SUMMARY OF THE Ph-D THESIS

GASTRIC PATHOLOGY ASSOCIATED WITH DIFFERENT SPECIES OF Helicobacter in Pets

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SUMMARY

*Helicobacter pylori* is one of the most common bacterial pathogens in humans. The infection with this bacteria is now recognized as a worldwide problem. It causes active chronic gastritis, peptic ulcer disease, acute erosive gastritis, and is a major risk factor for gastric carcinoma and MALT lymphoma.

Different gastric *Helicobacter* species were frequently found in animals, especially in dogs and cats, being present in both asymptomatic dogs (60-100%) and those with chronic vomiting (74-90%). In dogs the gastric *Helicobacter* infection is produces by *H. felis*, *H. Bizzozeronii*, *H. salomonis* and *H. heilmannii*. Coinfection with one or more bacterial species, usually *H.bizzozeronii* and *H. heilmannii* occurs in 15-20% of dogs (Kenneth et al., 1999).

Natural infection with *H.pylori* in dogs is disputed, but there are authors who have identified this bacteria by PCR method (Buczolits et al., 2003; Ogawa et al., 2005).

In cats, gastric infection with *Helicobacter* species is also common, ranging between 86-100%, being identified *H. felis*, *H. heilmannii*, *H. pamatensis* and *H.pylori* (Handt et al., 1994).

The presence of the *H. pylori*, *H. heilmannii* and *H. felis* in dog, cat and human, according to the literature, motivates the study to investigate the prevalence and the pathogenic mechanisms of the gastric infection with *Helicobacter* species in dogs and cat, these animals can be sources of *Helicobacter* spp. for human.

**The importance of researches**

Our results will bring new arguments about spontaneous *Helicobacter* gastric infection in pets and human, and experimental infection in guinea pigs.

**The structure of researches.** The thesis contains a number of 272 pages and is structured in accordance with nowadays legal provisions; it contains a number of 172 figures (pictures, graphics, charts) and 43 tables. The first part describes the most important and conclusive data from the literature on etiopathogenic aspects related to helicobacter gastric infection in humans, dogs and cats, the main gastric lesions associated with this infection, methods of diagnosis and aspects of experimental infection with *Helicobacter* spp. in different experimental animals. The second part of the researches are focused on spontaneous gastric pathology associated with helicobacter infection in dogs and cats (chapters II.2, II.3, II.4 and II.5), comparative helicobacter gastric infections in humans, dog and cat (chapter II.6) and finally, experimental gastric pathology of *H. pylori* in Guinea pigs (chapter II.7). Bibliographic part includes a number of 227 titles of scientific papers from romanian and foreign literature.

**The aim of these studies** was to evaluate both, spontaneous gastric infection with different bacterial species belonging to *Helicobacter* spp. in dogs and cats from the epidemiological and
pathogenesis point of view, and experimental gastric infection with *H. pylori* in Guinea pigs in terms of pathogenetic mechanisms from the gastric level and in vivo bacterial sensitivity to a new therapeutic protocol associated with probiotics.

**Spontaneous gastric pathology in dogs and cats**

In chapter II.2. „Epidemiological and morphological aspects of spontaneous gastropathies in dogs and cats” are described the main gastric lesions present in dogs and cats bodies during October 2007 - August 2010, and correlations between these lesions and helicobacter infection.

From a total number of 643 dogs and 133 cats examined, 73 (11.35%) dogs, respectively 25 (18.79%) cats presented different gastric lesions.

Assessing the age groups with gastric lesions in dogs, it was observed that chronic lesions, including chronic gastritis and gastric polyps were found in adults and old dogs; in cats there was no uniformity of the gastric lesions according to age. In dogs and cats, it was noted that gastric infection with *Helicobacter* spp. increases with age, being highest between 10-15 years old.

Acute erosive gastritis has been identified both in dogs (n=8/73 cases; 10.95%) and cats (n=8/25 cases; 32%), being characterized by superficial necrosis of the gastric mucosa and a mild neutrophilic infiltrate. In three cases it has been described gastric dilation and volvulus with gastric venous infarction. Hemorrhagic gastritis has been found in four cases (two dogs and two cats) associated with anticoagulant rodenticide poisoning (3 cases) and anaerobiosis (one cases).

