Co-infection of the Siberian hamster (*Phodopus sungorus*) with a novel *Helicobacter* sp. and *Campylobacter* sp.

Claude M. Nagamine,† Zeli Shen,‡ Richard H. Luong,* Gabriel P. McKeon,† Norman F. Ruby* and James G. Fox*

1Department of Comparative Medicine, Stanford University School of Medicine, Stanford, CA 94305, USA
2Division of Comparative Medicine, Massachusetts Institute of Technology, Cambridge, MA 02139, USA
3Department of Biology, Stanford University, Stanford, CA 94305, USA

We report the isolation of a novel helicobacter isolated from the caecum of the Siberian hamster (*Phodopus sungorus*). Sequence analysis showed 97% sequence similarity to *Helicobacter ganmani*. In addition, we report the co-infection of these Siberian hamsters with a *Campylobacter* sp. and a second *Helicobacter* sp. with 99% sequence similarity to *Helicobacter* sp. flexispira taxon 8 (*Helicobacter bilis*), a species isolated previously from patients with bacteraemia. Gross necropsy and histopathology did not reveal any overt pathological lesions of the liver and gastrointestinal tract that could be attributed to the *Helicobacter* or *Campylobacter* spp. infections. This is the first helicobacter to be identified in the Siberian hamster and the first report of co-infection of *Helicobacter* spp. and *Campylobacter* sp. in asymptomatic Siberian hamsters.

INTRODUCTION

The genus *Helicobacter* comprises Gram-negative, anaerobic or microaerophilic, fastidious, flagellated, spiral bacteria that inhabit the gastrointestinal and hepatobiliary tracts of many mammals and birds (Fox et al., 2006; Whary & Fox, 2004). Many *Helicobacter* spp. have been associated with diseases in humans (De Groote et al., 2000), pets and research animals (Whary & Fox, 2004). In humans, *Helicobacter pylori* infects 50% of the world’s population, causing peptic ulcers in 10–15% and gastric adenocarcinomas in 1–2% of infected individuals (Fox & Wang, 2007). To date, five *Helicobacter* spp. have been isolated from laboratory hamsters, all from the Syrian hamster (*Mesocricetus auratus*). *Helicobacter aurati* was isolated from the caeca and inflamed stomachs of hamsters with gastritis (Patterson et al., 2000a) and *Helicobacter cholecystus* was isolated from the gallbladders of hamsters with cholangiobiosis and cirrhotic pancreatic lesions (Franklin et al., 1996). *Helicobacter cinaedi* (Gebhart et al., 1989) and *Helicobacter mesocricetorum* (Simmons et al., 2000) were isolated from the faecal pellets of normal hamsters. *H. cinaedi* has also been isolated from human patients, raising the possibility of zoonotic transmission to susceptible humans (Kiehlbauch et al., 1994). More recently, a novel *Helicobacter* sp., most closely related to *Flexispira* taxon 8 within the *Helicobacter bilis/H. cinaedi* group, was isolated from the livers and caeca of aged (18–24 months) hamsters with chronic hepatitis, hepatic dysplasia and fibrosis, and/or biliary hyperplasia (Fox et al., 2009). These reports show that helicobacters, under certain conditions, can cause lesions in hamsters, which may serve as a model for certain human conditions, such as chronic hepatitis, gastritis, cholangiobiosis and cirrhotic pancreatic lesions. There have been no reports of the isolation of helicobacters from other hamster species.

Similar to *Helicobacter* spp., *Campylobacter* spp. are Gram-negative, anaerobic or microaerophilic, fastidious, spiral-, curved- or rod-shaped, flagellated bacteria that inhabit the gastrointestinal tract of mammals and birds (Man, 2011). *Campylobacteriosis* is one of the leading causes of foodborne bacterial gastroenteritis in the USA (Scallan et al., 2011) and is a potential zoonotic pathogen from pets and laboratory animals (Beisele et al., 2011; Fox, 1982; Kaur et al., 2011; Rossi et al., 2008; Shen et al., 2001). Indeed, the Syrian hamster can serve as a reservoir for *Campylobacter jejuni* (Fox et al., 1981), and *Campylobacter* spp. DNA, along with
H. aurati, has been identified in the stomachs of Syrian hamsters with gastritis (Patterson et al., 2000b), highlighting the zoonotic risk. We found no reports on whether Campylobacter spp. exist in other hamster species.

