Isolation and characterization of a novel Helicobacter species, Helicobacter jaachi sp. nov., from common marmosets (Callithrix jaachus)

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Purpose-bred common marmosets from domestic sources housed in a US research facility, and used in multiple drug discovery programmes, were noted to have a high incidence of spontaneous inflammatory bowel disease and sporadic cholecystitis and cholangiohepatitis. Inflammatory infiltrates increased in incidence and severity with age. Because Helicobacter spp. have been linked to gastrointestinal diseases, samples from the gastrointestinal tracts of 39 marmosets were screened for Helicobacter spp. by culture and PCR. Helicobacter spp. were frequently detected in marmosets; 28.2 % of the marmosets were positive for a proposed novel species, Helicobacter jaachi sp. nov., by culture, and 48.7 % were positive by Helicobacter genus-specific PCR. Seventeen strains of Helicobacter sp. from 11 marmosets were cultured from various gastrointestinal sites. Older animals (age 6–11 years) had a higher helicobacter prevalence rate (57.1 %) compared with younger animals (age 3–5 years), which had a 27.2 % prevalence rate. Cells of H. jaachi sp. nov. were catalase, urease and oxidase positive and had fusiform morphology, with periplasmic fibres and multiple bipolar, sheathed flagella. All isolates had similar 16S and 23S rRNA sequences, which clustered as representatives of a novel Helicobacter species closely related to ‘Helicobacter sanguini’ (97 %), a species isolated from cotton-top tamarins and ‘Helicobacter callitrichis’ (96 %) isolated previously from the faeces of common marmosets. The whole genome sequence of one of the liver isolates, H. jaachi sp. nov. MIT 09-6949T, had a 1.9 Mb genome length with a 41 mol% DNA G+C content. The type strain of Helicobacter jaachi sp. nov., MIT 09-6949T, has been deposited in the BCCM/LMG Bacteria Collection as LMG 28613T. These findings add to the increasing number of animal species with gastrointestinal disease in which novel enterohepatic Helicobacter spp. have been isolated.

INTRODUCTION

The common marmoset (Callithrix jacchus) has been utilized as a non-human primate model for nonclinical pharmaceutical development (Orsi et al., 2011). The common marmoset’s importance as a model in biomedical research will be enhanced with the recent publication of its genome (Worley et al., 2014). An illustration of the marmoset’s usefulness is its use in neuroscience research and genome editing technology (Kishi et al., 2014). A small common marmoset colony was established at GlaxoSmithKline to serve as a preclinical model for drug discovery and development. Common marmosets develop diet-induced obesity phenotypes when placed on high fat diets and were employed in studies to help develop anti-obesity compounds (Tardif et al., 2009). Over the course of several years, complete necropsies were performed on 46 marmosets originating from this colony. The spectrum of spontaneous pathology documented was similar to that reported in the literature for common marmosets (Chalmers et al., 1983; David et al., 2009; Kasparetz et al., 2006; Ludlage et al., 2005)

Abbreviations: EHS, enterohepatic Helicobacter species; IBD, inflammatory bowel disease.

The GenBank/EMBL/DDBJ accession numbers for the 16S rDNA sequences of MIT 09-6948 and MIT 09-6949T are KP701327 and KP701326, respectively. Those for the 23S rRNA and hsp60 gene sequences of MIT 09-6949T are KP701328 and KP701329. The accession number for the whole genome sequence of MIT 09-6949T is JRPR00000000.
and revealed a high incidence of inflammatory bowel disease and hepatobiliary inflammation.

