

2 - ORIGINAL ARTICLE

Models, Biological

Studies of distribution and recurrence of *Helicobacter* spp. gastric mucosa of dogs after triple therapy¹

Estudos da distribuição e recorrência do *Helicobacter* spp. na mucosa gástrica de cães após terapia tríplice

Thiago Pires Anacleto^I, Luiz Roberto Lopes^{II}, Nelson Adami Andreollo^{III}, Walter Octaviano Bernis Filho^{IV}, Maria Cristina Costa Resck^V, Adriano Macedo^{VI}

^I Research performed at the Faculty of Veterinary Medicine, FEPI, Itajuba-MG, Faculty of Veterinary Medicine, UNIFENAS, Alfenas-MG and Laboratory of Enzymology and Experimental Carcinogenesis of the Experimental Surgery and Medicine Division, Faculty of Medical Sciences, UNICAMP, Campinas-SP, Brazil. *Part of thesis Master degree. Tutor: Prof. Dr. Luiz Roberto Lopes.*

^I Fellow Master degree in Surgery, Faculty of Medical Sciences, UNICAMP, Campinas-SP, Brazil. *Conception and design.*

^{II} Associate Professor, Surgery Department, Digestive Disease Division, Faculty of Medical Sciences, UNICAMP, Campinas-SP, Brazil. *Supervisor; conception and design.*

^{III} Full Professor, Digestive Disease Division, Department of Surgery, Faculty of Medical Sciences, UNICAMP, Campinas - SP, Brazil. *Co-Supervisor; conception and design.*

^{IV} Assistant Professor, Surgery Department, Faculty of Veterinary Medicine, UNIFENAS, Alfenas-MG, Brazil. *Acquisition, analysis and interpretation of data.*

^V Assistant Professor, Anatomy Department, Faculty of Veterinary Medicine, UNIFENAS, Alfenas-MG, Brazil. *Acquisition, analysis and interpretation of data.*

^{VI} Assistant Professor, Pathology Department, Faculty of Medicine, UNIFENAS, Alfenas-MG, Brazil. *Acquisition of data, analysis and interpretation and histopathological studies.*

ABSTRACT

Purpose: To analyze the triple antimicrobial therapy in positive *Helicobacter* spp. dogs and to investigate recurrence. **Methods:** A total of 20 dogs underwent endoscopy followed by gastric biopsy using the rapid urease test and histopathology stained with Giemsa. Ten animals were treated with triple therapy recommended for humans and divided into control and experimental group. The control group was kept in isolation while the experimental group was placed in contact with positive animals during 60 days. **Results:** The prevalence of infection in animals in this experiment was 100%, and more frequent in the fundus and the gastric body. Therapy for 7 days using clarithromycin, amoxicillin and lansoprazole was effective in 100% of the animals. Recurrence of the infection in 80% of dogs in the experimental group, while the control group remained eradicated after 60 days. **Conclusion:** Crowded environments associated with close contact with dogs infected with helicobacter are a determinant for transmission of *Helicobacter* spp. between canines.

Key words: Helicobacter infections. Endoscopy. Gastric Mucosa. Dogs.

RESUMO

Objetivo: Avaliar a eficácia da terapia tríplice em cães naturalmente infectados pelo *Helicobacter* spp. e investigar a recorrência da infecção pelo contato com animais infectados. **Métodos:** Foram utilizados 20 cães, submetidos à endoscopia digestiva alta seguida de biópsia gástrica usando teste rápido da urease e histopatologia corada pelo Giemsa. Dez animais foram tratados com terapia tríplice preconizada para humanos e divididos em grupo controle e experimental. O grupo controle foi mantido em isolamento enquanto que o grupo experimento foi colocado em contato com os animais positivos durante 60 dias. **Resultados:** A prevalência da infecção nos animais deste experimento foi de 100%, e mais frequente no fundo e corpo gástrico. A terapia durante 7 dias empregando claritromicina, amoxicilina e lansoprazol foi eficaz em 100% dos animais. Houve recorrência da infecção em 80% dos cães do grupo experimental, enquanto que o grupo controle manteve-se erradicado após 60 dias. **Conclusão:** Ambientes aglomerados associado a íntimo contato com cães infectados por helicobactérias é fator determinante para transmissão do *Helicobacter* spp. entre caninos.

Descritores: Infecção por Helicobacter . Endoscopia. Mucosa Gástrica. Cães.