The Siberian hamster (Phodopus sungorus), also called the Russian or Djungarian hamster, is a small (head–body length 7.0–9.0 cm, weight 19–45 g) hamster whose discontinuous distribution in the wild encompasses the steppes of south-west Siberia and northern-eastern Kazakhstan and the steppes in the Minusinsk depression of the southern Krasnoyarsk Krai region of Russia (Ross, 1998). P. sungorus and its sibling species, Phodopus campbelli, have the most compressed reproductive cycles of the Eutheria, with females being able to wean a litter every 18 days (Newkirk et al., 1997). The Siberian hamster has a mean life span of 2.0–2.5 years (Ross, 1998) and is used in a variety of chronological studies (Butler & Zucker, 2009; Grone et al., 2011; Ruby et al., 2008; Schöttner et al., 2011; Tups et al., 2012) and in research on the evolution of parental behaviour (Ma et al., 2005; Stulberg & Wynne-Edwards, 1998; Wynne-Edwards & Lisk, 1989), parasite susceptibility (Ike et al., 2005; Uchida et al., 2003) and cancer (Kondo et al., 2008, 2009). As the popularity of the Siberian hamster increased as a household pet, there has been a corresponding increase in reports on Siberian hamster-associated respiratory allergic disease (Berto et al., 2002) and bite-induced anaphylaxis in humans (Lim et al., 2004; Niitsuma et al., 2003; Torres et al., 2012).

Given previous reports of the isolation of Campylobacter and Helicobacter spp. in Syrian hamsters, we hypothesized that Helicobacter spp. and/or Campylobacter spp. are present in Siberian hamsters. Here, we report the isolation of a novel Helicobacter sp. from the caeca of aged Siberian hamsters. This is the first Helicobacter sp. identified in the Siberian hamster. In addition, we report the co-infection of Siberian hamsters with a novel Campylobacter sp.

**METHODS**

Animals and husbandry. Founders for the Siberian hamster breeding colony were obtained from Dr I. Zucker (University of California, Berkeley, CA, USA) and maintained in an Association for the Assessment and Accreditation for Laboratory Animal Care, International-accredited facility. The animals were housed in solid-bottomed, static cages (9 x 7 x 6 in) with hardwood bedding (San-Chips; P. J. Murphy), maintained on a long-day photoperiod (16 h light: 8 h dark) at an ambient temperature of 22 °C, and were provided a commercial rodent diet (LabDiet 5015; Purina) and tap water ad libitum. The colony was monitored for pathogens transmissible to mice by the exposure of 5-week-old Crl: CD1 (ICR) female mouse sentinel to dirty bedding from the colony. Health reports from the vendor (Charles River Laboratories) indicated that the Crl: CD1 (ICR) colony was negative for Helicobacter spp. After exposure to hamster dirty bedding, the mouse sentinel was found to be negative for mouse hepatitis virus, Sendai virus, mouse parvovirus, minute virus of mice, ectromelia virus, lymphophoric choriomeningitis virus, mouse rotavirus, Theiler’s murine encephalomyelitis virus, mouse adenoviruses 1 and 2; and ecto- and endoparasites. In the non-barrier facility where the Siberian hamsters were housed, screening for helicobacter and campylobacter is not performed routinely on the mouse sentinels. Follicular mites, presumably Demodex sp., were identified in the hair of asymptomatic hamsters from this colony (McKeon et al., 2011). The research was approved by the Stanford University Institutional Animal Care and Use Committee.

