Short communication

Detection of Helicobacter spp. DNA in the oral cavity of dogs

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Abstract

The mode of acquisition of gastric Helicobacter spp. infection in dogs has not been determined. It is suspected that oral–oral and faecal–oral transmission may be involved. The present study sought to determine if Helicobacter spp. DNA is present in the oral cavity of healthy and vomiting dogs. Thirty-eight pet dogs (27 vomiting and 11 clinically healthy) were studied. The presence of Helicobacter spp. was determined by single and nested PCR evaluation of DNA extracted from saliva, dental plaque and gastric biopsy samples. Helicobacter spp. DNA was detected by nested PCR in 36 (94.7%) gastric biopsies, 17 (44.7%) dental plaque and 19 (50%) saliva samples out of the 38 dogs examined. Overall 27 (71.1%) dogs screened by nested PCR were found to harbour Helicobacter spp. DNA in the oral cavity (dental plaque and/or saliva). There was no significant difference in the prevalence of Helicobacter spp. DNA in the oral cavity of vomiting and healthy dogs, and the time from vomiting to oral sampling did not have significant impact. This study confirms the high prevalence of gastric Helicobacter spp. infection in dogs, and reveals that Helicobacter spp. DNA is detectable in the oral cavity of over 70% of dogs. These findings support the possibility of oral–oral transmission between dogs and that the canine oral cavity may act as source of non-pylori Helicobacter spp. infection for humans.

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1. Introduction

Spiral-shaped bacteria have been described in the stomach of dogs since the 19th century (Bizzozero, 1893), however it is only recently that a number of gastric Helicobacter spp. has been characterized in this animal species. Gastric Helicobacter spp.
infecting dogs are different and morphologically distinct from Helicobacter pylori since they appear as large, 5–15 μm long, tightly coiled bacteria (Jalava et al., 1997). To date, Helicobacter felis, Helicobacter bizzozeronii, Helicobacter salomonis, “Helicobacter heilmannii”, Helicobacter bilis and Helicobacter (Flexispira) rappini have been identified (Eaton et al., 1996; Hanninen et al., 1996; Jalava et al., 1997, 1998; Priestnall et al., 2004). Although gastric Helicobacter spp. infection is common in dogs, with reported prevalence rates ranging from 61% to 100% (Eaton et al., 1996; Happonen et al., 1998; Yamasaki et al., 1998; Wiinberg et al., 2005), the route of transmission remains unclear. The infection is probably acquired at an early age through oral contact between dams and puppies and among puppies, or the ingestion of vomitus and regurgitated food (Lee et al., 1991; Hanninen et al., 1998). This parallels the situation in people with H. pylori infection, where oral–oral and faecal–oral are considered likely routes of transmission (Brown, 2000). In people, H. pylori has been detected in saliva and dental plaque, and the oral cavity has been suggested as a reservoir and source of H. pylori infection.

A small percentage (approximately 0.25–1.7%) of human patients suffering from gastric disorders have been diagnosed with gastric Helicobacter spp. that are distinct from H. pylori and morphologically similar to the large spiral-shaped Helicobacter spp. of pet dogs (De Groote et al., 2005). Contact with dogs, cats, and pigs has been determined to be a risk factor for human non-pylori Helicobacter spp. infection (Meining et al., 1998). H. felis, H. bizzozeronii and H. salomonis have been detected by a PCR in 48.5% of gastric biopsy specimens from humans colonized by non-pylori Helicobacter spp. (De Groote et al., 2005). The zoonotic potential of gastric Helicobacter spp. from pet carnivores is further supported by the presence of identical strains of “H. heilmannii” in patients and their cats or dogs (Dieterich et al., 1998; Van Loon et al., 2003; Thomson et al., 1994). While the mode of acquisition of non-pylori Helicobacter spp. by people has not been determined, it has been suggested that licking by a pet could be a possible transmission route (Thomson et al., 1994).

It was the goal of this study to investigate the role of the oral cavity as a reservoir of Helicobacter spp. in pet dogs.

