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PREVALENCE OF Helicobacter spp. IN DOGS FROM CAMPO GRANDE-MS

PREVALÊNCIA DE Helicobacter spp. EM CÃES DE CAMPO GRANDE-MS

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Abstract
Helicobacter spp. is a spiral Gram-negative bacterium that has substantial clinical importance. It has been related to diseases such as gastritis and peptic ulcers, and more recently to gastric cancer in humans. Evidence suggests the potential of animals, particularly domestic ones, as the source of zoonotic infection of helicobacteria since bacteria with similar morphology to those found in animals were observed in the stomach of humans with gastritis. Thus, dogs have been identified to serve as an important host for infectious agents such as *Helicobacter* spp. From this perspective, the present study aimed to assess the prevalence of *Helicobacter* spp. in dogs from the Zoonosis Control Center of Campo Grande-MS. Samples of body, fundus, and gastric antrum from 96 dogs were collected to evaluate the presence of *Helicobacter* spp. through the rapid urease test and histological analysis. *Helicobacter* spp. was found in 94.7% of the dogs by rapid urease test and in 100% by histological analysis, with bacteria predominance in the stomach fundus region.
Keywords: dogs; *Helicobacter*; urease test.

Resumo
A *Helicobacter* spp. é uma bactéria Gram negativa espiralada, de grande importância clínica, que se relaciona a patogenias como gastrite e úlceras pépticas e, mais recentemente, com o carcinoma gástrico em humanos. Evidências sugerem o potencial dos animais, principalmente os domésticos, como fonte de infecção zoonótica das helicobactérias, já que bactérias com morfologia similar às encontradas em animais foram observadas no estômago de humanos com gastrite. Nesse contexto, os cães podem ser um importante reservatório de agentes infecciosos como a *Helicobacter* spp. O presente trabalho teve como objetivo avaliar a prevalência de *Helicobacter* spp. em cães do Centro de Controle de Zoonoses de Campo Grande/MS. Para tanto, foram utilizados 96 cães dos quais foram colhidas amostras do corpo, fundo e antro gástrico, para avaliação da presença da *Helicobacter* spp. por meio do teste rápido de urease e análise histológica. O teste rápido de urease permitiu a detecção de *Helicobacter* spp. em 94,7% dos cães; já a análise histológica indicou a presença de *Helicobacter* spp. em 100% dos animais avaliados com predominio da bactéria na

região do fundo do estômago.
Palavras-chave: cães; *Helicobacter*; teste de urease.

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Introduction

Helicobacter spp. is a helix-shaped, microaerophilic, Gram-negative bacterium, able to survive in a highly acid environment due to urease production[1-3].

Helicobacter spp. may colonize the gastrointestinal mucosa of humans, domestic animals (such as dogs, cats, pigs, and birds), besides wild animals, such as monkeys[4-7]. Around half of the world population might be infected, although only 5-10% present clinical cases[3]. The correlation of *Helicobacter* spp. in the pathogeny of gastritis and gastric ulcer has been demonstrated, and more recently the bacteria has been identified as the inducing-agent of gastric carcinoma in humans[8]. In dogs, studies on *Helicobacter* spp. prevalence are scarce; nevertheless, an infection rate around 67-100% is suggested. Studies comprising the histological evaluation of dogs' stomach revealed the presence of the bacterium as a predominant occurrence in the body and gastric fundus. However, the degree of colonization by these bacteria do not correlate directly with the diagnosis of mild to moderate gastritis in dogs[10-12].

This bacterium adheres to the gastric mucosa by an adhesine present on its surface, called BabA, facilitating the penetration of antigenic products to mucosa cells, compromising the immune response of the host[9,13]. Another mechanism of pathogenicity of *Helicobacter* spp. is the production of cytokines as CagA (cytotoxin-associated gene A) and VacA (vacuolating -associated cytotoxin). VacA behaves as a passive urea transporter and, thus, it increases the permeability of the epithelium to urea, which is broken into intermediate toxic products. The infection by CagA positive strains is associated with more serious epithelial lesions, acute or chronic severe inflammation, possibility of peptic ulceration, and risk of gastric cancer[14-16].