Gross necropsy and histopathology. Eighteen aged (17–27 month) Siberian hamsters (12 female, six male) were euthanized humanely by carbon dioxide asphyxiation and processed for macro- and microscopic pathological analyses (McKeon et al., 2011). All of the hamsters were retired breeders and were clinically healthy at the time of euthanasia. All major soft and hard tissues and organs were fixed in 10 % neutral buffered formalin for 48–72 h, trimmed and then embedded in paraffin following routine procedures. Blocks were sectioned at 4–5 mm and representative sections of each tissue were processed for haematoxylin and eosin (H&E) staining. Select slides were silver stained using the Warthin–Starry method to identify argyrophilic bacteria. For the liver, all lobes except the caudate lobe were assessed using both H&E and Warthin–Starry staining. All slides were evaluated by one of the authors who is a board-certified veterinary pathologist (R. H. L).

Samples and bacterial isolation. Of the 18 Siberian hamsters, caecal samples were obtained from five females and three males (17–20 months). Each caecum was divided into three samples: one was used for PCR analysis, a second sample was stored in sterile freeze medium (20 % glycerol in brain–heart infusion broth) at −80 °C and used for microbiological culture, and the third sample was fixed in 10 % neutral buffered formalin for histology. Faecal samples from a second group of 12 live hamsters were collected, placed into freeze medium and stored at −80 °C.

Caeal and faecal samples in freeze medium were homogenized and 100 μl aliquots of each slurry were placed on cefoperazone, vancomycin and amphotericin B (CVA) plates and passed through a 0.45 μm syringe filter onto blood agar plates (trypsinase soy agar plate with 5 % sheep blood; Remel Laboratories). The CVA and blood agar plates were incubated at 37 °C under micro-aerobic conditions in a vented jar containing N2, H2 and CO2 (80:10:10) and were checked every 2–3 days for 3 weeks. Suspected bacterial growth was identified as Helicobacter or Campylobacter sp. on the basis of colony morphology, phase microscopy and Gram staining (Shen et al., 2001).

Helicobacter and Campylobacter spp. PCR amplification, sequencing and sequence analysis. A portion of the caecal samples was sent to a commercial diagnostic laboratory for Helicobacter genus-specific and H. bilis- and H. hepaticus-specific PCR. DNA samples were extracted from Helicobacter and Campylobacter spp. culture isolates using a High Pure PCR Template Preparation kit (Roche Molecular Biochemicals) and from Siberian hamster faecal samples using a QIAamp DNA Stool Mini Kit (Qiagen). DNA samples were amplified by PCR using Helicobacter genus-specific and Campylobacter-specific primers as described previously (Fox et al., 2009; Shen et al., 2001, 2005). The Helicobacter genus-specific primers (C05: 5’-ACTTTACCCCCAGTCCTG-3’, and C097: 5’-GCTATGACGCGGTATCC-3’) amplified a 1.2 kb amplicon from the 16S rRNA gene (Fox et al., 1998). The Campylobacter-specific primers (C98: 5’-GATTITACCCCCACCA-3’, and C99: 5’-GGGTGAGGATGACACCT-3’) amplified a 300 bp PCR product from the 16S rRNA gene. The full-length 16S rRNA sequences of 1.6 kb from three helicobacter isolates and four campylobacter isolates were amplified using primers F24 and F25 (Dewhirst et al., 2005). The 1.6 kb 16S rRNA amplicons and 1.2 kb amplicons from helicobacter-specific PCR were purified, directly sequenced and compared with published helicobacter and campylobacter 16S rRNA genes in GenBank using a BLAST search. Lasergene software (DNASTAR) was used to reconstruct the 16S rRNA gene phylogenetic trees.
RESULTS

Gross necropsy and histopathology

Gross and histological examination of the stomachs, small intestines, large intestines (including caeca), livers and gallbladders failed to reveal any significant lesions that could be similar to pathologies associated with Helicobacter spp. colonization and/or infection in any of the Siberian hamsters in this study. This included absence of significant gastritis, adenocarcinomas of the glandular stomach, portal hepatitis, hepatic nodular dysplasia, cholangiohepatitis, cholangiofibrosis, pancreatitis or typhlocolitis (Fig. 1). Warthin–Starry staining also failed to reveal any observable spiral bacteria in the stomachs, caeca, liver and gallbladder in these Siberian hamsters. In addition, no pathology related to Campylobacter spp. infection was observed.