In addition to the inflammatory lesions found at necropsy, the colony was noted to have clinical gastrointestinal disease as evidenced by sporadic cases of diarrhoea and chronic weight loss. The finding of inflammatory bowel disease in conjunction with hepatobiliary inflammatory changes is reminiscent of findings reported in other non-human primates (NHP) infected with Helicobacter spp. A novel Helicobacter sp. was identified in cotton-top tamarins diagnosed with progressive colitis mimicking features of ulcerative colitis in humans (Saunders et al., 1999). A novel Helicobacter species, ‘Helicobacter callitrichis’, was isolated from the faeces of clinically normal, common marmosets (Won et al., 2007). An unclassified Helicobacter sp. was identified in the stomachs of three species of marmosets, based on immunohistochemistry of paraffin-imbedded tissues using a polyclonal Helicobacter pylori antibody (de Mello et al., 2005). Helicobacter macaceae and Helicobacter cinaedi have also been isolated from the faeces of clinically affected macaques with chronic colitis or from animals without clinical disease (Fox et al., 2001a, b, 2007; Lertpiriyapong et al., 2014; Marini et al., 2010). These prior findings prompted us to survey a common marmoset colony for the presence of helicobacters. In this study, 39 marmosets from the colony were screened by culture and PCR for the presence of Helicobacter spp. We characterized 17 strains representing Helicobacter jaachi sp. nov., a novel Helicobacter species isolated from clinical and pathology specimens from 11 of the animals.

METHODS

Animals. A total of 39 common marmosets (Callithrix jacchus), 37 males and 2 females, from a pharmaceutical company research colony of approximately 140 animals, were screened for helicobacter. Animals were originally obtained from a breeding colony formulated from multiple domestic sources (Harlan, Madison, WI, USA). All marmosets were housed singly due to scientific study considerations. All marmosets were from multiple domestic sources (Harlan, Madison, WI, USA). All marmosets were housed singly due to scientific study considerations. All marmosets had direct access to corncob bedding (Bed-o’Cob, The Andersons) and raw wooden sticks made of ash as well as numerous other enrichment items. The rooms were set on a 12:12 h light:dark cycle, and temperature and humidity were controlled at 75 ± 3 °F (23.9 ± 1.67 °C) and 50% ± 10%. The regular diet was Mazuri’s 5M15 (Mazuri), and a high fat variant containing 12% lard and 1% fructose added on a weight basis. All marmosets were offered small portions of various fresh and dried fruits, yogurt, pudding and cereal daily to add variety.

Marmoset ages ranged from 3 to 11 years of age; 11 younger animals were age 3–5 years, and 28 were older animals with ages ranging from 6 to 11 years. Seventeen animals were maintained intermittently on a high fat diet; eight animals were maintained on a regular diet, and 14 animals had an unknown dietary history. Sixteen animals were known to have been treated with one or more investigational test compounds, while nine were untreated controls, and 14 had unknown treatment-status. Five marmosets had diarrhoea, 20 without diarrhoea, and 1 were of unknown status at the time of culture.

From these animals, 30 faecal samples, 15 liver samples, 14 gallbladder samples, 13 stomach samples, 2 jejunum samples, 2 colon samples and 1 caecum sample were collected at necropsy in freeze media [20% glycol in Brucella broth (BD, Franklin Lakes, NJ)] and kept at −80 °C, prior to shipping to MIT for analysis.

Pathology examination. Selected animals were euthanized by exsanguination under isoflurane anaesthesia and subjected to a complete necropsy. Tissues from all major organs were fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned at 5 μm, and stained with haematoxylin and eosin. Prior to the initiation of the helicobacter culture and PCR studies, tissues were examined from 46 marmosets that were removed from the colony. Hepatobiliary and gastrointestinal tissues were examined from an additional 27 marmosets from the group of 39 animals subjected to helicobacter culture and/or PCR. Sections of liver, gallbladder, stomach, small intestine, caecum and colon were reviewed from all animals.

Bacterial isolation. Samples from liver, gallbladder, gastrointestinal tissues and faecal samples were homogenized in tissue grinders with 1.5 ml of freeze media containing 20% glycerol in Brucella broth. One millilitre of the slurry was gently pushed through a 0.65 μm-pore size syringe filter, and the homogenate was streaked onto a trypticase soy agar (with 5% sheep blood) plate (Remel Laboratories). The slurry (0.1 ml) was also directly added onto CVA plates which contained cepoerezan, vancomycin and amphotericin B (Remel). The cultures were then incubated at 25, 37 and 42 °C under microaerobic conditions in vented jars containing N2, H2 and CO2 (80:10:10). Biochemical characterization was performed on five isolates as previously described (Shen et al., 2005).

