uruguay

^cPlataforma de Investigación en Salud Animal, Instituto Nacional de Investigación Agropecuaria Estación Experimental La Estanzuela, Colonia, Uruguay

ABSTRACT *Campylobacter fetus* is an important reproductive pathogen of ruminants that occasionally infects humans. Here, we describe the complete circularized genome of a strain of *Campylobacter fetus* subsp. *fetus* isolated from a sheep. The final assembly consisted of a unique contig with a length of 1,849,237 bp.

Campylobacter species are *Epsilonproteobacteria* adapted to vertebrate hosts. Many of them cause disease in a wide range of livestock species and have extensive reservoirs in wildlife. *Campylobacter fetus* is an important animal pathogen and an opportunistic human pathogen. It produces considerable economic losses as a major reproductive pathogen of cattle and sheep. This species is currently divided into *C. fetus* subsp. *fetus*, *C. fetus* subsp. *venerealis* (1), and *C. fetus* subsp. *testudinum* (2). *C. fetus* subsp. *fetus* causes abortion mainly in sheep (3) and, to a lesser extent, cattle.

Here, we describe the closed whole genome of a strain of *C. fetus* subsp. *fetus* isolated from a sheep, representing an important resource for evolutionary and epidemiological studies of the species.

Fresh placenta was received at the Instituto Nacional de Investigación Agropecuaria (INIA) Animal Health Platform (Uruguay) for bacteriological analyses. The sample was spiked into Skirrow agar and incubated in a microaerobic atmosphere for 48 h at 37°C using CampyGen (Oxoid) (4). Biochemical testing was performed, including catalase, oxidase, and hydrogen sulfide production (triple-sugar iron medium), growth in the presence of 3.5% sodium chloride or 1% glycine, and growth at 25 and 42°C in Skirrow agar (4). Results were consistent with those of *Campylobacter fetus* subsp. *fetus*.

Bacterial colonies from five petri dishes of pure culture were suspended in 500 μ l of phosphate-buffered saline solution (pH 7.4), and DNA was extracted using the QIAamp DNA minikit (Qiagen, Inc., Valencia, CA). Whole-genome sequencing (WGS) was performed with Illumina and Pacific Biosciences (PacBio) sequencing technologies. An Illumina library was prepared with the Nextera XT library prep kit without modifications. Illumina reads were obtained using a MiSeq platform at Institut Pasteur de Montevideo, obtaining 1,443,064 2 \times 150-bp paired-end (PE) reads. All bioinformatic analysis software was set to default values. Read quality was assessed using FastQC v0.11.7 (5). Bases at the ends of the reads with a quality score lower than 20 and reads shorter than 50 bases were trimmed using Trimmomatic v0.39 (TRAILING:20 MINLEN:50) (6). A total of 1,421,381 PE reads remained after filtration (mean length, 145 bases) (Table 1).

Long reads were obtained using PacBio RS II single-molecule real-time (SMRT) technology with P6-C4 chemistry (Pacific Biosciences) at MacroGen, Inc. (Seoul, South Korea). A library was prepared with the 20-kb SMRTbell template library kit without modifications. DNA was sheared, and 20-kb fragments were selected. Preprocessing

Citation Costa D, Aráoz V, Barcellos M, Caffarena RD, Fraga M, Giannitti F, Monesiglio C, Pérez R, da Silva Silveira C, Calleros L. 2020. Complete genome sequence of *Campylobacter fetus* isolated from a sheep. Microbiol Resour Announc 9:e01008-20. <https://doi.org/10.1128/MRA.01008-20>.

Editor Irene L. G. Newton, Indiana University, Bloomington

Copyright © 2020 Costa 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 Lucía Calleros, calleros@fcien.edu.uy.

Received 28 August 2020

Accepted 20 October 2020

Published 5 November 2020

TABLE 1 Short- and long-read preprocessing summary

Sequencing platform	Metric	Prefilter	Postfilter
Illumina MiSeq	No. of reads	1,443,064	1,421,381
	Read length range (bp)	30–151	50–151
	Read Q-score range	21–36	22–36
PacBio RS II	No. of polymerase read bases	1,608,855,393	1,563,018,757
	No. of polymerase reads	150,292	101,447
	Polymerase read N_{50} (bp)	21,336	21,464
	Polymerase read length (bp)	10,704	15,407
	Polymerase read quality	0.583	0.844

was done using the SMRT Portal (smrtanalysis_2.3.0.140936.p5.167094) RS Subreads 1 protocol. It obtained 1,608,855,393 bases from 150,292 reads, with an average read length of 10,704 bp and an N_{50} value of 16,155 bp. After filtering, 97% of these data remained (Table 1).