Incidental findings in the stomachs, small intestines, large intestines (including caeca), livers and gallbladders included a squamous papilloma in the non-glandular stomach (n=1) and hepatic hemangiosarcoma (n=1), as reported previously (McKeon et al., 2011).

PCR and sequence analysis

PCR analysis of the eight caecal samples at the commercial diagnostic laboratory showed that all were positive using a Helicobacter genus-specific PCR assay but were negative for H. bilis- or H. hepaticus-specific PCR assays. Culture of the same eight caecal samples was performed on both CVA and blood agar plates. All eight samples produced positive growth on the CVA plates, but only two samples produced positive growth on the blood agar plates (Table 1). PCR amplification of the cultures from all CVA plates and one blood agar plate were positive for Helicobacter spp.; the culture from one blood agar plate was positive for Campylobacter spp. Direct sequencing of all nine Helicobacter-positive reactions identified a previously undescribed Helicobacter sp. that had 97 % sequence similarity to H. ganmani (Fig. 2a, samples 10-5747, 10-5750 and 10-5753). Sequence analysis of the single Campylobacter-specific reaction showed a 99 % sequence similarity to a previous Campylobacter isolate, MIT 97-5311, originally isolated from a Syrian hamster (Fig. 2b).

PCR amplification of the 12 faecal samples using Helicobacter genus-specific primers showed five (41.7 %) to be helicobacter positive (Table 2). All helicobacter-positive faecal samples were also campylobacter positive (Table 2). An additional three faecal samples (25 %) were campylobacter positive only. Overall, eight faecal samples (66.7 %) were campylobacter positive. The 1.6 kb 16S rRNA sequence analysis of four campylobacter isolates showed 99 % sequence similarity to MIT 97-5311 (Fig. 2b, samples 10-7217 and 10-7222). Sequences of three 1.2 kb helicobacter-specific PCR products showed 99 % sequence similarity to Helicobacter sp. flexispira taxon 8 (=H. bilis) (Table 2, Fig. 2a, sample 10-7218), a helicobacter isolated previously from bacteraemic human patients (Iten et al., 2001; Sorlin et al., 1999; Tee et al.,...
Both strains had a 184 bp intervening sequence region. This strain also had over 99% identity with an *H. bilis* isolate previously isolated from Syrian hamsters (Fox *et al.*, 2009), although the intervening sequence was not present in Syrian hamster *H. bilis* strains. Table 3 shows a summary of the helicobacter and campylobacter samples.

**DISCUSSION**

The Siberian hamster is a laboratory research animal and a common household pet. Knowledge of its microbiota allows laboratory animal medicine veterinarians to take the necessary precautions to avoid the transmission of potential pathogens to other laboratory animals and humans. Our data showed that the Siberian hamster colony sampled harboured at least two *Helicobacter* spp., a novel helicobacter with 97% sequence similarity to *H. ganmani* (Fig. 2a, samples 10-5747, 10-5750 and 10-5753) and a helicobacter with 99% sequence similarity to *Helicobacter* sp. flexispira taxon 8 (=*H. bilis*) (Fig. 2a, sample 10-7218). In addition, this colony also harboured a novel campylobacter with 99% sequence similarity to campylobacter isolate MIT 97-5311 (Fig. 2b, samples 10-7217 and 10-7222). It is unclear why the novel *H. ganmani*-like species was not amplified from faecal samples (Table 3). The fact that the caecal and faecal samples were obtained at different times may have contributed to this discrepancy. PCR of the caecal samples sent initially to the commercial diagnostic laboratory failed to identify *H. bilis*, whereas *H. bilis* was identified in the three faecal samples that were sequenced (Table 2). One explanation for this discrepancy is that, although mouse and Siberian hamster *H. bilis* share 99% sequence similarity overall, mouse and Siberian hamster *H. bilis* sequences in the region where the PCR primer were designed are different. Consequently, mouse *H. bilis* primers may not amplify Siberian hamster *H. bilis*.