Electron microscopy. Isolates identified as H. jaachi sp. nov. were examined by electron microscopy. Cells grown on blood agar for 48 h were gently suspended in 10 mM Tris/HCl buffer (pH 7.4) at a concentration of about 10^8 cells ml⁻¹. Samples were negatively stained with 1% (w/v) phosphotungstic acid (pH 6.5) for 20 to 30 s. Specimens were examined with a JEOL model JEM-1200EX transmission electron microscope operating at 100 kV.

DNA extraction for PCR analysis. A High Pure PCR Template Preparation kit (Roche Molecular Biochemicals) was used for extraction of DNA from the bacterial isolates and the tissue samples according to the manufacturer’s directions.

Helicobacter genus-specific primers 9F (5’-GAGTTTGATYCTGGCTCAG-3’) and C05 (5’-ACTTACACCCAGTGCT-3’) were used to amplify a 1.2 kb PCR product from the 16S rRNA gene (Fox et al., 1998). PCR amplifications were performed using the Expand High Fidelity PCR System (Roche Molecular Biochemicals). The following conditions were used for amplification: 35 cycles of denaturation at 94 °C for 1 min, annealing at 58 °C for 1.5 min and elongation at 72 °C for 2 min, followed by an elongation step of 7 min at 72 °C.

RFLP analysis. PCR-amplified 1.2 kb fragments (20 μl) were digested with 10 U of restriction endonucleases AliI and XbaI (New England BioLabs) in the appropriate buffer recommended by the manufacturer at 37 °C for 3 h. Restriction patterns were compared after the digested PCR products were separated on a 3% agarose gel.

16S rRNA gene sequencing. Eleven strains of the marmoset isolates were subjected to 16S rRNA gene sequence analysis. The conserved primers 9F 5’-GAGTTTGATYCTGGCTCAG-3’ and 1541R 5’-AAGGAGGTGWTCCARC-3’ from 16S rRNA genes were used to amplify the 1.5 kb products (Fox et al., 2007). The amplicons were purified with a QIAquick PCR Purification kit (Qiagen) and directly sequenced using an ABI Prism BigDye terminator cycle sequencing ready reaction kit on a genetic analyser 3500 (Applied Biosystems).
A phylogenetic tree was reconstructed by the neighbour-joining method.

**23S rRNA gene sequencing.** The 23S rRNA gene sequence of liver isolate 09-6949 was analysed by assessment of the 23S region in the whole genome of this strain.

**Whole genome sequence analysis.** The 09-6949 liver isolate was sequenced using Illumina MiSeq sequencing technology, as described previously (Sheh et al., 2013). The 250 bp paired-end sequencing reads generated by MiSeq were assembled into contigs using Velvet (Zerbino & Birney, 2008). Sequences were annotated using the NCBI Prokaryotic Genomes Automatic Annotation Pipeline (Klimke et al., 2009).

**Fluorescent in situ hybridization for Helicobacter species.** Paraffin sections of colons were deparaffinized and rehydrated. *Helicobacter* genus-specific probes, HEL274 and HEL717, labelled with Cy3 were used (Integrated DNA Technologies) (Chan et al., 2005). Hybridization buffer (0.9 M NaCl, 100 mM Tris/HCl, 0.1 % SDS, 30 % formamide) with 5 ng probe ml⁻¹ was used with 5 ng probe ml⁻¹ was preheated for 10 min at 74.5 °C; 80 µl of this solution was added to each slide. Slides were covered in Parafilm and placed in a dark humidification chamber overnight at 48 °C. After incubation, slides were rinsed in double-distilled water and serially washed in pre-warmed rinsing buffers for 15 min each (Buffer 1: 0.9 M NaCl, 100 mM Tris/HCl, 0.01 % SDS; buffer 2: 0.9 M NaCl, 100 mM Tris/HCl). Slides were air-dried, mounted in Vectashield with DAPI (Vector Laboratories) and examined under a Zeiss Axioskop 2 fluorescent microscope. Tissues were considered positive for *Helicobacter* spp. if fluorescent spiral organisms were observed under the Rhod filter.

