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PROKARYOTES

Closed Genome Sequences of Seven *Histophilus somni* Isolates from Beef Calves with Bovine Respiratory Disease Complex

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ABSTRACT *Histophilus somni* is a fastidious Gram-negative opportunistic pathogenic *Pasteurellaceae* that affects multiple organ systems and is one of the principal bacterial species contributing to bovine respiratory disease complex (BRDC) in feed yard cattle. Here, we present seven closed genome sequences isolated from three beef calves showing sign of BRDC.

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Bovine respiratory disease complex (BRDC) outbreaks in feed yard cattle are the primary drivers of disease-related antibiotic treatments and have a global impact estimated to be greater than $3 billion/year (1). In addition to respiratory infections, *H. somni* has been reported to be associated with meningoencephalitis, myocarditis, arthritis, and other systemic cattle infections (2). There is a need for more publicly available closed *H. somni* genome sequences because only two were available in GenBank as of January 2016.

Nasopharyngeal (NP) and bronchoalveolar lavage (BAL) isolates were cultured from animals exhibiting clinical signs of BRDC at a Kansas feed yard during a 28-day study of 180 calves originating from 3 different southeastern U.S. states (3–6). These isolates were collected from the animals in the feed yard hospital upon hospital entry (day 0) and on days 1 and 5. Closed genome and methylome sequences of these isolates were obtained using Pacific Biosciences (PacBio) single molecule real-time sequencing.

The *H. somni* isolates were grown overnight at 37°C in veterinary fastidious medium and shaken at 190 rpm. Genomic DNA was isolated using an in-house protocol to lyse the cells and purify on Qiagen Genomic-tip 100/G columns according to manufacturer directions. From the genomic DNA, PacBio 20-kb insert libraries were created and sequenced on a PacBio RS II system using C4/P6 (chemistry/polymerase) to at least 131-fold coverage. The genome sequences were assembled using PacBio HGAP3 to generate a single contig with overlapping 3′ and 5′ ends of at least 1 kb with greater than 99% identity. For each isolate's contig, the overlapping region was deleted from the 3′ end, the two ends were joined to circularize the chromosome, and GenSkew (http://genskew.csb.univie.ac.at) was used to localize the approximate origin of replication so that the base pair numbering could be reindexed to reflect the origin of replication at base pair position 1 (7). The PacBio RS_Resequencing pipeline was used to map the reads back to the references to generate final consensus concordance assemblies that were manually inspected to ensure that the assemblies were free from low-quality read mapping regions and inadequate read coverage (8). The genomes

TABLE 1 Chromosome and BioSample accession numbers with metadata

GenBank accession no.	BioSample accession no.	Day	Animal	Tissue	Fold coverage	No. of genes
CP018803	SAMN06141920	0	212	NP	250	2,005
CP018805	SAMN06141921	1	212	NP	131	2,008
CP018806	SAMN06141918	1	212	BAL	298	2,005
CP018802	SAMN06141919	5	127	BAL	498	1,913
CP018807	SAMN06141923	5	127	NP	236	1,918
CP018804	SAMN06141917	5	209	BAL	290	2,007
CP018808	SAMN06141922	5	212	NP	169	2,007

were annotated with NCBI Prokaryotic Genome Annotation Pipeline (released 2013). The use of cattle in this study was approved by the Kansas State University Institutional Animal Care and Use Committee.

Accession number(s). GenBank genome and BioSample accession numbers, with links to important metadata, are given in Table 1.

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