Evidence suggests the potential of animals, especially domestic ones, to be a source of zoonotic infection by helicobacteria, since bacteria with similar morphology to that found in animals were observed in the stomach of humans with gastritis[17]. This fact deserves close attention because most of the world population presents direct contact with a domestic animal species, mainly dogs[18]. However, the exact way the transmission of this microorganism occurs remains unknown. The isolation of *Helicobacter* spp. from saliva, dental plaque, and feces of dogs reinforces the hypothesis of transmission by these animals, oro-oral or oro-fecal via[2].

In this perspective, the present research aimed at evaluating the prevalence of *Helicobacter* spp. in dogs from the Zoonosis Control Center of Campo Grande, Mato Grosso do Sul.

Material and Methods

Biological samples collection was carried out from August 2007 to October 2007, at the Zoonosis Control Center (ZCC), Campo Grande-MS, using dogs destined to euthanasia. Laboratory analyses were performed at the Pharmacology and Mutagenesis Laboratory of Universidade Católica Dom Bosco - UCDB.

The biological samples were obtained from 96 dogs of undefined breed that came from the ZCC. The animals were anesthetized with Thiopental (7.7mg/kg) and then euthanized with 10 mL of potassium chloride, IV. The procedures were carried out according to the bioethics guidelines and appropriate authorizations. Samples were collected from the gastric fundus, separating the mucous from serous tissue. Only the mucous tissue was used for bacterium detection.

For the bacterium detection, fragments of approximately 6 mm obtained from the gastric fundus of the animals were used. These fragments were submitted to rapid urease test (URETEST, Renylab, PR) that comprises a qualitative colorimetric test to identify the bacterium. It is a highly specific and sensitive test, being more frequently used for the endoscopic diagnosis due to the potent urease activity of the bacterium[3,19].

For the histological evaluation, tissues from the gastric fundus, body, and antrum were randomly collected by open technique from 29 dogs, belonging to the group of 96 sampled animals. After the collection, the biopsies were immersed in formaldehyde solution at 10%. The production of blades with histological material followed this procedure sequence: (a) dehydration and diaphonization of samples with different alcohol concentrations and time; (b) inclusion of the obtained material in paraffin for two hours; (c) 6 µm histological cuts with the aid of a microtome; (d) rehydration of blades with different xylol and alcohol concentrations; (e) finally, staining by modified Giemsa method, to submit the blades material to the solution A (0.4 g basic fuchsin, 2 g of phenol, 4 mL of absolute alcohol, and 100 mL of distilled water) for 5 minutes, and to solution B (45 mL of distilled water; 5 mL of formaldehyde, and 5 mL of acetic acid) also for 5 minutes. By examining the blades (optical microscopy, 1000 x increase) stained or slightly reddish bacteria were observed.

The quantification of the prevalence of *Helicobacter* spp. in the stomach of dogs was carried out by analyzing of the histological blades. Three fields were randomly evaluated on each blade, where the bacteria were counted. A score (1 to 5) was attributed to each blade according to the mean number of bacteria. The scores were determined according to the following intervals: 1-60 bacteria (score 1); 61-120 (score 2); 121-180 (score 3); 181-240 (score 4), above 241 (score 5). The results were expressed as median of the scores in each group.

For the statistical analysis of the histological data, the analysis of variance test (ANOVA) was applied. In all cases, individual comparisons were tested with Bonferroni t-test (multiple comparisons). The number (*n*) of animals per experimental group is described in the figures. The differences were considered significant when $p<0.05$.

Results

The evaluation of the presence of *Helicobacter* spp. in dogs by the rapid urease test showed 94.7% of the animals were positive for the bacterium (Table 1).