Introduction

The *Helicobacter* gender was defined by ribossomic RNA composition studies of sequential hybridation of the bacteria DNA. Belonging to the VI gram-negative bacteria superfamily, they are microaerophyle bacteria, curved and spiral, of smooth surface, measuring close to 0,1µm to 0,5µm wide by 3µm length having round extremities followed by flagellated tufts¹.

After stating the relation between the presence of the *Helicobacter pylori* bacteria and the gastritis genesis, gastric and duodenal ulcer in human by Marshall and Warren², other species of spiral bacteria were identified and described in many animal species including dogs³.

More than half of the world population is infected by *Helicobacter pylori*. It happens in about 40% in developed countries, 70% in developing countries and in low socioeconomical communities with low hygiene it is higher^{4,5}.

In Brazil, recent studies using the urease test, histopathology and sorology on street dogs show 80% to 100% *Helicobacter* spp. prevalence, found with higher frequency at the fundus and gastric body^{6,7}.

The gastric *Helicobacter* commonly found in dogs are *Helicobacter bizzozeroni*, *Helicobacter felis*, *Helicobacter heilmannii* and *Helicobacter salomonis*⁸.

The *Helicobacter* spp. transmission mechanisms are not clear yet. The fecal-oral and oral-oral ways are the most probable^{4,9}.

Epidemiologic studies suggest that poor hygiene conditions, including contaminated food and water can be determinant factors to the transmission among humans and animals¹⁰.

The current therapy scheme indicated to the *Helicobacter pylori* eradication in humans is the triple therapy, which includes an acid secretion inhibitor in combination with two antibiotics^{11,12}.

Metronidazole, clarithromycin, amoxicillin, tetracycline and furazolidone are the most used antimicrobial drugs¹¹. According to the II Brazilian Consense about *Helicobacter pylori*, the therapy associated with clarithromycin, amoxicillin and a proton bomb inhibitor, is an efficient therapy, being indicated as first option for bacteria eradication¹².

The bacteria recurrence seems to occur in a discreet parcel of patients^{13,14}. Scientific informations related to reinfection are still restricted and show that the levels are higher in developing countries¹³.

The understanding of the transmission ways seem to be fundamental to establish strategies to an efficient treatment and specially to control helicobacteriosis in humans and animals.

The objective of this research is to analyze, the *Helicobacter* spp. prevalence in the distinct stomach regions in the canine specie, the efficacy of the clarithromycin, amoxicillin and lansoprazole association to eradicate the bacteria in dogs and determine if there is infection recurrence by direct contact with infected dogs.

Methods

This research had previous permission of the UNICAMP Ethics Committee of Animal Use, Biology Institute (protocol nº 1801-1).

We included in the study 20 street dogs, adults, within close to 20kg, naturally infected with *Helicobacter* spp. confirmed diagnosis by the fast urease test and histology. The animals were maintained at the Veterinary Medicine Hospital Kennel from the Itajuba University Center – UNIVERSITAS, vermifugated and vaccinated according to veterinary medical protocol.

To perform the high digestive endoscopy, all animals received associated 0.04 mg/kg atropine sulfate, 0.004 mg/kg fentanil citrate, 1 mg/kg acepromazine acetate by intramuscular injection as preanesthetic medication. The propofol was used at a 3 mg/kg intravenous dosage as a general anesthesia inducer. The animals were left in spontaneous mechanical ventilation with oxygen at 100 vol%, electrocardiographic monitoring and wrist oximetry. Additional propofol doses were used when necessary.

Pentax Gastrofibroscope FG-29P was used with 250W halogen light source and permanent biopsy forceps.

Were collected two samples from the fundus, two from the body and two from the gastric antrum. One sample of each stomach region was immediately immersed in a standard solution for fast urease test. This study considered positive the sample which presented color change until 12 hours (Figure 1). The second sample was immersed in a 10% formol solution for following histopathologic exam stained with Giemsa and modified Giemsa and bacteria identification increased 400x and 1000x.

For each procedure the endoscope equipment was immersed in a 2% glutaraldehyde solution for a minimum time of 30 minutes after mechanical cleaning with enzymatic solution. The forceps biopsy was treated by autoclave cycles at 134°C.

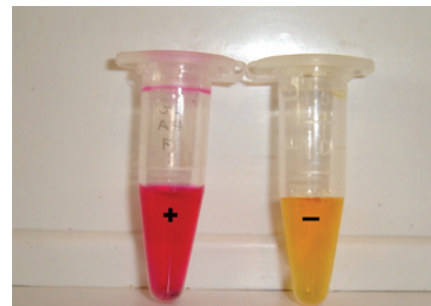


FIGURE 1 – Urease test showing positive and negative sample.

