The draft genome sequences of eight enteroheliohepatic Helicobacter species, *H. muridarum*, *H. trogontum*, *H. typhlonius*, and five unnamed helicobacters, are presented here. Using laboratory mice pervasively infected with helicobacters, we characterized the presence of known virulence factors.

Enteroheliohepatic *Helicobacter* species (EHS) are Gram-negative, microaerophilic, spiral-shaped bacteria that colonize the mucosa of the gastrointestinal tract and/or the livers of mammals, including humans, and birds (1, 2). Natural enteroheliohepatic *Helicobacter* sp. infection is prevalent in 88% of research mouse colonies worldwide (3). Our previous work reported the high prevalence of *Helicobacter hepaticus*, *Helicobacter rodentium*, *Helicobacter bilis*, and *Helicobacter typhlonius* in research mouse facilities (3). Previouly, we have sequenced multiple EHS, including *H. bilis*, *Helicobacter pullorum*, *H. hepaticus*, *Helicobacter cinaedi*, and *Helicobacter canadensis* (4, 5). While most infected mice develop minimal pathological changes, susceptible strains exhibit typhlocolitis and hepatitis, which can progress to colon cancer and heated cellular carcinoma (6). Previous studies have shown that *Helicobacter* infections can affect experimental outcomes in cancer studies and confound study results (7–9).

Furthermore, studies have highlighted the potential zoonotic nature of EHS species, as EHS isolated in rodents or birds, such as *H. cinaedi*, *H. canadensis*, *H. bilis*, and *H. pullorum*, have been identified in patients with diarrhea, cholecystitis, and biliary neoplasia (10–12), and it is well-documented that EHS can also infect other animal species, such as dogs, cats, geese, rhesus macaques, hamsters, gerbils, guinea fowl, and chickens (13–31).

In this report, we announce the whole-genome sequencing of eight EHS, including *Helicobacter muridarum* ST1, *Helicobacter trogontum*, *H. typhlonius*, as well as unnamed *Helicobacter* species (Massachusetts Institute of Technology [MIT] strains 01-6451, 03-1614, 03-1616, 05-5293, and 11-5569). These isolates were obtained from cecal, colon, and fecal samples of either laboratory or wild mice and rats. The isolates were sequenced using Illumina MiSeq sequencing technology, as described previously (32). The 250-bp paired-end sequencing reads generated by MiSeq were assembled into contigs using Velvet (33). The sequences were annotated using the NCBI Prokaryotic Genomes Automatic Annotation Pipeline (34). The G+C contents ranged from 33 to 39%, and between 1,922 and 2,520 genes were annotated per genome (Table 1).

Due to the ability of EHS to interfere with biomedical research involving rodents, we evaluated the presence of known *Helicobacter* virulence determinants, such as gamma-glutamyl transpeptidase (ggt), cytolethal distending toxin subunit B (cdtB), and components of both the type IV and type VI secretion systems. Both

### TABLE 1 Genome characteristics and accession numbers of eight rodent helicobacters

<table>
<thead>
<tr>
<th>Strain</th>
<th>GenBank accession no.</th>
<th>Host</th>
<th>Fold coverage</th>
<th>G+C content (%)</th>
<th>Estimated genome length (bp) using Velvet</th>
<th>No. of contigs using PGAP</th>
<th>No. of genes using PGAP</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>H. muridarum</em> ST1</td>
<td>JRPD0000000000</td>
<td>Mouse</td>
<td>56</td>
<td>33</td>
<td>2,354,445</td>
<td>92</td>
<td>2,351</td>
</tr>
<tr>
<td><em>H. trogontum</em> (“Flexispira rappini taxon 6“)</td>
<td>JRPL0000000000</td>
<td>Rat</td>
<td>48</td>
<td>34</td>
<td>2,762,714</td>
<td>129</td>
<td>1,922</td>
</tr>
<tr>
<td><em>H. typhlonius</em> MIT strain 97-6810</td>
<td>JRPF0000000000</td>
<td>Mouse</td>
<td>62</td>
<td>38.5</td>
<td>1,899,179</td>
<td>25</td>
<td>2,520</td>
</tr>
<tr>
<td><em>Helicobacter</em> sp. MIT strain 01-6451</td>
<td>JRMC0000000000</td>
<td>Mouse</td>
<td>89</td>
<td>37.5</td>
<td>2,056,937</td>
<td>48</td>
<td>2,064</td>
</tr>
<tr>
<td><em>Helicobacter</em> sp. MIT strain 03-1614</td>
<td>JRMS0000000000</td>
<td>Mouse</td>
<td>36</td>
<td>37.5</td>
<td>1,927,676</td>
<td>172</td>
<td>2,057</td>
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<td><em>Helicobacter</em> sp. MIT strain 03-1616</td>
<td>JROY0000000000</td>
<td>Mouse</td>
<td>37</td>
<td>39</td>
<td>1,890,582</td>
<td>176</td>
<td>1,974</td>
</tr>
<tr>
<td><em>Helicobacter</em> sp. MIT strain 05-5293</td>
<td>JROZ0000000000</td>
<td>Wild mouse</td>
<td>65</td>
<td>38</td>
<td>2,016,563</td>
<td>101</td>
<td>2,097</td>
</tr>
<tr>
<td><em>Helicobacter</em> sp. MIT strain 11-5569</td>
<td>JRBP0000000000</td>
<td>Mouse</td>
<td>80</td>
<td>35</td>
<td>2,024,356</td>
<td>83</td>
<td>2,135</td>
</tr>
</tbody>
</table>
H. muridarum and H. trogontum ATCC 700144 possess gtt, a Helicobacter pylori virulence factor that leads to cell cycle arrest, necrosis, and apoptosis (35). cdtB is present in H. muridarum, H. typhlonius, and the unnamed MIT strains 01-6451, 03-1614, 03-1616, and 05-5293. The entire cdABC cluster was found in H. muridarum and the unnamed MIT strains 01-6451, 03-1614, 03-1616, and 05-5293. Multiple type IV secretion genes (virB2-virB11 or virD4) were found in all species presented, excluding H. muridarum and MIT strain 01-6451. Type VI genes (hcr, icmF, vasD, and vgrG), associated with pathogenicity (36, 37), were less common. icmF, vasD, and vgrG were found in H. trogontum ATCC 700114 and the unnamed MIT strain 03-1614. vgrG was found in H. typhlonius and several unnamed species (01-6451, 03-1616, and 11-5659).

Nucleotide sequence accession numbers. The genome sequences have been submitted to GenBank under the accession numbers listed in Table 1.

ACKNOWLEDGMENTS

This project has been funded in part with federal funds from the National Institutes of Health, under grants R01CA067529, R01OD011141, P01CA26731, and P30ES02019 (all to J.G.F.).

REFERENCES