**RESULTS**

**Helicobacter isolation**

After 4–7 days of incubation under microaerobic conditions, 17 strains of helicobacter-like organisms were isolated from the liver, gallbladder, stomach, jejunum, colon, caecum and faeces of 11 marmosets (Table 1). The Gram-negative bacteria grew as a thin, spreading film on the agar surfaces. The biochemical characteristics of five isolates did not have c-glutamyl transpeptidase activity and all were sensitive to nalidixic acid and resistant to cefalotin. The organism grew in 1 % glycine and at 37 and 42 °C, but not at 25 °C.

**Electron microscopy**

By electron microscopy, cells of *H. jaachi* sp. nov. measured 3–6 µm in length and 0.2–0.5 µm in width (Fig. 1). The organisms were fusiform with periplasmic fibres and bipolar-sheathed flagella.

**Phylogenetic analysis**

Full 16S rRNA gene sequences were determined for six strains, which shared over 99 % sequence similarity with each other. The most closely related species was *Helicobacter sanguini* a helicobacter species isolated from cotton-top tamarin (97 %) (Fig. 2a). The 16S rRNA gene sequence similarity with *H. callitrichis*, a *Helicobacter* species isolated from marmoset faeces, was 96 %.

The 23S rRNA gene sequence of one of the liver isolates, MIT 09-6949, was analysed and compared with the 23S rRNA gene sequences of other *Helicobacter* spp., revealing that it was closely related to *H. sanguini* with 97 % identity (Fig. 2b).

The *hsp60* protein coding gene obtained from the whole genome sequence of MIT 09-6949 was compared with the 600 bp conserved region of other *Helicobacter* spp. The most closely related species was *Helicobacter typhlonius*, which had 87 % identity (Fig. 2c).

**RFLP analysis**

The 1.2 kb *Helicobacter*-specific PCR products of the 16S rRNA gene were subjected to digestion by *Alu*I and *Xba*I. By *Alu*I and *Xba*I digestion, five of the marmoset isolates had identical banding patterns; *‘H. sanguini’* and *H. macacae* had different patterns from *H. jaachi* sp. nov. by both *Alu*I and *Xba*I digestion (Fig. 3).

**Whole genome sequencing**

Using 163 contigs with a median coverage depth of 26-fold, the Velvet program calculated a 1 900 614 bp genome length with a 41 mol% DNA G+C content. Sequences were annotated using RAST (Rapid Annotations based on Subsystem Technology) and found 1992 coding sequences. Genes commonly used to characterize *Helicobacter*

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**Table 1. *Helicobacter* prevalence in marmosets analysed**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Faeces (%)</th>
<th>Liver (%)</th>
<th>Gallbladder (%)</th>
<th>Stomach (%)</th>
<th>Jejunum (%)</th>
<th>Colon (%)</th>
<th>Caecum (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Culture</td>
<td>8/30 (27)</td>
<td>2/15 (13)</td>
<td>1/14 (7)</td>
<td>1/13 (8)</td>
<td>2/14 (14)</td>
<td>2/12 (17)</td>
<td>1/12 (8)</td>
</tr>
<tr>
<td>PCR (%)</td>
<td>11/30 (37)</td>
<td>2/15 (13)</td>
<td>5/14 (36)</td>
<td>4/13 (31)</td>
<td>2/14 (14)</td>
<td>5/12 (41)</td>
<td>2/12 (17)</td>
</tr>
</tbody>
</table>

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spp. biochemically were evaluated. Catalase (EC 1.11.1.6) and urease genes (EC 3.5.1.5) were identified. However, γ-glutamyl transpeptidase, typically found in other species of Helicobacter, was not found.

