

University of Nebraska - Lincoln

DigitalCommons@University of Nebraska - Lincoln

Papers in Veterinary and Biomedical Science

Veterinary and Biomedical Sciences,
Department of

2018

Complete Genome Sequence of *Moraxella bovis* Strain Epp-63 (300), an Etiologic Agent of Infectious Bovine Keratoconjunctivitis

John Dustin Loy

University of Nebraska-Lincoln, jdloy@unl.edu

Aaron M. Dickey

USDA, Agricultural Research Service, aaron.dickey@usda.gov

Michael L. Clawson

USDA, Agricultural Research Service, Mike.Clawson@usda.gov

Follow this and additional works at: <https://digitalcommons.unl.edu/vetscipapers>



Part of the [Biochemistry, Biophysics, and Structural Biology Commons](#), [Cell and Developmental Biology Commons](#), [Immunology and Infectious Disease Commons](#), [Medical Sciences Commons](#), [Veterinary Microbiology and Immunobiology Commons](#), and the [Veterinary Pathology and Pathobiology Commons](#)

Loy, John Dustin; Dickey, Aaron M.; and Clawson, Michael L., "Complete Genome Sequence of *Moraxella bovis* Strain Epp-63 (300), an Etiologic Agent of Infectious Bovine Keratoconjunctivitis" (2018). *Papers in Veterinary and Biomedical Science*. 299.

<https://digitalcommons.unl.edu/vetscipapers/299>

This Article is brought to you for free and open access by the Veterinary and Biomedical Sciences, Department of at DigitalCommons@University of Nebraska - Lincoln. It has been accepted for inclusion in Papers in Veterinary and Biomedical Science by an authorized administrator of DigitalCommons@University of Nebraska - Lincoln.



Complete Genome Sequence of *Moraxella bovis* Strain Epp-63 (300), an Etiologic Agent of Infectious Bovine Keratoconjunctivitis

 John Dustin Loy,^a Aaron M. Dickey,^b Michael L. Clawson^b

^aSchool of Veterinary Medicine and Biomedical Sciences (SVMBS), University of Nebraska—Lincoln (UNL), Lincoln, Nebraska, USA

^bU.S. Department of Agriculture, Agricultural Research Service (ARS), U.S. Meat Animal Research Center (USMARC), Clay Center, Nebraska, USA

ABSTRACT We report here the complete closed genome sequence of *Moraxella bovis* strain Epp-63 (300) (Epp63). This strain was isolated from an infectious bovine keratoconjunctivitis (IBK) case in 1963. Since then, Epp63 has been used extensively for IBK research. Consequently, the genome sequence of Epp63 should help elucidate IBK host-pathogen interactions.

Infectious bovine keratoconjunctivitis (IBK) is a significant disease of cattle worldwide, and nearly 50% of herds in the United States are affected. The disease can cause considerable impact on afflicted animals, including blindness (1, 2).

Moraxella bovis is an etiologic agent of IBK. One strain, Epp-63 (300) (Epp63), has been studied in a variety of clinical models (3–5). Virulence factors such as pili (including phase variations thereof), hemolysins, phospholipases, and plasmids have been characterized in Epp63 (6–10). However, the genome of this important strain had not been sequenced, and no complete, closed genome sequence of any *M. bovis* isolate was available in GenBank as recently as July 2018.

Strain Epp63 had been stored lyophilized from 1987 until its revival in 2018. Following two passages on 5% sheep blood Trypticase soy agar, the isolate was grown overnight in brain heart infusion broth at 37°C with 5% CO₂, and the DNA was purified over 20/G gravity-flow anion-exchange columns (Qiagen, Valencia, CA, USA). The same DNA source was used to construct both a single-molecule real-time (SMRT) DNA library (10 to 20 kb) with SMRT Bell version 1.0 (Pacific Biosystems, Menlo Park, CA, USA) and a paired-end (2 × 151-bp) DNA library with TruSeq PCR-free LT (Illumina, San Diego, CA, USA), according to the manufacturers' instructions. The SMRT Bell and TruSeq libraries were sequenced on a PacBio RS II sequencer with P6 chemistry and a 6-h movie and an Illumina NextSeq 500 sequencer with NextSeq V2 chemistry, respectively.

The PacBio sequencing yielded 59,873 reads with a mean read length of 16,744 nucleotides (nt). These reads passed filtering controls of a minimum subread length of 500 nt and a minimum polymerase read quality and length of 0.80 and 100 nt, respectively, and were assembled with the Hierarchical Genome Assembly Process version 3 (HGAP3), which yielded three unique contigs. All three contigs had overlapping ends of redundant sequences that were identified with self-dotplots in Geneious version 11.1.2 (11) and removed, yielding a preliminary chromosome sequence and two plasmid sequences. The PacBio sequencing coverage exceeded 300- and 24-fold for the chromosome and plasmids, respectively. Sequences of the two plasmids had previously been generated and were available in GenBank; accordingly, the two plasmids sequenced in this study were oriented to start at the same base as their counterparts in GenBank. The chromosome was oriented to start at an origin of replication that was

This document is a U.S. government work and is not subject to copyright in the United States.

Received 27 July 2018 **Accepted** 6 August 2018 **Published** 30 August 2018

Citation Loy JD, Dickey AM, Clawson ML. 2018. Complete genome sequence of *Moraxella bovis* strain Epp-63 (300), an etiologic agent of infectious bovine keratoconjunctivitis. *Microbiol Resour Announc* 7:e01004-18. <https://doi.org/10.1128/MRA.01004-18>.