Molecular data suggest that bacterial 16S RNA gene sequence homologies of <97.5% are unlikely to be from the same species (Stackebrandt & Goebel, 1994). Therefore, it is highly probable that the *Helicobacter* sp. isolated in the Siberian hamster represents a novel species. Additional studies are required to fully characterize this *Helicobacter* sp. (Dewhirst *et al.*, 2000). Also of interest is the identification of hepatic hemangiosarcoma in an aged female Siberian hamster (McKeon *et al.*, 2011). In B6C3F1 male mice with *H. hepaticus*-associated hepatitis, the incidence of both hepatocellular carcinoma and hemangiosarcoma was higher compared with control, uninfected mice (Hailey *et al.*, 1998). However, given that we did not observe any evidence of helicobacter-induced lesions in the Siberian hamsters, whether there is a causative relationship between *Helicobacter* spp. infection and hemangiosarcoma in Siberian hamsters requires further studies.

The *Campylobacter* sp. identified had 99% sequence similarity to a previous isolate, MIT 97-5311, that was isolated from the caecum of a Syrian hamster. Co-infection with *Campylobacter* spp. and *Helicobacter marmotae* was recently reported in wild-caught black-tailed prairie dogs (*Cynomys ludovicianus*) (Beisele *et al.*, 2011). Unlike the prairie dogs, the present Siberian hamster colony has been maintained in the laboratory for several generations and the identification of *Campylobacter* sp. was unexpected. The authors are unaware of any reports on the prevalence of *Campylobacter* spp. infection among academic rodent colonies. This may be due to the low prevalence of *Campylobacter* spp. in research rodent colonies and/or the

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Table 1. Sex and age of Siberian hamsters that provided the caecal samples, microbiology culture conditions and sequence analysis

<table>
<thead>
<tr>
<th>Sex/age (months)</th>
<th>MIT ID</th>
<th>Plate type</th>
<th>Growth</th>
<th>Sequence similarity</th>
</tr>
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<tbody>
<tr>
<td>F/17</td>
<td>10-5748</td>
<td>CVA</td>
<td>+</td>
<td>97% <em>Helicobacter ganmani</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Blood agar</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>F/17</td>
<td>10-5750</td>
<td>CVA</td>
<td>+</td>
<td>97% <em>Helicobacter ganmani</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Blood agar</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>M/18</td>
<td>10-5749</td>
<td>CVA</td>
<td>+</td>
<td>97% <em>Helicobacter ganmani</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Blood agar</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>M/18</td>
<td>10-5751</td>
<td>CVA</td>
<td>+</td>
<td>97% <em>Helicobacter ganmani</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Blood agar</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>F/19</td>
<td>10-5747</td>
<td>CVA</td>
<td>+</td>
<td>97% <em>Helicobacter ganmani</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Blood agar</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>F/20</td>
<td>10-5752</td>
<td>CVA</td>
<td>+</td>
<td>97% <em>Helicobacter ganmani</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Blood agar</td>
<td>+</td>
<td>97% <em>Helicobacter ganmani</em></td>
</tr>
<tr>
<td>F/20</td>
<td>10-5753</td>
<td>CVA</td>
<td>+</td>
<td>97% <em>Helicobacter ganmani</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Blood agar</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>M/20</td>
<td>10-5754</td>
<td>CVA plate</td>
<td>+</td>
<td>97% <em>Helicobacter ganmani</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Blood</td>
<td>+</td>
<td>99% <em>Campylobacter MIT 97-5311</em></td>
</tr>
</tbody>
</table>
**Fig. 2.** Phylogenetic trees of 16s rRNA gene sequences showing the relationship of the helicobacter (Fig. 2a) and campylobacter (Fig. 2b) isolates in the present report (arrows) to previously sequenced isolates. GenBank accession numbers for the sequences used are shown. Bars, nucleotide substitutions per site.