Gastric ulcers were present both in dogs (n=8/73; 10.95) and cats (n=11/25; 44%). They have been grossly characterized by solitary or multiple necrotic foci with lack of substance, having a crateriform (excavated) aspect.

Chronic gastritis has been described in 37/73 dogs (50.68%) and 3/25 cats (12%), as an irregular diffuse thickening of the pyloric gastric mucosa, sometimes with a brain convolution aspect (giant hypertrophy).

In dogs, the assessment of chronic inflammation revealed that 21/37 (56.75%) of cases had gastritis grade I, 10/37 (27.02%) gastritis grade 2 and 6 / 37 (16.21%) gastritis grade 3.

Microscopically, the following types of chronic gastritis have been diagnosed: chronic superficial gastritis, chronic diffuse gastritis, chronic atrophic gastritis, chronic hypertrophic gastritis and chronic follicular gastritis. Also, were present preneoplastic lesions, such as incomplete intestinal metaplasia, glandular atrophy and epithelial dysplasia.
In dogs, the correlation between the degree of *Helicobacter* spp. colonization and histological markers of inflammation in the antrum region, have had a statistical significance (p <0.01). In cats, in the pyloric region of the stomach, it has been noticed that bacterial density is associated with severe acute inflammatory infiltrate (p<0.01). No statistically significant correlation with the degree of follicular hyperplasia has been identified. The cats have not presented metaplasia and / or dysplastic lesions in the gastric mucosa level.

Gastric polyps were found in 15 dogs, but they will be described in a later chapter. The gastric tumors hev been identified in 5/73 dogs (6.84%) and 1/25 cat (4%).

In dogs, gastric tumors were classified as: *in situ* adenocarcinoma (n=1), tubulo-papillary adenoma (n=1) and leiomyoma (n=3). In cats it has been found one gastric tumor represented by MALT lymphoma. In these tumors, the histological exam has showed a severe *Helicobacter* spp. infection.

Clinically, gastric lesions developed different symptoms such as rapid dilation of the stomach associated with gastric volvulus, sudden death in intoxication with anticoagulants, chronic vomiting (8 / 15 cases), weight loss (5 / 15 cases) and gastric retention syndrome (2 / 15 cases) associated with gastric polyps. Haematemesis, melena and anemia are present in acute and chronic gastric ulcers. In most cases of chronic gastritis and gastric erosions, the owners have not reported symptoms of gastric distress in animals. Also, in animals with gastric adenocarcinoma, leiomyoma and gastric lymphoma there have been reported chronic symptoms such as vomiting, diarrhea, progressive weight loss and gastric retention syndrome.

Histological exam has showed an increased number of infected animals with *Helicobacter* spp., 62/71 dogs (87.32%) and 23/25 (92%) cats, similar data has been reported by some authors as Weber in 1958, Henry in 1987 and Eaton in 1996. Gastric helicobacters have been present in all regions of the stomach, but in dogs these bacteria have been found more frequently in the pyloric area (58/71, 81.69%), and in cats in the body region (23/25, 92%).

The present study has showed the role of *Helicobacter* spp. infection in the development of gastric lesions in dogs and cats. Secondly it was noted that chronic gastritis produced by *Helicobacter* spp. infection is associated with preneoplastic changes, the lesions can be areas of development of the gastric cancer associated with *H.spp* infection, especially in dogs.

Chapter II.3. "Diagnose methods of spontaneous gastric infection with Helicobacter spp. in dogs and cats" presents data from a comparative study regarding different invasive methods used in the diagnosis of gastric infection with *Helicobacter* spp. in dogs and cats. Only invasive
methods have been used, which required the removal of the gastric mucosa, because the study has been done only on cadavers of dogs and cats.