We performed BLAST analysis of 57 genes including RTX (haemolysin), cdtABC (*Campylobacter jejuni* and *Helicobacter hepaticus*), TcdAB (*Clostridium difficile*), BFT (*Bacteroides fragilis*), small pore-forming toxins (*Clostridium* spp.), Shiga toxins (*Shigella dysenteriae*), actin-targeting toxins (*Clostridium* spp. and *Yersinia pseudotuberculosis*), type I secretion systems (T1SS, *Escherichia coli* and *Vibrio cholerae*), T2SS (*E. coli*, and *V. cholerae*), T3SS (*Y. pseudotuberculosis* and *E. coli*) and T4SS (*Agrobacterium tumefaciens*). Using Megablast, there were no significant hits. However, using tblastx, we found homology with the following genes with putative virulence characteristics:

- *A. tumefaciens* virB operon (T4SS) (AB011800, E value, 1.08 e-18), enterohemorrhagic *E. coli* haemolysin B (EHEC hlyB) (AAC70117.1, 1.10 e-36), *E. coli* gspD (T2SS) (1.68 e-13), *E. coli* gspE (T2SS) (1.54 e-86), *E. coli* gspF (T2SS) (4.83 e-38), *V. cholera* rtxB (DQ774425, 5.56 e-22), *V. cholera* general secretion pathway cluster (5.14 e-96) and *V. cholera* Rtx toxin gene cluster (AF119150, 4.84 e-21).

Table 2. Biochemical tests for the novel *Helicobacter* species isolated from marmosets

<table>
<thead>
<tr>
<th>Taxon</th>
<th>Catalase</th>
<th>Oxidase</th>
<th>NO₃</th>
<th>Urease</th>
<th>IAH</th>
<th>GGT</th>
<th>PO₄</th>
<th>C content (mol%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Novel <em>Helicobacter</em> species</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>41</td>
</tr>
<tr>
<td><em>Helicobacter callitrichis</em></td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>39.6</td>
</tr>
<tr>
<td><em>Helicobacter sanguini</em></td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>40.6</td>
</tr>
<tr>
<td><em>Helicobacter macacae</em></td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>45</td>
</tr>
<tr>
<td><em>Helicobacter bilis</em></td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>40.6</td>
</tr>
<tr>
<td><em>Helicobacter marmotae</em></td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>45</td>
</tr>
<tr>
<td><em>Helicobacter canis</em></td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>35.9</td>
</tr>
<tr>
<td><em>Helicobacter hepaticus</em></td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>35.7</td>
</tr>
<tr>
<td><em>Helicobacter pylori</em></td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>ND</td>
</tr>
</tbody>
</table>

*Urease gene was detected in the two urease-negative strains.

**Table 2.** Biochemical tests for the novel *Helicobacter* species isolated from marmosets.

PO₄, phosphoric acid; NO₃, nitrate reduction; IAH, indoxyl acetate hydrolysis; NA, nalidixic acid; CE, cefalotin; GGT, γ-glutamyl transpeptidase; S, sensitive; R, resistant; ND, not determined.

**Fig. 1.** Transmission electron micrographs of negatively stained cells of *H. jaachi* sp. nov. MIT 09-6849. Bar, 500 nm.
Helicobacter prevalence in marmosets

Of the 39 marmosets screened for Helicobacter species, 11 (28.2%) had Helicobacter spp. isolated from at least one sample. Eight of the isolates were cultured from faeces and/or large intestine. Helicobacter spp. were also isolated from two liver samples and one gallbladder sample.

By PCR analysis, 19 of the 39 (48.7%) marmosets were positive for Helicobacter spp. in at least one sample (Table 1). Helicobacter spp. were detected in 36% of the gallbladder samples by PCR, which may be related to the finding that about 10% of the marmosets had cholecystitis or biliary inflammation.

Fig. 2. Phylogenetic analysis of 16S rRNA (a), 23S rRNA (b) and hsp60 (c) gene sequences. Neighbour-joining trees were based on the comparison of genes from different Helicobacter species. Bars, number of nucleotide substitutions.