2. Materials and methods

2.1. Animals and sampling procedures

Thirty-eight pet dogs (27 vomiting and 11 clinically healthy admitted for spay or neuter) of various breeds were evaluated in this study. None of the animals had been treated with antibiotics within 4 weeks before examination. Vomiting dogs ranged in age from 6 months to 13 years (mean 5.18 years), 12 were males and 15 females. The time interval from the last vomiting episode and the sampling was recorded. Healthy dogs ranged in age from 1 to 14 years (mean 4.77 years), six were males and five females. Endoscopy and biopsy sampling were performed as part of clinical investigation with the owners’ agreement. The study was approved by the Ethical Committee of the Veterinary School of the University of Milan.

Saliva, dental plaque and gastric biopsy samples were obtained while the dogs were under anesthesia. Saliva and dental plaques were collected prior to the endoscopy to avoid cross-contamination. Saliva was collected by swabbing buccal mucosa with a sterile cotton swab that was immediately transferred into a tube containing 1 ml of sterile phosphate buffered saline (PBS). Dental plaque was removed from the first upper premolar with a sterile curette and placed in 1 ml of sterile PBS. Gastric biopsies were taken from the fundic and antral regions using a pediatric endoscope (Pentax FX-29 or Pentax EG-290I) and sterile biopsy forceps. After each sampling, the endoscope and the biopsy forceps were thoroughly cleaned, then sterilized using an activated aldehyde solution. Two biopsies were taken from each site for PCR analysis and histology. One sample from fundic and one from pyloric mucosa were pooled for PCR analysis, frozen immediately and stored at −20 °C until further analysis. For histological examination, one sample from fundic and one from pyloric mucosa were fixed in 10% buffered formalin.

2.2. PCR

DNA was extracted from saliva, dental plaque and gastric biopsy samples using DNeasy tissue KIT® (Qiagen, Hilden, Germany) according to the manufacturer’s instructions. Helicobacter genus-specific
primers (C97, forward: 5'-GCT ATG ACG GGT ATC C-3'; C98, reverse: 5'-GAT TTT ACC CCT ACA CCA-3') (Fox et al., 1998) were used to amplified a 398 bp fragment of the 16S rRNA gene. All PCR products obtained with the C97/C98 PCR were amplified with an internal pair of primers (HelF, forward: 5'-CGT GGA GGA TGA AGG TTT TA-3', location within the 398 bp fragment 128–147; HelR2, reverse: 5'-AAT TCC ACC TAC CTC TCC C-3', location within the 398 bp fragment 360–379) (Priestnall et al., 2004) producing a 251 bp fragment. Both amplifications were performed in a final volume of 20 μl containing 2 μl of DNA extracted, 200 μM of each deoxynucleotide (Roche diagnostic), 0.5 μM of each primer (Invitrogen), 1× Taq polymerase buffer 1.5 mM MgCl2 (Roche diagnostic) and 0.8 U of Taq polymerase (Roche diagnostic); 2 μl of the first reaction, diluted 1:8 in ultra-pure distilled water, were used as template in the nested PCR. Samples were heated at 94 °C for 5 min, followed by 40 amplification cycles of denaturation at 94 °C for 30 s, primer annealing at 57 °C for 30 s, and extension at 72 °C for 30 s, with an additional final extension step at 72 °C for 7 min. For nested PCR the amplification conditions were the same as above except that 35 cycles were used. Negative controls in which DNA extract was replaced by sterile distilled water were included in every set of reactions and DNA extracted from a swine stomach sample positive for Helicobacter organisms and degree of colonization as follows: − = absence of Helicobacter in the gastric glands; + = presence of Helicobacter in <5% of the gastric glands; ++ = presence of Helicobacter in 5–50% of the gastric glands; +++ = presence of Helicobacter in >50% of the gastric glands.

2.3. Histology

Biopsy samples were embedded in paraffin, sectioned at 4 μm, and stained with hematoxylin and eosin (HE) and Warthin-Starry (WS) stain. Samples were examined in a blinded fashion by a single pathologist and scored for the presence of Helicobacter organisms and degree of colonization as follows: − = absence of Helicobacter in the gastric glands; + = presence of Helicobacter in <5% of the gastric glands; ++ = presence of Helicobacter in 5–50% of the gastric glands; +++ = presence of Helicobacter in >50% of the gastric glands.