The methods used for the diagnosis of *H. pylori* infection are classified depending on the chosen infection indicator in: direct methods, which aim to identify the bacteria from gastric mucosa biopsies or bacterial cultures (morphological examination, cytology, bacteriology and molecular - genetics) and indirect methods, which are based on the identification of antibodies induced by the presence of bacteria or the determination of products resulting from the bacterial urease activity (breath test, serological examination and rapid urease test from gastric biopsies).

Lack of consensus in the literature regarding the diagnosis of helicobacter gastric infection in dogs and cats and the lack of a standard "gold protocol", similar to the diagnosis of *Helicobacter pylori* infection in humans determined us to accomplish this study which aims to identify gastric infection with *Helicobacter* spp. in dogs and cats through various invasive diagnostic methods.

Comparison of different diagnostic methods in terms of sensitivity and specificity, namely the identification of gastric infection with *Helicobacter pylori* through immunohistochemical technique are the main objectives of the study.

For cytological exam, urease test and histopathology there have been used fragments of gastric mucosa (samples) collected from three regions of the stomach (cardia, body and antrum) from 71 dogs and 25 cat’s bodies. Samples for microbiological examination have been taken from 20 corpses of dogs and 10 cats. For Giemsa method there have been used 20 samples from dogs and 10 from cats and for immunohistochemical techniques anti-Ac *H.pylori* there have been used 30 samples from dogs and 10 from cats. To analyze the effectiveness of different diagnostic methods there have been taken into consideration the following issues: Sn%, Sp%, PPV% and PNV%.

*Helicobacter* spp. infection has been found in fragments of gastric mucosa taken from the corpses of dogs and cats by all the methods of diagnosis used in this study, but in different percentages.

The interpretation of urease test for infection with Helicobacter spp it has been performed at different time intervals, which have been associated with different degrees of infection of 1 to 4 in: grade 0 (negative at 48 hours), grade 1 (positive at 48 hours), grade 2 (positive at 24 hours), grade 3 (positive at 4 hours), grade 4 (positive at 1 hour). Using this method, *Helicobacter* spp. infection has been found in 66/71 dogs (92.95%) and 100% cats.

In cytological exam, *Helicobacter* spp. were colored in dark blue on a light background, when Diff-Quick Panoptic was used. In Gram stain, bacteria have been colored in different shades
of red (acidophil type). Using this method *Helicobacter* spp. infection has been identified in a number of 66/71 dogs (92.95%) and 21/25 cats (84%), achieving similar results for urease test. *Helicobacter* spp. from gastric area have been pointed in dogs at a rate of 7.5% in cardia region, at 57.74% in the region of stomach body and 87.32% in the pyloric region. Gastric helicobacters have been found in cats in the cardia region at 20% cases, in the gastric body at 84% cases and in the pyloric region and 76% of cases through cytological exam.

In dogs, reported to the cytological examination, we have noted that the urease test has excellent sensitivity but its specificity ranges from 55.55% in the antrum to 96.96% in the cardia. This could be due to the presence in the two regions of the stomach of other urease-producing microorganisms such as *Proteus, Citrobacter, Enterobacter* and *Staphylococcus*, causing false positives results. Predictive value (-) of the UT (urease test) is 100%, making it the first choice to be recommended as a means of diagnosis, a negative value excluding infection and a positive test requires confirmation by cytology.

In cats UT sensitivity is increased, but the specificity varies between 50 and 100%, with small values in antrum, where there could be encountered other urease-producing microorganisms, which lead to an increase number of false positive tests. UT value consists in 100% PPV being used as first screening test, a negative value resulting in the exclusion of the infection and a positive value requires further investigation for the diagnostic approach of *Helicobacter* spp. infection.

Histopathology, as a method of diagnosis of *Helicobacter* spp. infection, has used fragments of gastric mucosa fixed in 10% formalin at neutral pH, included in paraffin and stained with H&E and M&T. *Helicobacter* - like Organisms were colored in shades of red and/or dark blue on the surface of foveolar epithelium or in the lumen of gastric glands, under the mucus layer. Various morphology of this bacteria, limit the diagnosis just for GHLO (Gastric *Helicobacter* - like Organisms), the differentiation between these species being accomplished through more specific methods. By this method, *Helicobacter* spp. infection has been identified in a proportion of 87.32% (62/71) in dogs and 92% (23/25) in cats.