The animals were divided into two 10 positive animal groups: Group I and Group II (Figure 2).

The group II animals were isolated in individual kennels and received therapy at 25 mg/kg clarithromycin, 50 mg/kg amoxicillin and 1mg/kg lansoprazole (Helicopac® - EMS) every 12 hours during 7 days (Figure 2).

One day after the end of the therapy, the animals were submitted to a new upper digestive endoscopy examination to verify the bacteria eradication. Then, the animals were subdivided

into two subgroups (experimental and control) composed of five treated animals each (Figure 2).

The experimental group was placed again in contact with group I and the control group was maintained in individual isolation kennels, both during 60 days, when a third upper digestive endoscopy examination was performed followed by *Helicobacter* spp. search (Figure 2).

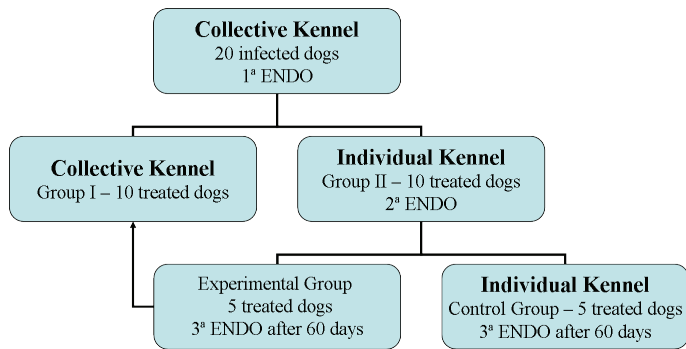


FIGURE 2 – Division of the groups.

Weekly assessment was performed, during all research period. The exception occurred during the seven days medication therapy, when they received daily clinical assessment, within the intention to check possible adverse effects.

The *Helicobacter* spp. prevalence, its distribution along the gastric mucosa and the triple therapy accuracy were determined by the percentage descriptive analysis. To analyze the reinfection occurrence was performed the Fisher exact test. The Kappa coefficient was utilized at the concordance of the urease test and histopathology.

Results

In the analyzed samples were seen spiral bacteria in the mucus, over the tissue surface, at the crypt bottom and inside the gastric glands (Figure 3).

The bacteria morphology found was similar in all dogs (Figure 4A). Both experimental group and control group, were seen long spiral bacteria similar to the species *Helicobacter heilmannii* e *Helicobacter bizzozeroni* (Figure 4B).

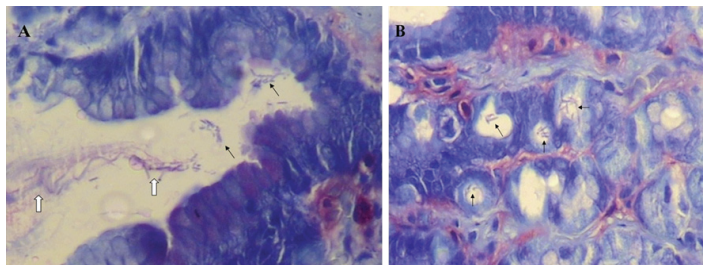


FIGURE 3 – A) Spiral bacteria in the mucus (white arrows), over the tissue surface and at the crypt (black arrows) (Giemsa 400x). B) Spiral bacteria in inside the gastric glands (black arrows) (Giemsa 400x).

According to the urease fast test, the fundus region and the gastric body showed 100% positivity, whereas the infection at the antrum was 60%. The positivity frequency for histopathologic *Helicobacter* spp. was 100% at the fundus, 90% at the body and only 50% at the antrum (Figure 5).

After group division, the isolated experimental group analysis resulted in 100% frequency at the fundus and body, and 80% at the gastric antrum according to the urease fast test (Figure 6). The histopathology was seen 100% prevalence at the fundus and 40% at the antrum (Figure 7).

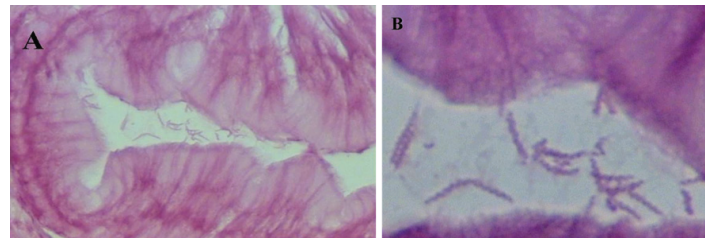


FIGURE 4 – A) First endoscopy: Spiral bacteria at the gastric crypt (modified Giemsa 400x). B) Bacteria with morphological evidence of long spiral (modified Giemsa 1000x).

