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

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Abstract

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 cholangiofibrosis and centrilobular pancreatitis (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, cholangiofibrosis and centrilobular pancreatitis. 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 north-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 chronobiological 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 (Bertó *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 (9×7×6 in) with hardwood bedding (Sani-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 sentinels 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 sentinels were found to be negative for mouse hepatitis virus, Sendai virus, mouse parvovirus, minute virus of mice, ectromelia virus, lymphocytic 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 haired skin 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 µm 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.

Caecal 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 (trypticase 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'-ACTTCACCCAGTCGCTG-3', and C97: 5'-GCTATGACGGGTATCC-3') amplified a 1.2 kb amplicon from the 16S rRNA gene (Fox *et al.*, 1998). The *Campylobacter*-specific primers (C98: 5'-GATTTTACCCCTACACCA-3', and C99: 5'-

GCGTGGAGGATGACACCT-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.

Go to:

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.*, 1998). 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 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 bacteraemia (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.

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Notes

Abbreviations:

H&E

haematoxylin and eosin

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Table 1.

Sex and age of Siberian hamsters that provided the caecal samples, microbiology culture conditions and sequence analysis. These same samples were positive in a *Helicobacter* genus-specific PCR assay but negative for *H. hepaticus*- and *H. bilis*-specific PCR assays at an independent diagnostic laboratory. All blood agar cultures were passed through a 0.45 µm filter. F, female; M, male.

Sex/age (months)	MIT ID	Plate type	Growt h	Sequence similarity
F/17	10- 5748	CVA	+	97% <i>Helicobacter ganmani</i>
Blood agar	–			
F/17	10- 5750	CVA	+	97% <i>Helicobacter ganmani</i>
Blood agar	–			
M/18	10- 5749	CVA	+	97% <i>Helicobacter ganmani</i>
Blood agar	–			
M/18	10- 5751	CVA	+	97% <i>Helicobacter ganmani</i>
Blood agar	–			
F/19	10- 5747	CVA	+	97% <i>Helicobacter ganmani</i>
Blood agar	–			
F/20	10- 5752	CVA	+	97% <i>Helicobacter ganmani</i>
Blood agar	+	97% <i>Helicobacter ganmani</i>		
F/20	10- 5753	CVA	+	97% <i>Helicobacter ganmani</i>
Blood agar	–			

Sex/age (months)	MIT ID	Plate type	Growt h	Sequence similarity
M/20	10-5754	CVA plate	+	97% <i>Helicobacter ganmani</i>
Blood	+	99% <i>Campylobacter</i> MIT 97-5311		

Table 2.

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

MIT ID	<i>Helicobacter</i> PC R	Sequence similarity	<i>Campylobacter</i> PC R	Sequence similarity
10-	+		+	99%

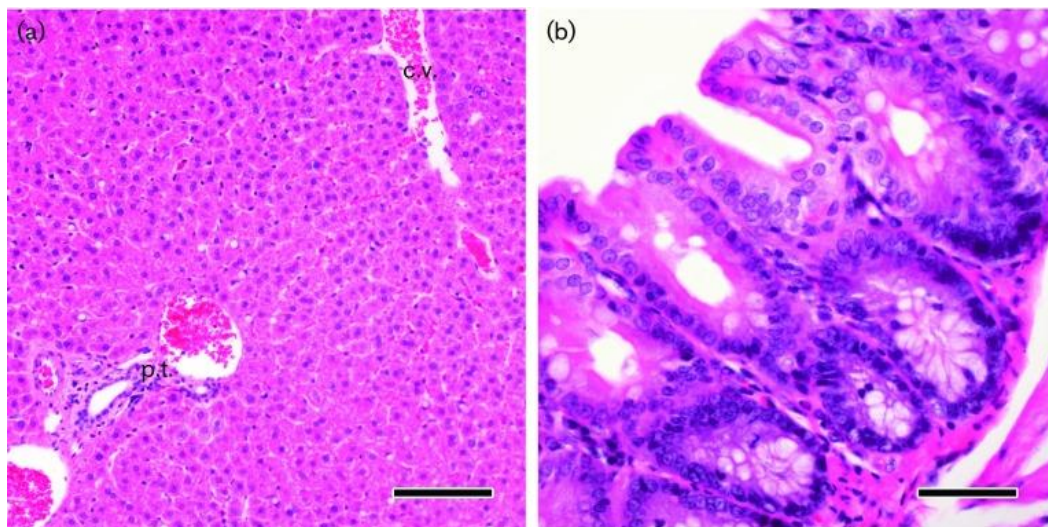
MIT ID	<i>Helicobacter</i> PCR	Sequence similarity	<i>Campylobacter</i> PCR	Sequence similarity
7214				MIT97-5311
10-7215	+		+	99% MIT97-5311
10-7216	-		-	
10-7217	-		+	99% MIT97-5311
10-7218	+	99% <i>Helicobacter</i> sp. flexispira and 99% <i>H.bilis</i>	+	
10-7219	+	99% <i>Helicobacter</i> sp. flexispira and 99% <i>H.bilis</i>	+	
10-7220	-		-	
10-7221	-		-	
10-7222	-		+	99% MIT97-5311
10-7223	-		-	
10-7224	-		+	
10-7225	+	99% <i>Helicobacter</i> sp. flexispira and 99% <i>H.bilis</i>	+	

Table 3.