Editor John J. Dennehy, Queens College

This is a work of the U.S. Government and is not subject to copyright protection in the United States. Foreign copyrights may apply. Address correspondence to John Dustin Loy, jdloy@unl.edu.

identified with Ori-Finder (12). The chromosome and plasmid sequences were then polished and error corrected with PacBio sequences using RS Resequencing version 1. The Illumina TruSeq library sequencing yielded 3.8×10^6 million reads that were mapped onto the chromosome and plasmid sequences in Geneious with “map to reference” and used to correct homopolymer errors. This yielded an *M. bovis* Epp63 chromosome of 2,839,913 bp and two plasmids, pMBO-1 and pMBO-2, of 44,217 and 27,077 bp, respectively.

The chromosome and plasmids were annotated with the NCBI Prokaryotic Genome Annotation Pipeline (PGAP) (https://www.ncbi.nlm.nih.gov/genome/annotation_prok). A total of 2,816, 34, and 38 protein-coding genes were identified in the chromosome, pMBO-1, and pMBO-2, respectively.

Data availability. The genome sequence of the chromosome and plasmids of strain Epp63 have been deposited in DDBJ/ENA/GenBank under the accession numbers CP030241 to CP030243. The version of the chromosome described in this paper is the first version. The plasmid sequences were previously reported in GenBank under accession numbers AB169976 and AB169977.

ACKNOWLEDGMENTS

Moraxella bovis strain Epp-63 (300) was supplied by Douglas Rogers (SVMBS, UNL), who received it from G. W. Pugh, Jr. (National Animal Disease Center, USDA-ARS, Ames, IA, USA). We thank Gennie Schuller for technical help.

Funding was provided in part by the USDA-ARS as well as the Nebraska Agricultural Experiment Station with funding from the Hatch Act (number 1007070) from the Animal Health and Disease Research (section 1433) capacity funding program (number 1002196) through the USDA National Institute of Food and Agriculture. The use of product and company names is necessary to accurately report the methods and results; however, the 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

- Webber JJ, Selby LA. 1981. Risk factors related to the prevalence of infectious bovine keratoconjunctivitis. *J Am Vet Med Assoc* 179: 823–826.
- Brown MH, Brightman AH, Fenwick BW, Rider MA. 1998. Infectious bovine keratoconjunctivitis: a review. *J Vet Intern Med* 12:259–266. <https://doi.org/10.1111/j.1939-1676.1998.tb02120.x>.
- Rogers DG, Chevillat NF, Pugh GW, Jr. 1987. Pathogenesis of corneal lesions caused by *Moraxella bovis* in gnotobiotic calves. *Vet Pathol* 24:287–295. <https://doi.org/10.1177/030098588702400401>.
- Hughes DE, Pugh GW, Jr, McDonald TJ. 1965. Ultraviolet radiation and *Moraxella bovis* in the etiology of bovine infectious keratoconjunctivitis. *Am J Vet Res* 26:1331–1338.
- Gould S, Dewell R, Tofflemire K, Whitley RD, Millman ST, Opiessnig T, Rosenbusch R, Trujillo J, O'Connor AM. 2013. Randomized blinded challenge study to assess association between *Moraxella bovoculi* and infectious bovine keratoconjunctivitis in dairy calves. *Vet Microbiol* 164: 108–115. <https://doi.org/10.1016/j.vetmic.2013.01.038>.
- Farn JL, Strugnell RA, Hoyne PA, Michalski WP, Tennent JM. 2001. Molecular characterization of a secreted enzyme with phospholipase B activity from *Moraxella bovis*. *J Bacteriol* 183:6717–6720. <https://doi.org/10.1128/JB.183.22.6717-6720.2001>.
- Clinkenbeard KD, Thiessen AE. 1991. Mechanism of action of *Moraxella bovis* hemolysin. *Infect Immun* 59:1148–1152. <https://iai.asm.org/content/59/3/1148>.
- Marrs CF, Schoolnik G, Koomey JM, Hardy J, Rothbard J, Falkow S. 1985. Cloning and sequencing of a *Moraxella bovis* pilin gene. *J Bacteriol* 163:132–139. <https://jbs.asm.org/content/163/1/132>.
- Kakuda T, Sarataphan N, Tanaka T, Takai S. 2006. Filamentous-haemagglutinin-like protein genes encoded on a plasmid of *Moraxella bovis*. *Vet Microbiol* 118:141–147. <https://doi.org/10.1016/j.vetmic.2006.06.024>.
- Marrs CF, Ruehl WW, Schoolnik GK, Falkow S. 1988. Pilin-gene phase variation of *Moraxella bovis* is caused by an inversion of the pilin genes. *J Bacteriol* 170:3032–3039. <https://doi.org/10.1128/jb.170.7.3032-3039.1988>.
- Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, Buxton S, Cooper A, Markowitz S, Duran C, Thierer T, Ashton B, Meintjes P, Drummond A. 2012. Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics* 28:1647–1649. <https://doi.org/10.1093/bioinformatics/bts199>.
- Gao F, Zhang C-T. 2008. Ori-Finder: a Web-based system for finding *oriC* in unannotated bacterial genomes. *BMC Bioinformatics* 9:79. <https://doi.org/10.1186/1471-2105-9-79>.