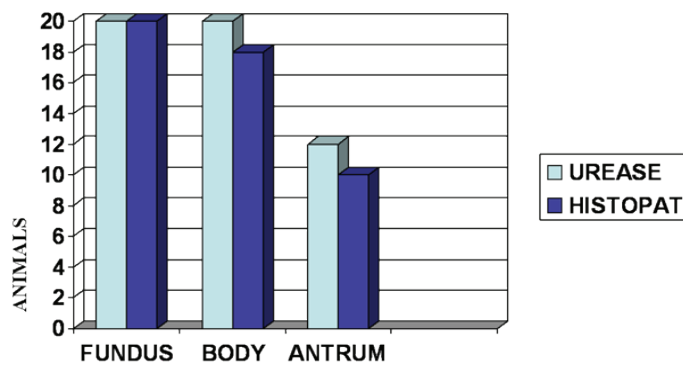


FIGURE 5 – Frequency of *Helicobacter* sp in the distinct stomach regions at the first endoscopy.

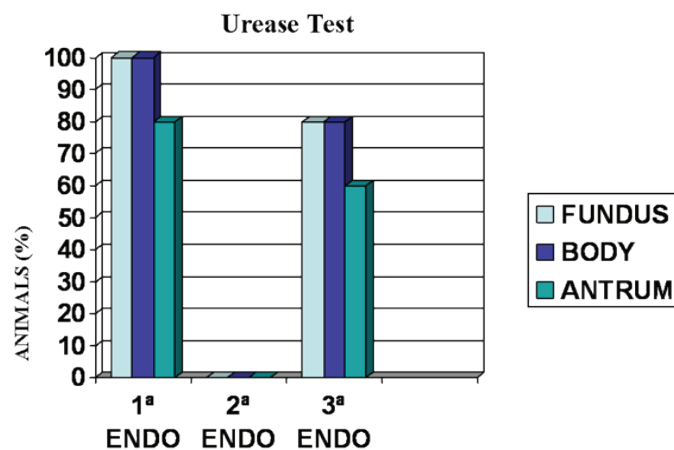


FIGURE 6 – Frequency *Helicobacter* sp in the distinct stomach regions in the experimental group according to the urease fast test.

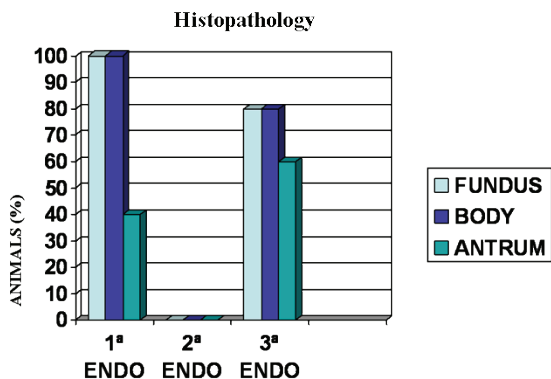


FIGURE 7 – Frequency of *Helicobacter* sp in the distinct stomach regions in the experimental group according histopathology.

The control group presented 100% positivity at the fundus and 60% at the gastric antrum (Figure 8). By the histopathology 100% at the fundus, 80% at the body and 60% at the antrum (Figure 9) were positives.

The triple therapy used was efficient in 100% of the animals after 7 days. The results were negatives at the urease fast tests and histopathologies in both experimental and control groups (Figures 8 and 9). Were not seen *Helicobacter* spp. at the tissues, at the gastric glands and at the mucus of the biopsied regions (Figure 10).

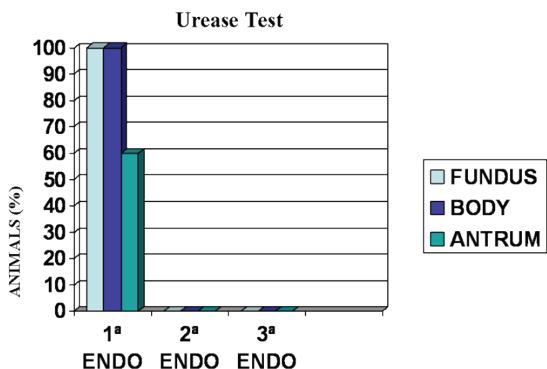


FIGURE 8 – Frequency of *Helicobacter* sp in the distinct stomach regions in the control group according to urease fast test.