Fig. 3. RFLP analysis of marmoset Helicobacter sp. isolates amplified by PCR with Helicobacter genus-specific primers, digested with AluI and XbaI and electrophoresed on a 3% agarose gel. Lane M, 100 bp DNA ladder; lanes 1–5, marmoset isolates; lane 6, ‘H. sanguini’; lane 7, H. macacae.
Animals aged 6 years and older had higher helicobacter-positive prevalence rates than animals younger than 5 years old (39% by culture and 57% by PCR vs 0% by culture and 27% by PCR) (Table 3). Helicobacter species were isolated from animals ingesting a high fat diet, from primates treated with compounds, as well as from animals without diarrhoea (data not shown). The diarrhoeic animals reflect only the clinical state of the animal at the time of sampling; in addition, the diarrhoeic state of 14 marmosets was unknown.

**Helicobacter** species were isolated from animals ingesting a high fat diet, from primates treated with compounds, as well as from animals without diarrhoea (data not shown). The diarrhoeic animals reflect only the clinical state of the animal at the time of sampling; in addition, the diarrhoeic state of 14 marmosets was unknown.

**Histopathology findings**

Numerous lesions were noted in this population of marmosets and these findings recapitulated the spectrum of survey pathology previously reported. These changes included glomerulo- and interstitial nephritis, amyloidosis, extramedullary haematopoesis and metabolic bone disease. However, for the purposes of this study, we focused on lesions of the hepatobiliary and gastrointestinal systems where inflammatory changes were prominent in a relatively high incidence of animals (Table 4). In the liver, the inflammatory lesions consisted of lymphoid infiltrates in hepatic portal regions as well as mild to moderate infiltrates in the mucosa and submucosal tissues of large bile ducts and gallbladder. Occasional animals had mild to moderate cholangiohepatitis (Fig. 5a). Several marmosets had cholecystitis with transmural lymphocytic infiltration of the gallbladder (Fig. 5b). A high incidence of cholecystitis was not found, but many animals in this study had small focal accumulations of lymphoid cells within the gallbladder submucosa. Lymphoid infiltrates increased in incidence and severity with age. Enteric pathology consisted of a continuous spectrum of inflammatory bowel disease (IBD) changes ranging from relatively minimal focal to segmental lymphoid infiltrates in the lamina propria to severe lymphoid cell accumulations with effacement of the normal bowel architecture in both large and small intestine. Severe accumulations of chronic inflammatory cells found in the lamina propria (Fig. 5c) were associated with changes in villous and crypt architecture, with intraepithelial inflammatory cell accumulations.

**DISCUSSION**

Since the isolation and characterization of *H. pylori* in 1982 as the aetiologic agent of peptic ulcer disease, and

**Table 3. Ages of marmosets and Helicobacter detection**

<table>
<thead>
<tr>
<th>Helicobacter detection</th>
<th>Age (years)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3–5</td>
<td>6–11</td>
</tr>
<tr>
<td>Culture-positive (%)</td>
<td>0/11 (0)</td>
<td>11/28 (39.3)</td>
</tr>
<tr>
<td>PCR-positive (%)</td>
<td>3/11 (27.2)</td>
<td>16/28 (57.1)</td>
</tr>
</tbody>
</table>