In dogs, the specificity of histopathological exam used to identify the gastric infection with *Helicobacter* spp. has had values between 80 and 100% depending on the gastric region. In cats, histopathology has had an increased sensitivity, moderate specificity ranging between 50 and 100%, explained by the irregular distribution of *Helicobacter* spp. infection and the small number of animals from the study group. Identification of gastric infection with *Helicobacter* spp. on histological sections has had a high positive predictive value but a negative test does not exclude infection *H.* spp because its irregular distribution.
Microbiologically, *Helicobacter* spp. have been isolated from the pyloric region of the stomach in 6/20 dogs, but not in cats.

Giemsa method confirmed the *Helicobacter* spp. infection in all cases which have been identified by common stains, such as H&E and M&T, staining in dark blue the spiral organisms. The negative samples obtained by common stains, have also been negative by Giemsa method. The organisms marked by Giemsa method have been pleomorphic in size and number of curves, which invalidates the strict utilisation of this method to identify only *H. pylori* (wrong diagnose).

For immunohistochemical method, polyclonal primary antibodies anti-*Helicobacter pylori* were used. The samples used for this method have been positive by histopathology exam.

*H. pylori* has been identified as a spiral-shape organism, brown marked, with 3-5/0.5 µm diameter, present on the surface of the foveolar epithelium and inside of gastric glands, in 6/30 dogs (20%) and 4/10 cats (40%); they have also been confirmed by PCR technique. The gastric organisms with multiple large curves have not been marked in this way.

The presence of *Helicobacter pylori* in dogs and cats, highlighted through more specific diagnostic tests, such as PCR and IHC, has concluded that these animals can be a source of this infection for human.

In chapter II.4 “The identification of the different species of *Helicobacter* from gastric biopsies from dogs and cats using the PCR molecular technique” we have aimed to identify three of the main *Helicobacter* species from a series of gastric biopsies from dogs and cats who have presented lesions at this level by PCR technique, using specific primers for *H. pylori*, *H. felis* and *H. heilmannii*.

The identification of *H. pylori* and the correlation of the bacteria with the developed of gastric lesions are the main objectives of this study, especially because this type of infection in dogs is contested by other authors. The presence of this bacteria in dogs and cats could explain the *Helicobacter pylori* infection sources in humans, given its zoonosis potential.

For this study there have been used gastric mucosa fragments from 15 dog bodies and 15 cat bodies with gastric lesions. The samples have been separately prelevated from the three gastric regions (cardia, body and antrum) in Eppendorf tubes and they have been kept at -70 °C. For this study there where preleveted gastric samples only from those animals which have been positive at the urease test: in fact, another aim of this research is to establish the connection between a positive urease rapid test and the PCR results. DNA extraction and purification has been performed in the Patology Department and data amplification and interpretation have been carried out in the Genetics Department of the Horticulture Faculty.
Out of 15 samples collected from dogs, 4 positive cases of *H. pylori* have been identified, 8 positive results of *H. heilmannii*, whereas *H. felis* have not been found in any of these samples.

Despite the fact that the presence of Helicobacter felis-like bacteria has been revealed by histopatologic examination, PCR technique has not confirmed it. This is explained by the fact that bacteria found in the samples belong to other *Helicobacter* species. In gastric biopsies performed on cats, out of 15 samples collected, 5 positive cases of *H. pylori* have been identified, 7 positive results of *H. heilmannii* and 9 cases of *H. felis*.

In cats, bacterial coinfection with several *Helicobacter* species has been confirmed in 6 cases, as follows: a double infection with *H. felis* and *H. pylori* has been noticed in 2 out of 15 cases, a bacterial coinfection with *H. pylori* and *H. helimannii* has been identified in other 2 cases out of 15 and a coinfection with *H. felis* and *H. helimannii* has been identified only in one case out of 15. A triple coinfection with *H. felis*, *H. pylori* and *H. helimannii* has been identified only in one case out of 15. In dogs, a bacterial coinfection with several *Helicobacter* species has been confirmed only in one case, between *H. pylori* and *H. helimannii*. *Helicobacter* was identified only in two of the three regions of the stomach from where the samples have been collected.