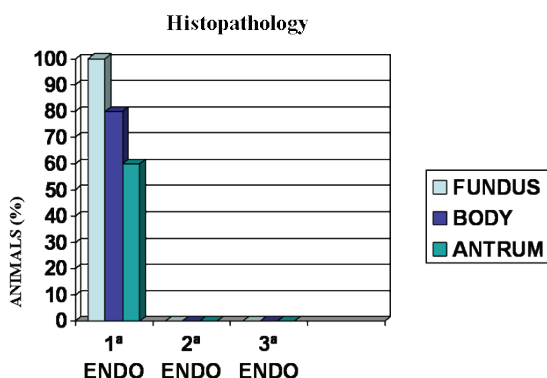


FIGURE 9 – Frequency of *Helicobacter* sp in the distinct stomach regions in the control group according to the histopathology.

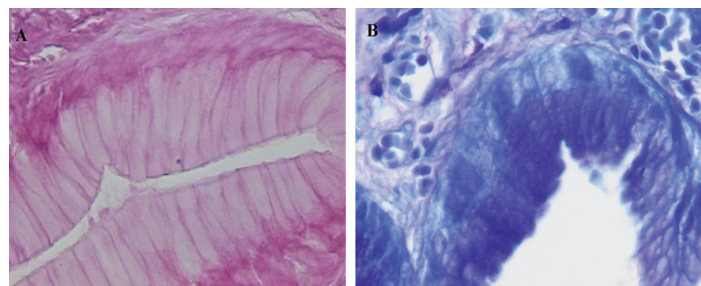


FIGURE 10 – A) Sample of the gastric fundus one day after the end of the triple therapy in control group (modified Giemsa 400x). B) Sample of the gastric fundus one day after the end of the triple therapy in experimental group (Giemsa 400x).

Third digestive endoscopy followed of biopsy 60 days after triple therapy showed that the control group continued eradicated (Figures 8 and 9). The *Helicobacter* spp. were absent in all samples (Figure 12). At the experimental group, 80% of the animals showed again *Helicobacter* spp. presence ($p < 0.05$). The dogs that had *Helicobacter* spp. reinfection the fundus and the body showed positive in 100% of the cases and the antrum in only 50%, according to the urease test (Figure 6). The histopathology identified the bacteria at the crypt bottom (Figure 11A), inside the glands (Figure 11B) and the mucus. The prevalence was 100% at the fundus and body, and 80% at the antrum (Figure 7).

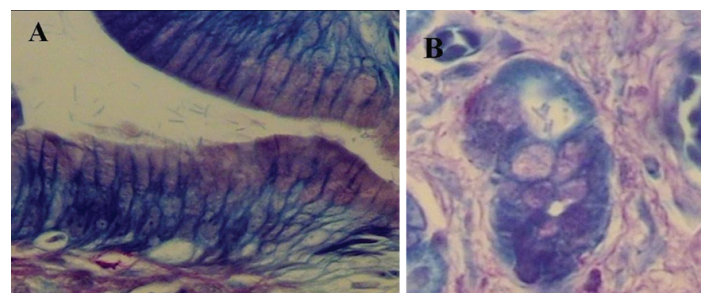


FIGURE 11 – A) Sample of the gastric fundus showing spiral bacterias at the third endoscopy in the experimental group (Giemsa 400x). B) Sample of the gastric fundus showing spiral bacterias inside the glands at the third endoscopy in the experimental group (Giemsa 400x).

Signs of gastrointestinal problems were not observed during the observation period. The possible adverse effects which followed the triple therapy used were not clinically detected.

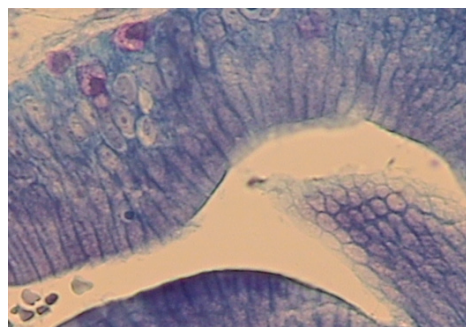


FIGURE 12 – Sample of the gastric fundus without *Helicobacter* sp in the control group 60 days after the end of the triple therapy (Giemsa 400x).

Discussion

Helicobacter spp. are frequently found in dogs^{6-8,15}, not only in healthy animals¹⁵, but also in dogs with gastropathy symptoms⁷.