2.4. Statistical methods

The relationship of Helicobacter spp. PCR (stomach and oral cavity: positive or negative) and vomiting was evaluated by use of the x²-test. The relationship of gastric colonization density (assessed by WS stain) and the time of vomiting, to the presence of Helicobacter spp. DNA in the oral cavity were determined by use of the Mann–Whitney test. Significance was set at P < 0.05.

3. Results

Helicobacter spp. DNA was detected by single PCR in gastric biopsies from 30 (78.9%) of the 38 dogs examined. Nested PCR yielded positive results in those 30 cases and in 6 gastric biopsies that were negative by single PCR, for a total of 36 (94.7%) positive cases (Table 1). No difference in prevalence of Helicobacter spp. DNA was found between vomiting (96.3%) and control dogs (90.9%) (Table 1). Helicobacter organisms were detected in WS-stained sections of gastric biopsies in 27 (71%) out of the

<table>
<thead>
<tr>
<th>Site</th>
<th>Positive dogs/total (%)</th>
<th>Non-vomiting, n = 11</th>
<th>Total, n = 38</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gastric mucosa</td>
<td>26/27 (96.3%)</td>
<td>10/11 (90.9%)</td>
<td>36/38 (94.7%)</td>
</tr>
<tr>
<td>Dental plaque</td>
<td>12/27 (44.4%)</td>
<td>5/11 (45.5%)</td>
<td>17/38 (44.7%)</td>
</tr>
<tr>
<td>Saliva</td>
<td>14/27 (51.9%)</td>
<td>5/11 (45.5%)</td>
<td>19/38 (50.0%)</td>
</tr>
<tr>
<td>Oral cavitya</td>
<td>21/27 (77.8%)</td>
<td>6/11 (54.5%)</td>
<td>27/38 (71.1%)</td>
</tr>
</tbody>
</table>

a Combination of dental plaque and saliva.
38 dogs examined. WS stain did not detect *Helicobacter* organisms in the 2 PCR-negative cases, and was negative in 9 PCR-positive cases.

*Helicobacter* spp. DNA was detected by single PCR in 8 (21.1%) dental plaque and 10 (26.3%) saliva samples from the 38 dogs examined. Nested PCR detected *Helicobacter* spp. DNA in additional cases, to yield a total of 17 (44.7%) and 19 (50%) positive dental plaque and saliva samples (Table 1). In Fig. 1, some representative results of single and nested PCR from gastric biopsies, dental plaque and saliva are shown. A total of 27 (71.1%) dogs were found to harbour *Helicobacter* spp. DNA in the oral cavity (dental plaque and/or saliva) (Table 1). PCR amplification products were obtained only from the oral cavities of dogs whose gastric biopsies were PCR positive. When *Helicobacter* spp. DNA was detected in gastric biopsies by nested PCR, but not by single PCR, the oral cavity was only positive by nested PCR (Table 2).

There was no significant difference in the prevalence of *Helicobacter* spp. DNA in the oral cavity of vomiting (77.8%) and healthy dogs (54.5%) (Table 1). The percentage of positive oral cavity samples was also quite constant regardless of the time interval between vomiting and sampling, being 70% (7/10) in dogs with a time interval of <7 h, 80.0% (8/10) with a time interval of 7–12 h and 85.7% (6/7) with a time interval of >12 h. There was no statistical relationship (P = 0.053) between the degree of gastric colonization assessed histologically and the presence in the oral cavity of *Helicobacter* spp. DNA.

### Table 2

<table>
<thead>
<tr>
<th>Oral cavity</th>
<th>Stomach</th>
</tr>
</thead>
</table>
|             | Nested+| Nested+/single+ | Nested+/
| Negative    | 2       | 4                | single+  |
| Nested+/single−| –   | 2                | –        |
| Nested+/single+| –   | –                | 13       |

* Combination of dental plaque and saliva.

