Eradication of gastric *Helicobacter* spp. by triple therapy in dogs

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ABSTRACT: The aim of this study was to determine the efficacy of a triple anti-*Helicobacter* therapy using omeprazole, amoxicillin and clarithromycin (OAC) in dogs. A total of 15 healthy adult stray dogs with naturally acquired *Helicobacter* infection were evaluated using polymerase chain reaction and rapid urease test. Subsequently, they received a 21-day triple regimen. One day after the discontinuation of treatment, a second molecular analysis of gastric biopsies revealed complete eradication of *Helicobacter* DNA with negative quantitative urease testing in all 15 dogs. Our results confirmed the high prevalence of gastric *Helicobacter*-like organisms (GHLOs) in the stray dog population of Shiraz, Iran, and the effectiveness of our therapeutic regimen for the complete eradication of these microorganisms in stray dogs. In conclusion, for the complete elimination of non-*pylori Helicobacter* spp. from the gastric mucosa of dogs, a 21-day three-drug regimen with omeprazole, amoxicillin and clarithromycin is suggested.

Keywords: *Helicobacter* spp.; dog; amoxicillin; clarithromycin; omeprazole

Many humans have close interactions with companion animals which leads to the sharing of many diseases. Gastric *Helicobacter* spp. is one of the common infections in dogs, which may occur without clinical signs of gastritis (Simpson 2010). However, some human patients suffer from gastric disorders due to Non-*pylori Helicobacter* spp. (Heilmann and Borchard 1991; Andersen 2001; De Groote et al. 2005; Van den Bulck et al. 2005a; Duquenoy and Luyer 2009). Unlike *H. pylori*, gastric *Helicobacter*-like organisms (GHLOs) induce milder degrees of gastritis in humans, but more severe disorders such as gastric erosion/ulcers and cancer are also reported (Morgner et al. 2000; Yoshimura et al. 2002; Alon et al. 2010). It is almost certain that such infections in humans originate from animals, including dogs, through direct contact (Meining et al. 1998; Haesebrouck et al. 2009). Therefore, eradication of *Helicobacter* infections in dogs that have close contact with humans should be considered as one of the methods to control this zoonotic infection. The efficacy of different therapeutic regimens has been evaluated in dogs, cats and humans. However, an optimal method has yet to be established (DeNovo and Magne 1995; Neiger and Simpson 2000; Graham et al. 2003; Fischbach et al. 2004; Gatta et al. 2005; Gisbert et al. 2005; Qasim et al. 2005; Khoshnegah et al. 2011; Georgopoulos et al. 2012). In fact, previous studies have shown that triple or quadruple therapies are not 100% effective and, thus, further studies are needed in this regard. The aim of this study was to evaluate a 21-day triple *Helicobacter* species therapy in dogs with naturally acquired infection.

MATERIAL AND METHODS

Animals. Gastric biopsies of 15 asymptomatic, adult (mean age = 1.5 years), stray dogs were used in this study. All dogs were isolated from different locations of Shiraz, Iran. The animals were kept under supervision for seven days for observation and to ensure their health status using clinical and laboratory examinations. The dogs then underwent a 12 h fast and were subsequently tranquillized

with an intramuscular injection of xylazine hydrochloride (Alfasan, Woerden, Holland) (0.5 mg/kg) and acepromazine maleate (Castran, Interchemie, Holland) (0.05 mg/kg), and anaesthetised with a combination of diazepam (Phoenix Pharma Ltd, Gloucester, England) (0.25–0.5 mg/kg), and ketamine hydrochloride (Alfasan, Woerden, Holland) (5–10 mg/kg). Gastroscopy was performed with a 7.9 mm diameter gastroduodenoscope (MEDIT, Canada) to obtain three pairs of biopsies from the cardia, fundic and antral regions of the stomach.

Quantitative urease test. One of the gastric biopsy specimens from each of the mentioned regions was placed into urea broth media (DIFCO, USA) and incubated at 37 °C for 24 h. Colour transformation from yellow to pink/red within 24 h was considered as a positive result with the following degrees: colour change within the first 2 h (+3), between 2 and 6 h (+2) and between 6 and 24 h (+1). No colour transformation within 24 h was considered negative (0) (Ricci et al. 2007).