http://jmm.sgmjournals.org 1069

Fig. 4. FISH: *Helicobacter* species in the lumen of an infected caecum stained with a *Helicobacter* genus-specific probe (in red). Magnification, ×630.
subsequently its classification as a class I gastric cancer carcinogen, over 40 other *Helicobacter* species have been isolated (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi); many of these *Helicobacter* spp. can produce serious non-gastric disease in their respective hosts (Fox, 2002). Enterohelobacteria have evolved to inhabit the mucus of the intestinal crypts and colonize intestinal crypts of a variety of animals, as well as humans. *Helicobacter* species that colonize the lower bowel and biliary tract of non-human primates have been routinely isolated from cases with chronic inflammation of the lower bowel (Fox et al., 2001a, b, 2007; Lertpiriyapong et al., 2014; Marini et al., 2010; Saunders et al., 1999; Won et al., 2007). The importance of these findings is augmented by experimental studies in mice which demonstrate that *H. hepaticus*, the prototype enterohelobacterial *Helicobacter* species (EHS), has been shown to play a central role in the development of chronic inflammation and cancer at both intestinal and extraintestinal sites, in both immunocompromised and immunosuppressed mice (Erdman et al., 2009; Fox et al., 1996, 2011; García et al., 2011; Knutson et al., 2013).

Several EHS have zoonotic potential. For example, other EHS isolated from animals and birds, such as *Helicobacter bilis* and *Helicobacter pullorum*, have been identified in patients with cholecystitis and biliary neoplasia (Fox et al., 1998; Matsukura et al., 2002; Segura-López et al., 2015). In 1984, a group of microaerobic *Campylobacter*-like organisms were isolated from rectal swabs of male homosexuals suffering from proctocolitis and enteritis (Fennell et al., 1984; Totten et al., 1985). Originally classified as *Campylobacter cinaedi* and *Campylobacter fennelliae*, they were later recognized to be *Helicobacter* spp. In an attempt to understand the pathogenesis of *H. cinaedi* and *Helicobacter fennelliae* infection, pigtailed macaques (*Macaca nemestrina*) were experimentally challenged by the oral route with the micro-organisms (Flores et al., 1990). Both *H. cinaedi* and *H. fennelliae* caused bacteraemia, diarrhoea and focal colonic lesions. We have isolated *H. cinaedi* from an inflamed colon, mesenteric lymph node and liver of a rhesus monkey (Fox et al., 2001a). This case and recent studies in humans highlight the ability of enteric helicobacters to translocate across the intestinal epithelia in both monkeys and humans (Araoka et al., 2014); translocation to the liver is also noted with *H. hepaticus* during the development of hepatitis and hepatocellular carcinoma in A/JCr and B6C3F1 mice (Boutin et al., 2004; Fox et al., 1996; Hailey et al., 1998). *H. cinaedi* has also been isolated from rhesus monkeys without clinical disease (Fernandez et al., 2002). However, this study did not perform colonic biopsies to ascertain if the *H. cinaedi*-positive monkeys had histological evidence of chronic colitis, a common finding in rhesus monkeys (Fernandez et al., 2002). Isolation of other novel helicobacters from inflamed colons of rhesus monkeys and cotton-top tamarins is also consistent with the increasing recognition of enteric helicobacters in

<table>
<thead>
<tr>
<th>Cohort or 27 animals from which culture and/or PCR performed</th>
<th>Liver</th>
<th>Gall bladder</th>
<th>Stomach</th>
<th>Small intestine</th>
<th>Caecum/colon</th>
</tr>
</thead>
<tbody>
<tr>
<td>Animals positive for <em>Helicobacter</em> by culture and/or PCR</td>
<td>2/7 (28.6 %)</td>
<td>4/7 (57.1 %)</td>
<td>0/7 (0 %)</td>
<td>10/20 (50 %)</td>
<td>6/20 (30 %)</td>
</tr>
<tr>
<td>Animals negative for <em>Helicobacter</em> by culture and/or PCR</td>
<td>10/27 (37 %)</td>
<td>14/27 (51.8 %)</td>
<td>2/27 (7.4 %)</td>
<td>7/27 (37 %)</td>
<td>14/27 (51.8 %)</td>
</tr>
</tbody>
</table>