Table 1- Distribution of dogs regarding the positivity for *Helicobacter* spp. determined by the rapid urease test

No. of animals	Positive	Negative
96	94.70% (n= 91)	5.20% (n=5)

Considering the characteristics of the animals (sex, age, clinical signs) that presented positivity to *Helicobacter* spp. and the possible correlations between them, we verified the distribution of animals regarding sex was homogenous (Figure 1, panel A), since both males and females presented bacteria similarly (Figure 1, panel B). The evaluation of the animals regarding the age range from 1 to 15 years (Figure 1, panel C) presented positivity for bacteria in all groups (Figure 1, panel D).

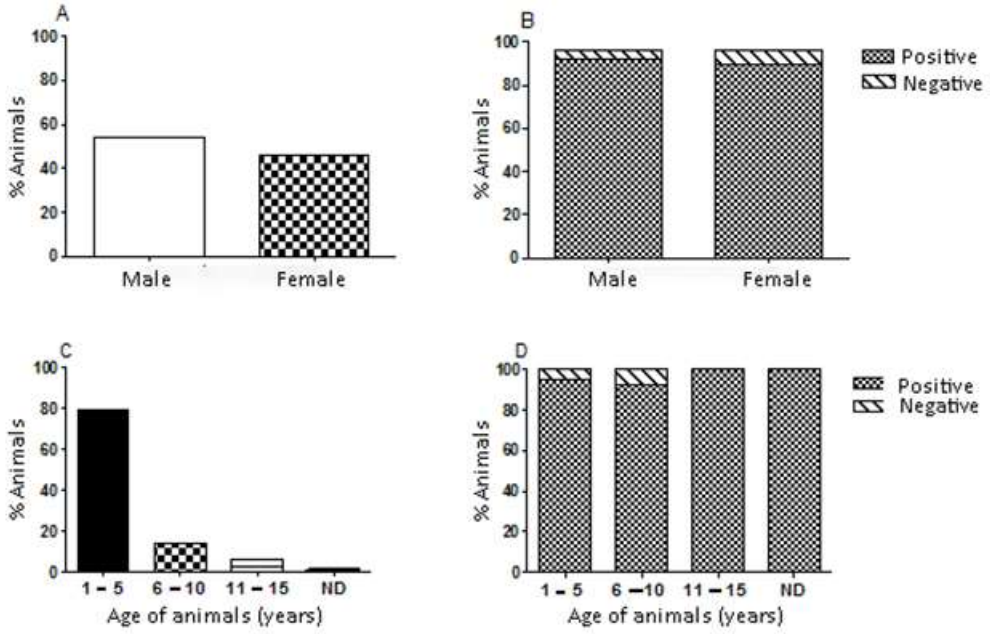

Figure 1. Panel A - Distribution of the dogs evaluated by age. **Panel B** - Representation of the distribution of animals by sex regarding positivity to *Helicobacter* spp. We evaluated 52 males and 44 females. **Panel C** - Distribution of dogs evaluated by age range. **Panel D** - Representation of the distribution of animals by age regarding positivity to *Helicobacter* spp. We evaluated 76 animals at 1-5 years of age, 13 animals at 6-10 years, 6 animals at 11-15 years, and 1 animal at unidentified age. Total: n (96). The bacteria prevalence was determined by the rapid urease test.

The evaluation of *Helicobacter* spp. by histological analysis showed 100% prevalence. The analysis was carried out by counting the bacteria on the randomly chosen fields and attributing values (scores) to the intervals, as described in the Methods section. The data showed a predominance of bacteria in the gastric fungus and followed by the body when compared with the stomach antrum (Figure 2). Besides, bacteria distribution was differentiated in the three stomach regions (Figure 3).

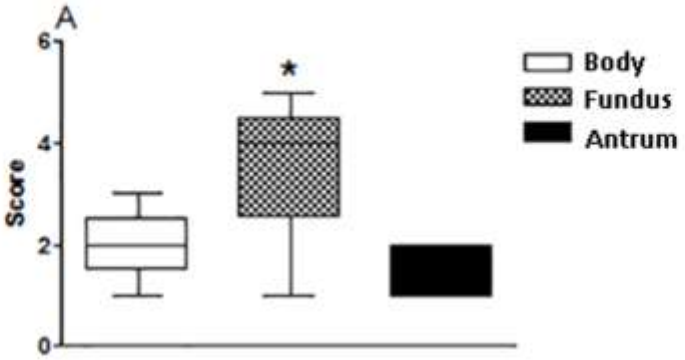

Figure 2. *Helicobacter* spp. distribution according to the stomach region. The stomach regions were classified as gastric fundus, body, and antrum. The animals were evaluated regarding the stomach regions in relation to symptomatology in dogs. The bacteria prevalence was determined by the histological analysis using the modified Giemsa staining method in 29 animals. Statistical analysis was considered significant for the fundus when compared with body and antrum $p<0.05$ (*) (ANOVA, followed by Bonferroni test).