**Table 2.** Helicobacter and Campylobacter PCR results of Siberian hamster faecal samples and sequence similarity of the amplicons

<table>
<thead>
<tr>
<th>MIT ID</th>
<th>Helicobacter PCR</th>
<th>Sequence similarity</th>
<th>Campylobacter PCR</th>
<th>Sequence similarity</th>
</tr>
</thead>
<tbody>
<tr>
<td>10-7214</td>
<td>+</td>
<td></td>
<td>+</td>
<td>99% MIT97-5311</td>
</tr>
<tr>
<td>10-7215</td>
<td>+</td>
<td></td>
<td>+</td>
<td>99% MIT97-5311</td>
</tr>
<tr>
<td>10-7216</td>
<td>–</td>
<td></td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>10-7217</td>
<td>–</td>
<td></td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>10-7218</td>
<td>+</td>
<td>99% Helicobacter sp. flexispira and 99% H.bilis</td>
<td>+</td>
<td>99% MIT97-5311</td>
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<tr>
<td>10-7219</td>
<td>+</td>
<td>99% Helicobacter sp. flexispira and 99% H.bilis</td>
<td>+</td>
<td>99% MIT97-5311</td>
</tr>
<tr>
<td>10-7220</td>
<td>–</td>
<td></td>
<td>–</td>
<td></td>
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<td>10-7221</td>
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<td>–</td>
<td></td>
<td>+</td>
<td>99% MIT97-5311</td>
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<td>10-7223</td>
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<td></td>
</tr>
<tr>
<td>10-7224</td>
<td>–</td>
<td></td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>10-7225</td>
<td>+</td>
<td>99% Helicobacter sp. flexispira and 99% H.bilis</td>
<td>+</td>
<td></td>
</tr>
</tbody>
</table>
rarity of *Campylobacter* spp. diagnostic testing. Indeed, except for Syrian hamster colonies, most commercial and academic rodent colonies are not routinely screened for *Campylobacter* spp. (Livingston & Riley, 2003; Mähler et al., 2014; Pritchett-Corning et al., 2009; Yamamoto et al., 2001). *Helicobacter* sp. flexispira taxon 8 has been isolated from immunocompromised and immunocompetent patients with bacteremia (Iten et al., 2001; Sorlin et al., 1999; Tee et al., 1998), raising the possibility that this Siberian hamster *Helicobacter* sp. may have zoonotic potential. Additional studies are needed to determine the pathogenic potential of the Siberian hamster *Campylobacter* and the *Helicobacter* spp. to other laboratory animals and/or humans.

### ACKNOWLEDGEMENTS

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### REFERENCES


### Table 3. Summary of *Helicobacter* and *Campylobacter* results for Siberian hamster caecal and faecal samples

<table>
<thead>
<tr>
<th>Species</th>
<th>Test</th>
<th>Caecum</th>
<th>Faeces</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Helicobacter</em></td>
<td>Positive rate</td>
<td>8/8</td>
<td>5/12</td>
</tr>
<tr>
<td></td>
<td>16s rRNA sequences</td>
<td>Novel <em>Helicobacter</em></td>
<td><em>H. sp. flexispira taxon 8 or H. bilis</em></td>
</tr>
<tr>
<td><em>Campylobacter</em></td>
<td>Positive rate</td>
<td>1/8</td>
<td>8/12</td>
</tr>
<tr>
<td></td>
<td>16s rRNA sequences</td>
<td><em>Campylobacter</em> (MIT 97-5311)</td>
<td><em>Campylobacter</em> (MIT 97-5311)</td>
</tr>
</tbody>
</table>