### 4. Discussion

Bacteriological culture and PCR have been used for *Helicobacter* spp. detection in the oral cavity of humans, each of these methods having disadvantages. Culture has been reported only in few cases, due to the complexity of the oral microflora and the fastidious growth of *H. pylori* on synthetic media (Dowsett and Kowolik, 2003). In addition, *H. pylori* could be present in this site as viable, but non-culturale cocccid organisms (Bode et al., 1993). Since many *Helicobacter* spp. in dogs are either difficult to culture or have not been cultured, this initial investigation was performed using culture independent techniques. PCR offers greater sensitivity, specificity and rapidity than culture. PCR sensitivity can be increased by the use of a nested PCR (Bamford et al., 1998) as confirmed by the results of this study. In the present study gastric colonization by *Helicobacter* spp. was detected by nested PCR in 94.7% of the dogs examined, confirming the high prevalence of gastric *Helicobacter* spp. infection in pet dogs. The similar rate of detection of *Helicobacter* spp. DNA in vomiting and clinically healthy dogs is not surprising and in agreement with a previous study (Yamasaki et al., 1998).

We also found a high prevalence (71.1%) of *Helicobacter* spp. DNA in the oral cavity. To our
knowledge this is the first study that has analysed the presence of *Helicobacter* spp. DNA in the oral cavity of pet dogs. Our findings are similar to those observed in cats with *H. pylori* infection where it was detected in 50% of the saliva and 42% of dental plaque samples (Fox et al., 1996). In humans, the presence of *H. pylori* DNA in the oral cavity ranges from 0% to more than 90%. This high variability may be due to the lack of uniformity of laboratory procedures employed, for example with respect to DNA extraction and purification, and choice of primers for PCR (Dowsett and Kowolik, 2003). The oral cavity was positive for *Helicobacter* spp. DNA only in dogs with positive gastric biopsies, although dogs with negative gastric biopsies were too few (2/38) to draw final conclusions on this issue. When *Helicobacter* spp. DNA was detected in gastric biopsies only by nested PCR, but not by single PCR, the oral cavity resulted negative or positive only by nested PCR, suggesting a relationship between the degree of gastric colonization and the presence in the oral cavity of *Helicobacter* spp. DNA. However, no statistical relationship was found between the degree of gastric colonization assessed histologically and the presence in the oral cavity of *Helicobacter* spp. DNA, although the *P* value (0.053) was very close to be significant.

Saliva was found positive in a high percentage of cases (50.0%) despite it is considered an inhospital milieu for bacteria since it contains antimicrobial substances such as peroxidase enzymes, lysozyme and secretory IgA (Tenovuo, 1989; Fox et al., 1996). Also dental plaque was frequently positive (44.7%). Dental plaque represents a privileged niche for several organisms that acquire an increased resistance to environmental stress, antimicrobial agents and host defences (Marsh, 2005) and therefore dental plaque has been suggested as a permanent reservoir of *H. pylori* and a potential source of reinfection after antimicrobial treatment in humans (Miyabayashi et al., 2000; Suk et al., 2002). In this study, neither the presence of vomiting, nor the time since vomiting significantly impacted the detection of *Helicobacter* spp. DNA in the oral cavity, as might have been expected by the passive transfer through the vomitus to the oral cavity (Axon, 1995), suggesting that the oral cavity may represent a site of active *Helicobacter* spp. colonization and not just a site of passive contamination by gastric content.

In this study we employed *Helicobacter* genus-specific primers which do not permit to discriminate among different *Helicobacter* spp. Future studies with more specific methods (i.e. species-specific PCR, DNA sequencing of amplicons) are necessary to identify the *Helicobacter* spp. at the species and strain level and therefore to speculate if the stomach and the oral cavity are colonized by the same organisms. The results of this study raise the possibility that the oral cavity of dogs may act as a reservoir or source of *Helicobacter* spp. infection for dogs and non-*pylori* *Helicobacter* spp. infection for humans. However, since PCR is not able to distinguish bacterial DNA from degraded/non-viable and viable organisms, and enables the detection of even small numbers of organisms that may be insufficient to cause infection, further investigation is required to ascertain the risk posed by contact with the oral cavity of dogs.

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**References**