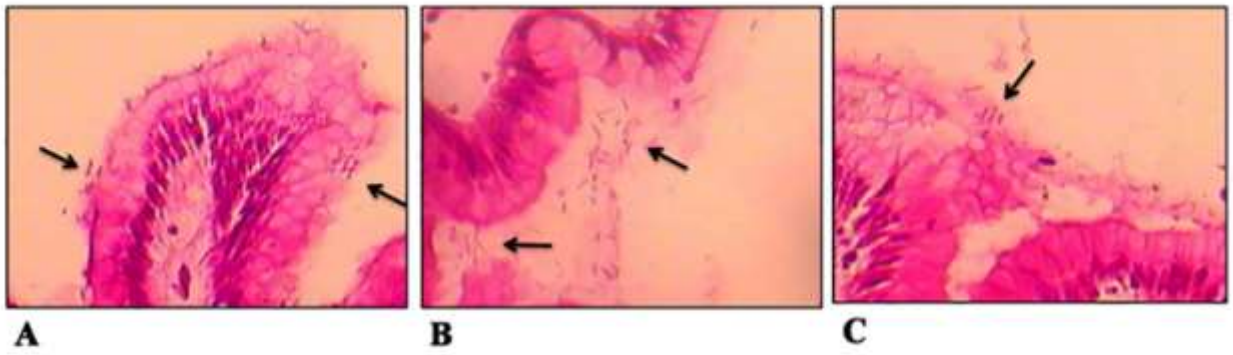

Figure 3. Representation of the stomach regions of dogs with presence of Helicobacter spp. bacterium. **Panel A:** Helicobacter spp. located in the gastric fundus region. **Panel B:** Helicobacter spp. located at the gastric body region. **Panel C:** Helicobacter spp. located at the gastric antrum region. 29 animals were submitted to histological evaluation by the modified Giemsa staining the bacteria were visualized by optical microscopy (1000X increase).

Discussion

The determination of the presence of *Helicobacter* spp. may be done by different methods. At least two

methods should be combined to obtain reliable results[2,20]. Invasive methods for the detection of *Helicobacter* spp. are still commonly used, involving gastroscopy and collection of a biopsy sample for the performance of rapid urease test and histopathological exam. Currently, the use of the detection of bacterial DNA by PCR[21] has been suggested for the identification of *Helicobacter* spp. in the feces. However, this method may be less precise due to a lower quantity of bacteria in the feces of the degradation of bacterial DNA in the large intestine[7].

The results obtained in this study allowed to observe the high prevalence of *Helicobacter* spp. by the rapid urease test, corresponding to 94.7% (91/96) of the animals. These results are compatible with other investigations found in the literature, that showed the presence of the bacteria is high in dogs, and it may reach 100% of these animals[9-12]. However, a study carried out in Poland by Jankowski and collaborators[21], using the technique of *Helicobacter* spp. PCR-detection revealed the presence of the bacterium in only 23.3% of the dogs. This result may be associated with the small number of bacteria in the feces or bacterial DNA degradation in the samples.

The high prevalence of *Helicobacter* spp. was not correlated to the parameters such as sex, age, and clinical signs since almost all the animals evaluated were positive to the bacterium.