DNA extraction and PCR assays. DNA was extracted from the gastric biopsy specimens using a DNeasy tissue kit (Qiagen, Germany) according to the manufacturer's instructions. Polymerase chain reaction (PCR) amplifications were performed in a final volume of 25 µl containing 100 ng of extracted DNA, 2.5 µl of 10× PCR buffer (Fermentas, Lithuania), 0.2mM of dNTP, 1.5mM $MgCl_2$, 25 pmol/µl of each primer and 0.2 IU of Taq DNA polymerase (Fermentas, Lithuania). The PCR was carried out using a MJ-Mini BioRad thermal cycler (BioRad, USA) with an initial denaturing cycle at 94 °C for 4 min, followed by 33 cycles of 94 °C for 1 min, 62 °C for 1 min, and 72 °C for 1 min. A final extension step was performed at 72 °C for 7 min. The resulting PCR products underwent gel electrophoresis (1.0% agarose gel with ethidium bromide (0.5 mg/l) and were visualised under a UV transilluminator. The primer sequences used in this study for the detection of *Helicobacter* spp. were (F): 5'-AAG GAT GAA GCT TCT AGC TTG CTA-3', (R): 5'-GTG CTT ATT CGT GAG ATA CCG TCA T-3'. The size of the expected fragment (398 bp) was compared to a 100 bp reference marker (Fermentas).

Therapeutic protocol. All *Helicobacter* spp.-positive dogs in this study received omeprazole 0.5–1 mg/kg, SID (one capsule per day for animals less than 15 kg body weight [BW], and two capsules for those with more than 15 kg BW), amoxicillin 20 mg/kg, BID, and clarithromycin 7.5 mg/kg, BID, for 21 days. All dogs were kept in isolation during this study.

The second molecular analysis and rapid urease testing (RUT) were performed on the day after the end of the treatment.

RESULTS

According to RUT, 15/15 (100%) of the gastric specimens were mild to moderately positive for *Helicobacter* spp. and no severe degrees were detected.

Genus-specific PCR also identified 15/15 (100%) of the subjects as *Helicobacter* spp.-positive (Figure 1).

After three weeks, the successful eradication rate was 100%, and the second analysis revealed no signs of gastric *Helicobacter* infection neither by PCR nor RUT in any of the 15 dogs (Figure 2).

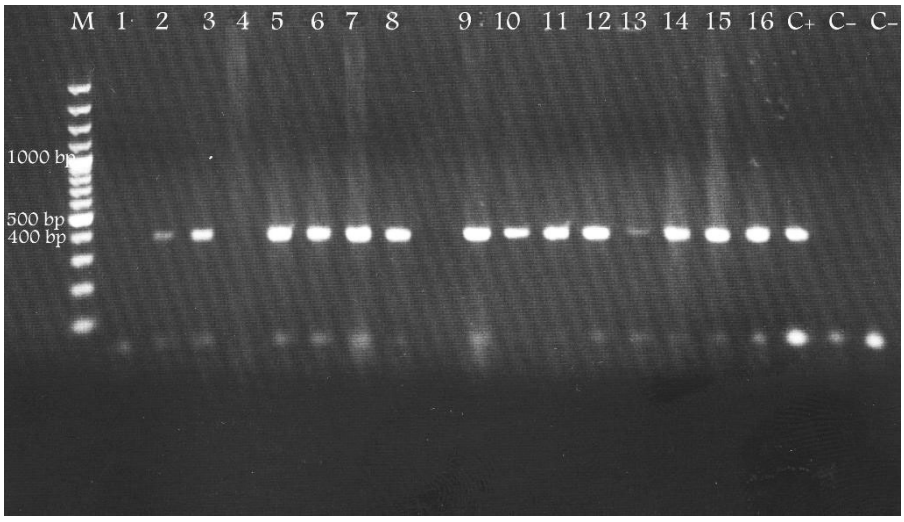

Figure 1. PCR amplification of DNA extracted from biopsy samples before treatment with species-specific *Helicobacter* primers. Lane M = 100 bp molecular ladder; lanes 1–16 = biopsy samples; lane C+ = positive control; lane C– = negative control

