EPIDEMIOLOGY OF INFECTION

Failure to isolate Helicobacter pylori from stray cats indicates that H. pylori in cats may be an anthroponosis – an animal infection with a human pathogen

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The recent isolation of Helicobacter pylori from cats obtained from a commercial supplier has potentially important public health implications. The present study investigated whether H. pylori infection was common in stray cats. Twenty-five cats were examined for the presence of H. pylori by histological examination, culture and two polymerase chain reaction (PCR) assays. Histologically, the gastric biopsy specimens from all cats showed large spiral organisms typical of H. felis and not H. pylori. Samples from 23 cats yielded bacterial growth and two had no growth. Colonies grossly similar to H. pylori were tested for catalase, oxidase, urease and Gram’s stain reactions. None was H. pylori. All samples tested as positive by the Helicobacter 16s rRNA genus-specific PCR assay and only six cats and a mouse stomach infected with H. heilmannii gave positive results with the adhesin subunit A (hpaA)-specific PCR assay, which is consistent with either H. pylori or H. heilmannii. The helicobacters identified in these samples by PCR were not cultivable and hence were probably H. heilmannii. H. pylori infection is uncommon in stray cats and owning pet cats should not be a threat to public health in relation to H. pylori infection.

Introduction

Helicobacter pylori infection is a major health problem as it is the cause of peptic ulcers and plays a major role in the development of gastric cancer [1]. Although current evidence suggests that the natural reservoir for infection is man, the primary route of transmission is unknown. H. pylori has been isolated or detected in faeces, saliva, dental plaque and, recently, in water [2–8]. The available data are consistent with faecal–oral, oral–oral or a common environmental source as possible mode(s) of transmission [8–14]. Understanding the mode(s) of transmission of H. pylori is essential for developing public health measures to control the spread of infection.

Recently the search for reservoir hosts and an animal model for H. pylori infection led to the isolation of the organism from the gastric antrum and corpus of domestic cats obtained from a single commercial source of research animals [15, 16]. The possibility that cats may be a zoonotic source of H. pylori infection in man was suggested. This development is significant with regard to public health implications; cats could represent a natural reservoir that transfers H. pylori to man. This study was designed to evaluate the prevalence of H. pylori infection in stray cats. Several procedures were used, including biochemical, histological and cultural analysis to detect H. pylori and differentiate it from other Helicobacter spp. To confirm the identity of the organism, two recently developed PCR assays were used; one based on the H. pylori 16S rRNA sequence, is Helicobacter-genus specific and the other is based on the H. pylori adhesin subunit A (hpaA) encoding gene.

Materials and methods

Animals and gastric specimens

Twenty-five stray cats scheduled to be killed by the Harris County Animal Control Center were studied.
Ages ranged between 4 weeks and 30 months. All cats were healthy. Gastric tissues were obtained immediately after death. The stomach was exposed by a midline abdominal incision and was removed by transection of the distal oesophagus and the proximal jejunum. It was opened along the greater curvature. The stomach mucosa was not rinsed to keep the bacteria attached to the mucosal surface. Sharp cut dissection was used to obtain long samples of mucosa, submucosa and muscularis propria. Several specimens were obtained from the fundus, body, antrum and duodenum of each cat's stomach. Two biopsy samples from each site of the stomach were placed separately in cysteine medium containing glycerol 20% and frozen at −70°C until analysed by culture and PCR. Additional biopsy samples from each site were fixed in buffered formalin 10% for histological analysis.

A snap frozen mouse stomach infected with *H. helminthii* was a gift from Dr A. Lee, University of New South Wales, Sydney, Australia. The stomach was obtained from a BALB/c mouse bred in a specific pathogen-free environment before infection.

