

Complete Genome Sequence of *Mycoplasma haemocanis* Strain Illinois

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***Mycoplasma haemocanis* is a blood pathogen that may cause acute disease in immunosuppressed or splenectomized dogs. The genome of the strain Illinois is a single circular chromosome with 919,992 bp and a GC content of 35%. Analyses of the *M. haemocanis* genome will provide insights into its biology and *in vitro* cultivation requirements.**

Mycoplasma haemocanis, together with certain mycoplasmal bacteria (class *Mollicutes*), have a particular tropism for red blood cells (RBC) of their vertebrate hosts. As a group, they represent a new clade of mycoplasmas and have been given the trivial name hemoplasmas (7, 9). *M. haemocanis* is a blood pathogen that may cause overt, hemolytic anemia in immunosuppressed (2, 6) or splenectomized (5, 6) dogs. Like other species of hemoplasmas, this bacterium cannot be cultivated *in vitro*. The prevalence of *M. haemocanis* infection can vary from 0.5% to 40% (12). The genome of this hemoplasma was entirely sequenced and assembled in order to better understand aspects of its biology: metabolic features, mechanisms of pathogenicity, and evolutionary relationships.

Genomic DNA was extracted from *M. haemocanis* isolated from the blood of a naturally infected dog at the peak of bacteremia (3) by using a Quick-gDNA miniprep kit (Zymo Research, Irvine, CA). Its whole genome was sequenced from paired-end libraries (TruSeq DNA sample preparation kit; Illumina, San Diego, CA) using 20% of an Illumina v3 chemistry lane (HiScanSQ). Sequencing resulted in 15.7 million high-quality filtered read pairs with an average read length of 2×100 nucleotides and a $>3,400\times$ genome equivalent coverage. Reads were assembled using ABySS-PE v1.2.7 utilizing 20% of the reads, with “kmer” set to 95 bases (11). Predicted scaffolds with significant BLAST matches to dog DNA were excluded, and the remaining mycoplasma scaffolds were then organized based on the orientation predicted in the assembly and on the genome sequence of *M. haemofelis* strain Ohio2. A total of 13 gaps were identified and closed using conventional PCR followed by Sanger sequencing. First-pass annotation was achieved using the NCBI annotation pipeline.

The complete singular circular chromosome has a size of 919,992 bp and G+C content of 35%, genomic features compatible with other hemoplasma species sequenced to date (1, 4, 8, 10). As described for all the mycoplasmas sequenced (22 species to date), *M. haemocanis* also uses the opal stop codon (UGA) for tryptophan. The 16S, 23S, and 5S rRNA genes are present as single copies and share the same operon. The genome annotation suggests the presence of 1,157 predicted protein coding sequences (CDS) and 31 tRNAs.

Putative functions of the *M. haemocanis* CDS were automatically assigned by the NCBI annotation pipeline. Most of the CDS are represented as hypothetical proteins (76%), which might be related to a large repertoire of paralog genes. By recombination of these genes, *M. haemocanis* might vary its surface antigens to successfully evade the host's immune system. As suggested for other hemoplasmas, it is likely that *M. haemocanis* takes advantage of the

RBC's metabolism by scavenging nutrients, which leads to diminished RBC life span and exacerbation of anemia during acute disease. Detailed analyses of this complete genome will provide not only information about the *M. haemocanis* lifestyle but also clues for *in vitro* cultivation of these selective and intriguing bacteria.

Nucleotide sequence accession number. The genome of *M. haemocanis* strain Illinois was deposited in GenBank under the accession number [CP003199](https://doi.org/10.1093/nuclemta/ctt003).

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