The *Helicobacter heilmannii*, *Helicobacter bizzozeroni*, species are morphologically similar bacteria, show spiral evident format and smaller than *Helicobacter pylori*^{16,17}. *Helicobacter felis* has periplasmatic fibrillations as a particularity^{18,19} and the *Helicobacter salomonis* are larger microorganisms with discreet wavy morphology²⁰.

The *Helicobacter* spp. were seen in the mucus, over the tissue surface, at the crypt bottom and inside the gastric glands, adding that in this research the bacteria density was higher in areas with more mucus^{6,7}.

The bacteria prevalence in street dogs and in dogs which live in crowded areas like, city kennels in Brazil is between 90% to 100%^{6,7,15,21}. In a retrospective study performed in Colombia shows that the *Helicobacter* spp. prevalence in pet dogs is 50% less²².

The results found in animals in this study were according to the veterinary literature agreeing to the high prevalence and it is supported by epidemiologic studies with human patients, infected with *Helicobacter pylori*, which live in collectivity, mainly in groups within a low socioeconomic level and within low hygiene, where it is observed high infection and reinfection average⁴.

Helicobacter pylori is more frequent at the gastric antrum region in humans¹, however in dogs the fundus and body are presented with high bacteria density and higher probability to find *Helicobacter* spp.^{6,7,15,21}.

The urease fast test can present false negative and false positive results, being the histopathology the most sensible and indispensable technique in detecting gastric helicobacter in dogs. Stained histopathology by Giemsa and modified Giemsa allowed us easy seeing and microorganism identification at the positive urease test. The urease fast test negative result and the tests which the color change time occurred above 2 hours were related to histopathologic exams with helicobacter absence or low colonization density^{7,21-24}. This work showed good concordance between the urease test and histopathology according Kappa coefficient.

The fact that the used therapy in this experiment was not followed by adverse effects, was observed using amoxicillin, metronidazole and colloidal bismuth subcitrate^{25,26}.

The omeprazole, pantoprazole and lansoprazole also can act, in vitro, as antimicrobial by inhibiting the bacteria urease²¹. And the lansoprazole seems to have pharmacological power and action time similar to omeprazole, and also present minimal side effects in North America dogs^{25,26}.

The therapeutic failure as the cause of major reinfection rate and peptic disease recurrence or recrudescence was described^{13,14}, demonstrating that the triple therapy based in clarithromycin, amoxicillin and lansoprazole reduced in an efficient way the human reinfection cases¹³.

Study with amoxicillin, metronidazole and bismuth subcitrate in dogs with gastropathy clinical signs, positive for

Helicobacter spp, scored clinic status improvement in 80% of the animals. On this study 57% of the treated dogs showed again the *Helicobacter* spp. presence in the gastric mucosa after three years²⁵.

According to this research, even with an efficient triple therapy, eradicated individuals can have a higher recurrence probability if maintained intimate contact with positive individuals, associated to low hygiene conditions, specially in crowded situations⁴.

The fecal-oral transmission was suggested at first for the *Helicobacter pylori* isolation in children diarrhea droppings⁴ meanwhile the microorganism detection in the saliva and dental plaque in infected patients strengthened the oral-oral transmission hypothesis²⁷.

Different helicobacter from *Helicobacter pylori* were also identified at healthy domestic animal oral cavity^{21,28,29}.

The *Helicobacter pylori* transmission among people was related after observing equal microorganism reinfection among married patients³⁰.

Literature reviews described in Brazil and abroad state that the natural infection by different bacteria from *Helicobacter pylori* in human could be a sign of interspecies transmission. These studies suggest, with a lot care, a probable power zoonotic^{3,21,28,29}.

This way the canine specie is, in our point of view, a good model to be used on experimental research, focusing on clarify and detail the real transmission mechanism and the bacteria control of this gender, in both animals and human.

Conclusions

The *Helicobacter* spp. is found in higher rate at the fundus and gastric body of the dogs. The triple therapy based in clarithromycin, amoxicillin and lansoprazole is an efficient treatment in eradicating *Helicobacter* spp. in dogs.

The recurrence by *Helicobacter* spp. occurs in eradicated dogs maintained in contact of 60 days with naturally infected dogs. Crowded situations and the intimate contact with infected dogs determine transmission of *Helicobacter* spp. in dogs.

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Correspondence:

Thiago Pires Anacleto
R. João Anny Rey, 232
37130-000 Alfenas – MG Brasil
tpanacleto@gmail.com

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