**Culture and histological analysis**

The culture procedure used routinely in this laboratory to isolate *H. pylori* from human gastric biopsies was applied to these feline biopsy samples [17, 18]. The tissue samples were thawed at room temperature, then dissected into smaller pieces. Multiple, representative sections of the stomach were taken for culture. The tissues were minced, then ground between the frosted ends of two sterile microscope slides; c. 50 μl of cysteine medium were used to resuspend the ground tissues for transfer to plated media and 25-μl volumes of the tissue suspension were used to inoculate one selective and one non-selective horse blood agar plate (HBA). The horse blood agar contained Brain Heart Infusion broth (Difco Laboratories, Detroit, MI, USA) with Bacto-agar (Difco Laboratories) 1.3% and horse blood (Cocalico Biologicals Inc., Reamstown, PA, USA) 10%. Selective plates were supplemented with amphotericin B, trimethoprim and vancomycin. All plates were incubated at 37°C in air with CO2 12% and 100% humidity for up to 14 days as described previously [17, 18]. Bacterial growth resembling *H. pylori* was picked and transferred to fresh, non-selective HBA plates. Identification of bacterial isolates as *H. pylori* was based on the results of the Gram’s stain reaction, cell morphology and positive reactions for catalase, urease and oxidase activity. Identification tests for other bacterial isolates were determined by the Clinical Microbiology Laboratory, VA Medical Center, Houston, TX, USA.

For histological examination, each formalin-fixed biopsy sample was processed, oriented on edge, embedded in paraffin and cut in sequential 4-μm sections. From each specimen, one slide (usually with 8–12 sections) was stained with haematoxylin and eosin (H & E), one with periodic acid Schiff (PAS), and one with the Genta stain for the visualisation of *Helicobacter* spp. Virtually all specimens included surface epithelium and muscularis mucosae. The presence of *Helicobacter* spp. was assessed morphologically and the results were scored as infected or uninfected based on the presence or absence of the organism.

**Nucleic acid preparation**

To prepare nucleic acid from the cat’s tissues and the mouse stomach, biopsy samples were processed as described previously [20]. Briefly, each specimen (50–100 mg) was thawed and washed in sterile saline, then homogenised in 300 μl of lysis buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0, 10 mM NaCl, Triton X-100 1%, and proteinate K 20 mg/ml final concentration) with a disposable pestle homogeniser (Kontes, Vineland, NJ, USA). After incubation for 3 h at 55°C, lysozyme was added to a final concentration of 50 mg/ml and incubation was continued overnight at 37°C. The mixture was then boiled for 20 min followed by multiple extractions with an equal volume of a mixture of phenol:chloroform:isoamyl alcohol (phenol:CIAA; 24:1, v:v) and CIAA, respectively. The nucleic acid templates were ethanol precipitated, dissolved in 100 μl of TE (10 mM Tris-HCl, 1 mM EDTA, pH 8.0) buffer, and stored at −20°C until used.

**PCR and hybridisation analysis**

The PCR assay of the *hpA4* adhesin-encoding gene was applied with the same primers – *hpA-1* primer (5’GAATTACCATCCAGCTAGCG-3’) and *hpA-2* primer (5’-GTAACCTTGACAAAACCGGC-3’) – and conditions as described previously [8]. These primers amplify a 375-bp fragment of the adhesin subunit gene [8, 21]. To confirm that any product was actually indicative of the presence of a *Helicobacter* sp., the samples positive with the above assay were also evaluated with a second PCR, a 16S rRNA RT-PCR, as described previously [8, 20, 22]. The two oligonucleotide primers derived from *H. pylori* 16S rRNA and designated HP1 (5’-TGCGAATCCGCTAGCGTAATG-3’) and HP2 (5’-GCTAAGAGATCAGCCTAGTATGCC-3’) were used in the RT-PCR assay. Ten μl of the extracted DNA from each gastric tissue sample were used as templates in each reaction. Positive and negative controls were also tested in parallel with each assay. The oligonucleotide primers were custom synthesised (Genosys Biotechnologies Inc., Houston, TX, USA).