The histological analysis by modified Giemsa staining of the biopsy of 29 dogs revealed the presence of *Helicobacter* spp. in 100% of the animals; however, the distribution of this bacterium was heterogeneous at the different gastric regions. The colonization pattern at the fundus was significantly higher when compared with the gastric body and antrum (Figure 3). Other studies showed similar results; however, Vieira[20] also observed a significant prevalence at the body region. The prevalence of bacteria diagnosed by the rapid urease test and the histological analysis was similar. Although both tests have demonstrated similar sensitivity for the detection of the bacterium, it is noteworthy that according to the literature[20], the histological analysis is a more reliable test and it can complement the results obtained by the rapid urease test, especially when there is a possibility of a false negative.

The high prevalence of *Helicobacter* spp. observed in dogs and humans is inversely proportional to the sanitary and economic standard[23]. The infection caused by *Helicobacter* spp. related to the pathologies is catastrophic in humans when compared to dogs. Although there is a significant presence of helicobacteria in dogs, it is not possible to relate it with gastric alterations in these animals[22,24,25]. Studies suggest several hypotheses to explain the factors related to the high prevalence of the bacteria and the infection pattern. An important factor among protection mechanisms is the rapid renewal rate of the gastric epithelium during an aggression. After being exposed to an aggressor agent, epithelial surface cells are exfoliated, and then there is an increase in the number of mitoses, with an increase in cellular input to recover the surface. Therefore, low-intensity infections may evolve to possible significant damages in the gastric mucosa[26,27].

Other studies suggest that the VacA gene codifies vacuolating cytokines and is present in approximately 50% of *Helicobacter* spp. species. This finding partly explains why only the minority of infected individuals develop ulcers and an even lower number evolve to gastric cancer, even when these individuals are infected by more virulent strains of *Helicobacter* spp.[28,29]. According to Israel and Peek[30], in humans all individuals that are *Helicobacter* spp. carriers present coexisting gastric inflammation; however, only a small percentage of colonized individuals develop any pathology. The increase in the risk may be related to differences in the expression of specific bacterial products, variations in the immune response of the host to the bacterium, or specific interactions between the host and the microorganism[31].

Studies have shown that *Helicobacter heilmannii*, *Helicobacter felis*, *Helicobacter salomonis*, *Helicobacter bizzozeronii*, and *Helicobacter pylori* have been found colonizing the stomach of dogs[6,31,32]. The prevalence of this species varies according to the geographic localization; however, a higher prevalence of *Helicobacter heilmannii* is suggested[21]. This bacterium has been proved to be pathogenic to humans[33].

Despite all the investigations, *Helicobacter* spp. infection vias remain unknown. *Helicobacter* spp. transmission may be direct, i.e., by oral, oro-fecal, or gastro-oral vias, as well as indirect, by contaminated food, water, or poorly disinfected endoscopic equipment[34-37]. Although there is no unequivocal demonstration, the high incidence of *Helicobacter* spp. in the stomach of the animals turn them into a risk factor in the transmission of the infection to humans[6,36,37].

Research carried out in the USA and Germany with Denmark dogs showed these animals offer small zoonotic risk because humans are usually affected by a specific subtype (1); in other words, they present higher risk of infection related to pathologies, different from what has been observed in dogs and cats (subtypes 2 and 4)[9]. However, pigs also present a higher risk because of the high frequency of infection by subtype (1)[38].

Kato et al.[39] reported the occurrence of *Helicobacter heilmannii* in children that did not have pets; however, Thomson et al.[40] and Van Loon et al.[41] observed the same species in children and their pets. Zoonotic transmission of the genus *Helicobacter* has been suggested due to the presence of the gastric microorganisms with similar morphology in the stomach of several animal species[42].

Considering the obtained data, we could conclude that in dogs from Campo Grande city (Mato Grosso do Sul State), the colonization rate by *Helicobacter* spp., evaluated by urease test is 94.7%, while the histological analysis obtained 100% rate. Despite the high prevalence, it was not possible to establish a correlation with sex, age, or clinical signs. The impact of these helicobacteria in dogs is still considered controversial because it is not possible to state whether they are part of the stomach microflora of these animals or not. Therefore, new studies should be carried out to try to identify the pathogenicity factors and/or factors in the bacteria/host relation that result in gastric diseases in dogs and other animals.

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