In veterinary medicine the term frequently used to define spiral-shaped bacteria in the stomach of animal species, especially in carnivores, is GHLO (Gastric *Helicobacter* - Like Organism). For this reason, many authors associate gastric lesions in carnivores with GHLO instead of different *Helicobacter* species, even though these were identified by high-performance techniques such as IHC and/or PCR. Despite this, we believe it is incorrect to use the term GHLO to define gastric *Helicobacter* species, as long as a high-performance technique was used to differentiate these bacteria. Associating gastric lesions with a bacterial species instead of a bacterial genus simply confirms the fact that only certain *Helicobacter* species have pathogenetic relevance for the gastric mucosa. Moreover, important data concerning the type of gastric lesion caused by a certain *Helicobacter* species can be found, starting from the hypothesis that *Helicobacter* species are different as far as pathogenicity is concerned.

Gastric lesions have been present both in the pyloric and in the body region. Acute gastritis and chronic active and inactive gastritis, gastric ulcer and MALT lymphoma are histopathologic gastric lesions found in cats and dogs in this research. In dogs gastric infections with *Helicobacter pylori* and *Helicobacter heilmannii* have been associated with acute and chronic gastritis found both in the pyloric and fundic region. In cats gastric infections with *H. felis*, *Helicobacter pylori* and *Helicobacter heilmannii* have been associated with acute and chronic gastritis, gastric ulcer and MALT lymphoma, found both in the pyloric and fundic region. *Helicobacter felis* has not been associated with acute gastric lesions in cats, this infection being
associated with chronic gastritis, chronic gastric ulcer and MALT lymphoma both in the fundic and in the pyloric region. From this point of view, data correspond with the information found in other studies in this field.

Despite having found a series of gastric lesions in which the presence of gastric *Helicobacter* (*H. felis, H. pylori, H. heilmannii*) has been identified both in dogs and in cats using the PCR technique, it is not possible to assert that these lesions were not caused by *Helicobacter* spp., since primers have been used only for 3 bacterial species. Considering the fact that gastric mucosa of cats and dogs is also colonised by other bacterial species belonging to the Helicobacter genus, such as *H. bizzozeroni, H. canis, H. rappini, H. billis*, the existence of gastric lesions could also be explained by their presence. Moreover, it is impossible to exclude the influence of other factors such as hyperacidity, nutrition, medical substances, nonbacterial biotic agents and dysmetabolisms in the development of these gastric lesions.

This research has provided evidence for the presence of *Helicobacter pylori* bacterium in dogs, in spite of the fact that the presence of this bacterium in the stomach of this species has been denied by other studies. The presence of these bacteria in dogs can explain one of the sources of infection with *Helicobacter pylori* in humans, dog owners and not only. The presence of *H. pylori* infection in cats corresponds with the data found in other studies.

In the chapter II.5. „*Gastric polyps in dogs and Helicobacter infection*” we firstly propose to evaluate the epidemiological, gross and histological aspects of the gastric polypoid masses in dogs, and secondly to achieve a link between them and *Helicobacter* spp. infection. In humans, the gastric polyps, especially those hyperplastic or regenerative are common, and there is a strong association with a various types of chronic gastritis and *H. pylori* infection. To our knowledge there are no literature data about the correlation between gastric polyps, chronic gastritis and *Helicobacter* spp infection in dogs, aspect which prompted us to expand the area of research in this direction.

The investigations have been performed in the Department of Pathology, Faculty of Veterinary Medicine from Cluj-Napoca, Romania. During four years (2007-2010), 643 complete necropsies on non putrefactive cadavers of dogs were made. From the total cases, 15 of them (2, 33%) revealed focal, intraluminal, solitary or multiple outgrowth of gastric mucosa (polypoid masses). From these dogs, 8 male and 7 females, aged between 9-17 years, six have died because of euthanasia at the request of the owner because of advanced cancer or irreversible chronic diseases, and the other nine dogs have died from various conditions.
The patients’ history have revealed digestive symptoms such as chronic vomiting (8/15 cases), gastric retention (2/15 cases) and weight loss (5/15 cases).