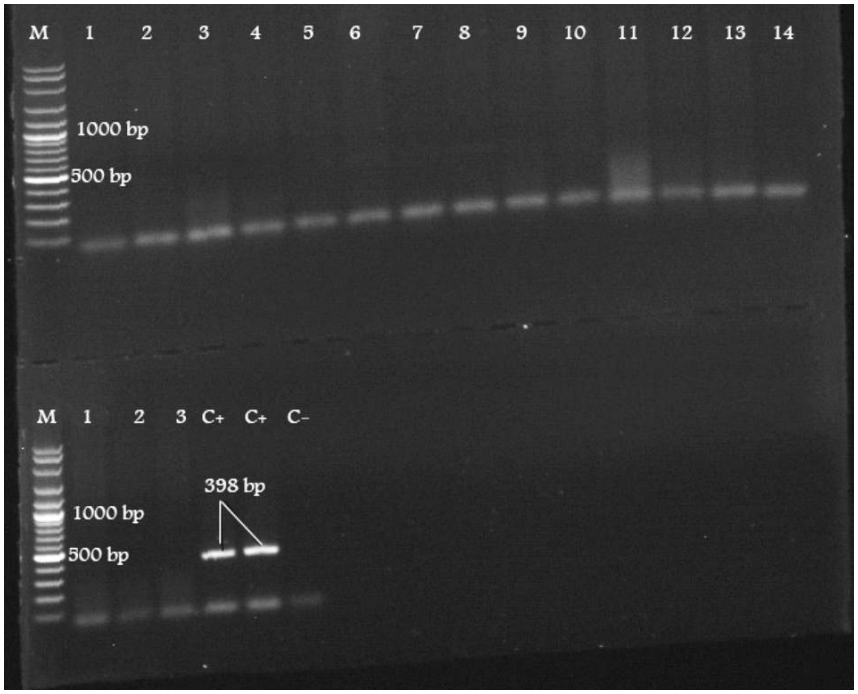

Figure 2. PCR amplification of DNA extracted from biopsy samples after treatment with species-specific *Helicobacter* primers. (Up) lane M = 100 bp molecular ladder; lanes 1–14 = biopsy samples. (Down) lane M = 100 bp molecular ladder; lanes 1–3 = biopsy samples; lane C+ = positive control; lane C– = negative control

DISCUSSION

Gastric infection with non-*pylori Helicobacter* spp. is rare, but is known to be associated with chronic active gastritis, peptic ulcers, and low-grade mucosa-associated lymphoid tissue lymphoma in humans (Morgner et al. 2000; Yoshimura et al. 2002; Van den Bulck et al. 2005; Alon et al. 2010). In contrast to *H. pylori*, various *Helicobacter* species colonise the stomachs of domestic animals, which might be a reservoir for transmission to humans. Although the exact route of this zoonotic transmission is not clearly understood, maintaining close contact with dogs and cats may put people, especially immunocompromised persons, at high risk for this infection (Alon et al. 2010). Therefore, to prevent this zoonotic infection, an efficient therapeutic protocol for dogs and cats is required.

In the current study, a high prevalence of *GHLO* infection (100%) was detected, which is in agreement with another study conducted in our area by (Shabestari et al. 2008) (93%).

The susceptibilities of *Helicobacter* spp. isolated from the stomach of cats and dogs to 10 antimicrobial agents were estimated by Van den Bulck et al. (2005b) via determination of the minimal inhibitory concentration (MIC) using the agar dilution method (Van den Bulck et al. 2005b). *Helicobacter* species were all highly susceptible to ampicillin, clarithromycin, tetracycline, tylosin, enrofloxacin, gentamicin, and neomycin, as demonstrated by low MICs. In a veterinary study, clinical signs in 90% of 63 dogs and cats were resolved by using a combination of metronidazole, amoxicillin and famotidine, and 74% of 19 animals had no evidence of *Helicobacter* species in gastric biopsies (DeNovo and Magne 1995). The outcome is obviously better if agents to which the organism is susceptible are used for treatment. Therefore, in the current study we used omeprazole (as a proton pump inhibitor), clarithromycin and amoxicillin.

The variability of treatment success with an individual regimen has been related to the occurence of antimicrobial resistance, compliance with the drug regimen, and duration of therapy (Vakil et al. 2004).

Recent studies suggest that the success rate of triple regimens can be improved if the duration is extended to 14 days or if an additional antibiotic is given (Nakayama and Graham 2004; Vilaichone et al. 2006).

Khoshnegah et al. (2011) assessed a quadruple therapy (omeprazole, amoxicillin, metronidazole and clarithromycin for 14 days) in 13 asymptomatic, naturally infected cats, but they suggested that antibiotic regimens that are effective against *Helicobacter pylori* in people cannot eliminate *Helicobacter* species in cats with naturally acquired infections (Khoshnegah et al. 2011). However, our results showed the effectiveness of a 21-day three-drug regimen in dogs using omeprazole, amoxicillin and clarithromycin.

Our results show that extending the duration of therapy can be more effective than using additional

antibiotics in the therapeutic protocol such as was carried out in the study of Khoshnegah et al. (2011). Although, a previous study has shown that triple therapy for seven days using clarithromycin, amoxicillin and lansoprazole was effective in 100% of dogs (Anacleto et al. 2011), in human medicine, therapies of shorter duration are no longer recommended since it has been demonstrated that a 14-day triple therapy has an approximately 12% better cure rate than a 7-day therapy (Vilaichone et al. 2006). Our study confirms this finding in dogs. Consequently, a 21-day triple or quadruple therapy can be considered a reasonable therapeutic period for the eradication of *Helicobacter* spp. from gastric mucosa of dogs.

The results of PCR and RUT were not positive in any of the dogs in the second evaluation suggesting that recrudescence is unlikely. However, to more definitively determine this, recrudescence and/or reinfection should be assessed for longer periods of time after drug therapy.

In conclusion, for complete elimination of non-*pylori Helicobacter* spp. from the gastric mucosa of dogs, a 21-day three-drug regimen with omeprazole, amoxicillin and clarithromycin is suggested. However, further controlled trials of antibiotic therapy with different durations of treatment in infected dogs and evaluation of re-infection are obviously required before guidelines regarding the treatment of gastric *Helicobacter* species in dogs can be made.

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