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

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

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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.


RESUMO

Objetivo: Avaliar a eficácia da terapia triplice em cães naturalmente infectados pelo Helicobacter spp. e investigar a recorrência da infeção pelo contato com animais infectados. Métodos: Foram utilizados 20 cães, submetidos à endoscopia digestiva alta seguida de biopsia gástrica usando teste rápido da urease e histopatologia corada pelo Giemsa. Dez animais foram tratados com terapia triplice preconizada para humanos e divididos em grupo controle e experimental. O grupo controle foi mantido em isolamento enquanto que o grupo experimental foi colocado em contato com os animais positivos durante 60 dias. Resultados: A prevalência da infeção nos animais deste experimento foi de 100%, e mais frequente no fundo e o corpo gástrico. A terapia durante 7 dias empregando claritromicina, amoxicilina e lansoprazol foi eficaz em 100% dos animais. Houve recorrência da infeção 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.

Introduction

The **Helicobacter** gender was defined by ribosomic RNA composition studies of sequential hybridization 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 0.1um to 0.5um wide by 3um length having round extremities followed by flagellated tufts.

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 Warren, other species of spiral bacteria were identified and described in many animal species including dogs.

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 hygine it is higher.

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

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

The **Helicobacter** spp. transmission mechanisms are not clear yet. The fecal-oral and oral-oral ways are the most probable.

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

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.

Metronidazole, clarithromycin, amoxicillin, tetracycline and furazolidone are the most used antimicrobial drugs. 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 eradication.

The bacteria recurrence seems to occur in a discreet parcel of patients. Scientific informations related to reinfection are still restricted and show that the levels are higher in developing countries.

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 pylori**, 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 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.](image)

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).

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).

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).
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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).

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).

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.
Discussion

*Helicobacter* spp. are frequently found in dogs\(^6\)\(^-\)\(^8\),\(^15\), not only in healthy animals\(^13\), but also in dogs with gastropathy symptoms\(^7\).

The *Helicobacter heilmannii*, *Helicobacter bizzozeroni*, species are morphologically similar bacteria, show spiral evident format and smaller than *Helicobacter pylory\(^{16,17}\)*. *Helicobacter felis* has periplasmatic fibrillations as a particularity\(^18,19\) and the *Helicobacter salomonis* are larger microorganisms with discreet wavy morphology\(^20\).

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\% less\(^22\).

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 pylorii*, which live in collectivity, mainly in groups within a low socioeconomic level and within low hygiene, where it is observed high infection and reinfection average\(^4\).

*Helicobacter pylorii* is more frequent at the gastric antrum region in humans\(^1\), 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\(^21\)-\(^24\). 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 urease\(^21\). 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\(^9\)-\(^14\), demonstrating that 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.

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.

References

3. Carvalho GD, Pinto PSA, Vilória MIV, Nero LA. Aspectos
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