Summary of *Helicobacter* and *Campylobacter* results for Siberian hamster caecal and faecal samples

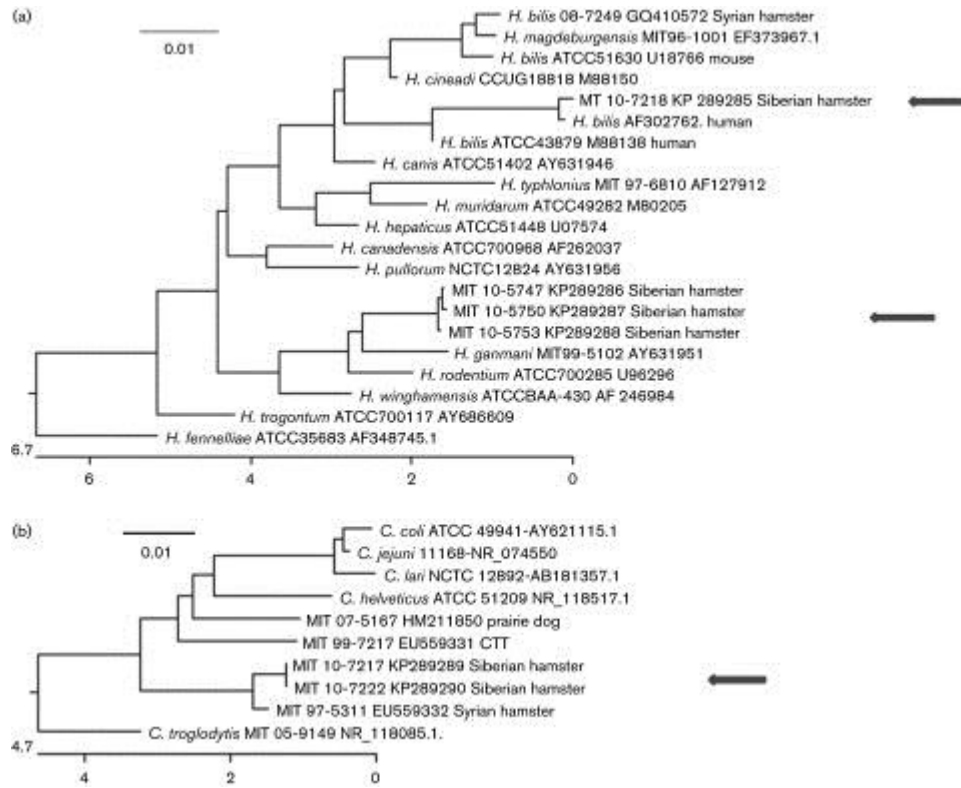
Species	Test	Caecum	Faeces
<i>Helicobacter</i>	Positive rate	8/8	5/12
	16s rRNA sequences	Novel <i>Helicobacter</i>	<i>H. sp. flexispira</i> taxon 8 or <i>H. bilis</i>
<i>Campylobacter</i>	Positive rate	1/8	8/12
	16s rRNA sequences	<i>Campylobacter</i> (MIT 97-5311)	<i>Campylobacter</i> (MIT 97-5311)

Fig. 1.



Photomicrographs of liver (a) and caecum (b) from a study Siberian hamster. The liver displayed a normal hepatic lobular histology, with observable portal triads (p.t.) and central veins (c.v.), and minimal to no evidence of inflammation, fibrosis or neoplasia. Similarly, the caecum also displayed normal histology, with the mucosa containing normal intestinal glands of regular height, with minimal inflammation, fibrosis or neoplasia. Additionally, the remainder of the gastrointestinal tract (including non-glandular stomach, glandular stomach, small intestines and colon) and gallbladder were also histologically normal (not shown). Warthin–Starry-stained sections also failed to reveal the presence of any spiral bacteria in these sections. Overall, these photomicrographs are representative of the gastrointestinal tracts and hepatobiliary system in all the Siberian hamsters examined in this study, except for the presence of a squamous papilloma in the non-glandular stomach in one hamster, and hemangiosarcoma in the liver of another hamster. H&E stain. Bars, 100 μ m (a); 50 μ m (b).

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.