To improve sensitivity, Southern blots and subsequent hybridisation were performed. The target sequences (375 bp and 506 bp amplified from the reference strain *H. pylori* RD26) generated by both assays were used as probes in Southern blot hybridisations. The probes
were labeled with digoxigenin-11-dUTP by the hexanucleotide priming technique with the Genius labelling kit following the manufacturer’s instructions (Boehringer Mannheim, Indianapolis, IN, USA). For gel and Southern blot analysis, 20-μl volumes of PCR samples were electrophoresed through agarose 2% gels containing ethidium bromide 0.5 μg/ml and photographed under UV light. The amplified fragments were then transferred overnight on to nylon membranes (Hybond-N+; Amersham, Arlington, IL, USA) by the alkaline transfer method with 0.4 N NaOH [22]. Hybridisations with digoxigenin-labelled probes were performed with the Genius kit as directed by the manufacturer (Boehringer Mannheim).

Results

Histology and cultures

Large spiral organisms morphologically similar to *H. felis* or to *H. heilmannii* (previously known as *Gastrospirillum hominis*) and not morphologically similar to *H. pylori* were seen in biopsy samples from all cats. Cultures from 23 of 25 cats showed bacterial growth resembling *Pasteurella* spp., as well as mycoplasma and other bacteria; two had no growth. Colonies grossly similar to *H. pylori* were selected for further study and tested for catalase, oxidase, urease and Gram’s stain. None was *H. pylori*.

Amplification analysis

The specificity and sensitivity of the *hpaA*-PCR assay and the 16S rRNA RT-PCR assay has been demonstrated previously [8,20]. The expected 375-bp fragment from the *hpaA*-PCR assay was detected from as few as one bacterium per reaction and the 506-bp fragment from the 16S rRNA RT-PCR assay was detected from two bacterial cells/reaction. Samples from all the cats tested gave positive results for *Helicobacter* by the 16S rRNA genus-specific RT-PCR assay and samples from six of the cats and the mouse gave positive results by the adhesin subunit A (*hpaA*)-specific PCR assay. The specificity of these results was confirmed by Southern hybridisation with the amplified fragments as specific probes (Fig. 1). Taken together with the failure in cultivation, it is likely that positive results by the *hpaA*-specific PCR assay were due to *H. heilmannii*.

Discussion

*H. pylori* is a significant gastro-intestinal pathogen because of its association with digestive diseases in man such as peptic ulcer, gastric cancer and MALT lymphoma [1,23–25]. Experimental animal models are needed to study the role of the micro-organism in these diseases and the mechanism(s) of disease formation and prevention. During the process of development of animal models, several *Helicobacter* species have been discovered and each one has been found to naturally colonise a specific animal host. For example, the natural hosts of *H. felis*, *H. mustelae*, *H. muridarum*, *H. nemestrinae* and *H. acinonyx* are cats and dogs [26,27], ferrets [28], rodents (rats and mice) [29], primates and cheetahs [30,31].

Although some experimental animals, such as piglets, dogs and mice, have been infected successfully with *H. pylori*, *H. pylori* does not naturally colonise the stomach of non-primate animals [32–34]. Hence, until recently, zoonotic transmission has not been considered as a risk factor in the epidemiology of *H. pylori* infection in man. The recent isolation of *H. pylori*
from a colony of domestic cats [15,16,26] suggested the possibility of zoonotic transmission because of human exposure to cats. Although in this communication, H. felis-like organisms and presumably H. heilmannii were found in stray cats, H. pylori infection was not identified. Recently, the hpaA-PCR assay was shown to amplify same size fragments (375 bp) from H. pylori [8], H. nemestrinae and H. acinonyx [35], and now H. heilmannii (from the infected mouse stomach). The Helicobacter organisms identified in the samples by hpaA specific-PCR were not cultivable and were probably H. heilmannii or another uncultivable Helicobacter species. The results of the present study agree with previous studies, indicating that H. heilmannii is responsible for causing chronic gastritis in cats [36] and with studies reporting on the isolation of different Helicobacter-like organisms, but not H. pylori, from cats [27,29,37–39].

The report of the isolation of H. pylori from cats obtained from commercial vendors may represent a special circumstance. This is consistent with previous studies showing that owning pets was not associated with an increased prevalence of H. pylori infection [13,40,41]. Recently, it has been confirmed that owning cats is not associated with an increased risk of acquiring H. pylori infection [42,43]. While H. pylori infection is unlikely to be a zoonosis, infection in cats may represent an anthroposon.

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References