In 14 cases, the gastric polyps have been found in the pyloric region of the stomach and in one case the polypoid mass was present at corpus and pyloric junction. Multiple gastric polyps (between 2 and 9) were found in 8 dogs, while solitary polypoid masses were present in 7 cases. The size of gastric polyps ranged from 5 to 30 millimeters in diameter with an average of 12 mm.

In 14 cases the histopathology exam of the gastric mass has confirmed polyps, and only in a single case a polyp-like structure has been revealed.

In the structure of the gastric polyps there have been observed different preneoplastic lesions, such as intestinal metaplasia, chronic atrophic gastritis and low epithelial dysplasia of the gastric mucosa. In one case there has been found a benign neoplastic lesion, classified as tubule-papillary adenoma.

10 out of 15 cases have revealed chronic hypertrophic gastritis in the pyloric region. In other two cases, the stomach wall has been deformed by a polypoid growth originated from submucosal mesenchymal tissue.

All, Giemsa for *H. pylori* staining, urease test, cytopathology have revealed the presence of *Helicobacter* spp. in 93.33 % of samples (14/15). The IHC method has revealed the presence of *H. pylori*-like helix shaped bacteria, of brown colour at positive sites, in 20 % of gastric polyps (3/15).

The inflammatory infiltrate (macrophages, lymphocytes and plasma cells), glandular atrophy, fibrous tissue proliferation, lymph follicle hypertrophy and minimal change with goblet cell hyperplasia have been the main histological aspects of the chronic gastritis.

In the background gastric mucosa of hyperplasic and inflammatory polyps, Kendall test analysis has showed a significant correlation between GHLO colonization and mononuclear cells degree, glandular atrophy, intestinal metaplasia and lymphoid follicle hyperplasia (p< 0.01). On the other hand, Kendall test has showed no correlation between GHLO colonization and neutrophils score, epithelial necrosis and epithelial dysplasia (p>0.05).

In the background gastric mucosa of adenomatous polyps, Kendall test has showed a significance correlation between GHLO colonization and mononuclear cells score, lymphoid follicle hyperplasia and epithelial dysplasia (p<0.01).

In conclusion, the present study has found a correlation between the presence of GHLO and the histopathological changes in the background gastric mucosa (around of polyps) and development of the gastric polyps.
In chapter II.6. “Comparative aspects of the spontaneous gastric infection with *Helicobacter spp.* in humans, dogs and cats” we have followed the investigation of the *Helicobacter pylori* infection in humans from The 3rd Medical Department of the Cluj-Napoca Hospital which have been suffering from gatroduodenal distress and in the end we have compared the results of this study with those from the dogs and cats from the previous chapters.

The objectives of the study are represented by the evaluation of the gastric lesions in humans induced by * Hp* infection, comparison between different diagnosis methods in *Hp* infections, correlations between the degree of bacterial infection with the intensity of the lesions and finally, developing a comparative study of the gastric * Hp* infection in humans, dogs and cats, from an etiological, pathogenetic and diagnosis methods aspects.

The study has been made on a number of 32 patients, 15 men and 17 women, aged between 21 and 69 years, which have been diagnosed with * H. pylori* following the respiratory test with C14 marked urea. The respiratory test was positive in all the patients. Later on, they have been examined by UDE. Through this method the esophageal, gastric and duodenal mucosa have been examined with the purpose of identifying the different inflammatory or neoplastic lesions at this level and also performing gastric mucosa biopsies (cardia, body and antrum) for the urease test, microbiology, histopathology, immunohistochemistry (polyclonal Ab anti-*H. pylori*) and PCR method (specific primers for *H. pylori*, Vac A and Cag A genes).

At UDE, from the 32 patients, 4 have been identified with chronic lesions and 5 with acute lesions, 23 patients had not gastric lesions. At the urease test, from 32 patients, 29 have been positive and 3 negative.