**Table 4.** Lesion incidences for inflammatory infiltrates in marmosets.

**Fig. 5.** (a) Lymphohistiocytic inflammatory infiltrate in perportal region of liver from a marmoset with chronic cholangiohepatitis. Inflammatory cells circumscribe the portal triad including the bile duct. Haematoxylin and eosin (H&E), × 400. (b) Chronic cholecystitis characterized by diffuse submucosal infiltrate of lymphoid cells in the wall of the gallbladder. H&E, × 200. (c) Section of jejunum from a marmoset with inflammatory bowel disease characterized by loss of crypts and effacement of normal villous structure by severe diffuse lymphohistiocytic infiltrate in the lamina propria. H&E, × 200.
children with gastroenteritis who reside in developing countries (Fox et al., 2001b; Lastovica & le Roux, 2000; Saunders et al., 1999) and the identity of enteric helicobacters in the lower bowel of children with inflammatory bowel disease (Thomson et al., 2011; Zhang et al., 2006). Our findings in this study are consistent with previous NHP studies, in that we identified *Helicobacter jaachi* sp. nov. from 49 % of the marmosets by PCR and were able to culture the organism from 28 % of the marmosets, mostly from the faeces and lower bowel, but also from the upper gastrointestinal tract, liver and gallbladder.

The novel *Helicobacter* species *H. jaachi* sp. nov. shared many biochemical properties with those of ‘*H. callitrichis*’, previously isolated from the faeces of clinically normal marmosets, with the exception that *H. jaachi* was urease-positive and alkaline phosphate hydrolysis (PO₄) negative, whereas ‘*H. callitrichis*’ is urease-negative and PO₄-positive. By 16S rRNA analysis, they are 4 % different, which warrants separate species designation. Specific pathological changes could not be attributed to *H. jaachi* in this study due to differences in age, housing, treatment, diet, other unidentified intestinal microbial organisms, limited number of cases, and the fact that it was an open colony examined over a long period of time. The overall health of this population of domestic colony-bred marmosets, despite the occasional case of persistent diarrhoea and weight loss, was deemed excellent. Over the course of several years, there were few cases of ‘wasting syndrome’, severe anaemia or severe metabolic bone disease similar to those reported previously in experimental colonies of *Callithrix jacchus*. The spectrum and relative incidence of major findings in the present colony were similar to that reported by others (Ludlage & Mansfield, 2003; Ludlage et al., 2005).

In summary, this marmoset colony had a relatively high incidence of hepatobiliary and inflammatory bowel lesions. Importantly, these conditions have been associated with helicobacter infections in different animals, including other species of non-human primates (Fox, 2002; Fox et al., 1998, 2001a, b, 2007; Lertpiriyapong et al., 2014). Further, numerous experimental *Helicobacter* sp. infections in mice have reproduced salient features of IBD and hepatobiliary disease observed in marmosets (Erdman et al., 2009; Fox, 2002; Fox et al., 1996, 2011; Hailey et al., 1998; Knutson et al., 2013; Maggio-Price et al., 2005; Mangerich et al., 2012; Meeker et al., 2014).

**Description of Helicobacter jaachi sp. nov.**

*Helicobacter jaachi* [N.L. gen. masc. n. of (*Callithrix*) jacchus (systematic name of the marmoset species) from which the bacterium was first isolated].

Cells are fusiform (0.2–0.5 μm) with periplasmic fibres. The bacterium is Gram-negative and non-sporulating. The organism is motile, having multiple, bipolar, sheathed flagella. The organism grows slowly and appears on solid agar as a spreading film on the surface. The bacterium grows at 37 and 42 °C, but not at 25 °C, under microaerobic conditions, but not aerobically or anaerobically. The bacterium is oxidase, catalase, urease and indoxyl acetate hydrolysis positive, but alkaline phosphatase hydrolysis and γ-glutamyl transpeptidase negative. It grows on 1 % glycine and is resistant to cefalotin and sensitive to nalidixic acid.

The type strain MIT 09-6949ᵀ, a liver isolate, has been deposited in the BCCM/LMG Bacteria Collection as LMG 28613ᵀ. It has a DNA G+C content of 41 mol%, and its genome is 1 900 614 bp. 16S rRNA and 235 rRNA gene sequences of the type strain have been deposited in GenBank under accession numbers KP701326 and KP701328, respectively.

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