A hybrid assembly approach was performed with Unicycler v0.4.7 assembler (7), using *bold* mode. The quality of the assembly was addressed using Quast v4.3 (8).

Assembly resulted in a unique 1,849,237-bp contig with an average G+C content of 33.3%, as expected for *C. fetus* subsp. *fetus* genomes.

The sequences were annotated with NCBI Prokaryotic Genome Annotation Pipeline (PGAP v4.13) (9). The genome contains 1,884 coding sequences: 1,811 hypothetical proteins, 44 tRNA genes, 3 rRNA operons as in all other *Campylobacter* species, and 2 CRISPR arrays.

Data availability. These whole-genome sequencing, assembly, and raw data have been deposited in DDBJ/ENA/GenBank under the BioProject number [PRJNA554155](https://www.ncbi.nlm.nih.gov/bioproject/PRJNA554155).

ACKNOWLEDGMENTS

This work was supported by the Uruguayan Agencia Nacional de Innovación e Investigación (grant ANII FSSA-105252 and Ph.D. fellowship for D.C.) and the Instituto Nacional de Investigación Agropecuaria (grants N-15156_PL_15_0_00 and N-23398).

The funders had no role in study design, data collection and interpretation, or the decision to submit the work for publication. We declare no conflict of interest.

L.C., F.G., M.F., and R.P. conceived the study. V.A. and R.D.C. collected the sample. M.B., C.M., C.D.S.S., and L.C. performed the experiments. D.C. assembled the genome. L.C. and D.C. wrote the manuscript. All the authors revised the manuscript.

REFERENCES

- Veron M, Chatelain R. 1973. Taxonomic study of the genus *Campylobacter* Sebald and Veron and designation of the neotype strain for the type species, *Campylobacter fetus* (Smith and Taylor) Sebald and Veron. *Int J Syst Bacteriol* 23:122–134. <https://doi.org/10.1099/00207713-23-2-122>.
- Fitzgerald C, Tu ZC, Patrick M, Stiles T, Lawson AJ, Santovenia M, Gilbert MJ, van Bergen M, Joyce K, Pruckler J, Stroika S, Duim B, Miller WG, Loparev V, Sinnige JC, Fields PI, Tauxe RV, Blaser MJ, Wagenaar JA. 2014. *Campylobacter fetus* subsp. *testudinum* subsp. nov., isolated from humans and reptiles. *Int J Syst Evol Microbiol* 64:2944–2948. <https://doi.org/10.1099/ijs.0.057778-0>.
- Sahin O, Yaeger M, Wu Z, Zhang Q. 2017. *Campylobacter*-associated diseases in animals. *Annu Rev Anim Biosci* 5:21–42. <https://doi.org/10.1146/annurev-animal-022516-022826>.
- OIE. 2008. Bovine genital campylobacteriosis, p 661–670. *In* Manual of diagnostic tests and vaccines for terrestrial animals (mammals, birds and bees), 6th ed, vol 2. OIE, Paris, France.
- Andrews S, Lindenbaum P, Howard B, Ewels P. 2010. FastQC high throughput sequence version 0.11.7. <http://www.bioinformatics.babraham.ac.uk/projects/fastqc/>.
- 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>.
- Wick RR, Judd LM, Gorrie CL, Holt KE. 2017. Completing bacterial genome assemblies with multiplex MinION sequencing. *Microb Genomics* 3: e000132. <https://doi.org/10.1099/mgen.0.000132>.
- Gurevich A, Saveliev V, Vyahhi N, Tesler G. 2013. QUAST: quality assessment tool for genome assemblies. *Bioinformatics* 29:1072–1075. <https://doi.org/10.1093/bioinformatics/btt086>.
- Tatusova T, Dicuccio M, Badretdin A, Chetvernin V, Nawrocki EP, 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>.