Following the histopathological exam performed on the 19 gastric biopsies, there have been revealed several lesions at this level, 16 of which suggesting active chronic gastritis of different degrees. Other two biopsies have shown incomplet intestinal metaplasia and only one biopsy has shown low epithelial dysplasia. Evaluating both the gastric lesion intensity and the degree of the *Helicobacter* colonization, we have observed that there is no significant statistical correlation between these two characteristics.

After IHC, used to investigate the infection with *H. pylori*, it was demonstrated the presence of marked bacteria in all 19 cases, having a range of gastric mucosa colonization.

PCR amplification for *H. pylori*, using the same nucleotide sequences for dogs and cats, has been done in all 4 of the bacterial cultures studied. The PCR amplification for the cagA gene (297 bp) has been done at all of the 4 initial samples, while the samples from the bacterial cultures which have been amplified the vacA gene (259 bp) have been positive in ¾ of the patients.
In humans, most epidemiological studies are done in the direction of the *H. pylori* bacteria. By comparison, in animals most epidemiological studies include the identification of *Helicobacter* spp. Although it is a comparative study, it is normal to find a higher occurrence of the *Helicobacter* infection in dogs and cats that in humans, precisely because of this perspective.

If in humans we have identified *H. pylori* in all of the 32 gastric biopsies, in animals we have identified this bacterium in a smaller number of cases (in dogs 4 positives for *H. pylori* out of 15 gastric biopsies and in cats 5 positive for *H. pylori* out of 15 biopsies), in contradiction with other studies that infirm the presence of this bacteria, especially in dogs.

From the human gastric biopsies there had not been identified other spiral bacteria from the *Helicobacter* spp., such as *H. felis* and *H. heilmannii*, bacteria also with pathogenic potential in humans. Instead, we have identified these two bacteria in dogs and cats using the PCR technique. In dogs, the coinfection with several species of *Helicobacter* has been identified in 6 cases, thus a double infection between *H. felis* and *H. pylori* has been observed in 2 out of 15 cases, the co-infection between *H. pylori* and *H. heilmannii* in other 2 out of 15 cases and the co-infection between *H. felis* and *H. heilmannii* has been present in only one case of 15. In cats the co-infection between several species of *Helicobacter* has been identified in only one case, between *H. pylori* and *H. heilmannii*.

Most gastric infections with *H. pylori* in humans had chronic lesions, while in dogs and cats the inflammatory lesions associated with *Helicobacter* spp gastric infections have been various, but similar with human patients. Erosive gastritis, gastric ulcers, chronic gastritis and gastric neoplasm have been identified in the dogs and cats studied. From a histological point of view, the chronic gastritis has coincided with those described in humans and animals by other specialists.

The gastric lesions induced by *H. pylori* have been found both in the antrum and in the body of the stomach. In dogs, the gastric infections with *H. pylori* and *H. heilmannii* have been associated with acute and chronic gastritis found both in the antrum and in the fundus. In cats the gastric infections with *H. felis*, *H. pylori* and *H. heilmannii* have been associated with acute and chronic gastritis, gastric ulcer and MALT lymphoma, these being found both in the antrum and in the body regions.

Both in animals and in humans there can be used several methods of diagnosis of the gastric infection with *Helicobacter* spp. If the respiratory test is done with difficulty in animals, in humans the sensibility of the test varies between 90-98%, and the specificity reaches 99-100%. Used in human patients from whom the gastric biopsies have been taken for this study, the
respiratory test has been positive in all 32 cases. We couldn’t use non-invasive methods due to the fact that the study has been made on dog and cat cadavers.

The cytopathological exam has not been used in human patients studied mainly because in clinical practice is rather preferred the urease test. On the contrary, in dogs and cats the cytopathological exam could be considered as the “gold standard” for the identification of the helicobacterial infection gastritis. Through this test we have identified gastric helicobacter in dogs in a proportion of 92.95% and in cats 84%.

The sensibility of the urease test reported to the cultural test has been 90%. The specificity of the test is 100% only when the color change of the culture medium is observed until 4 hours. In humans, the use of this test on all 32 gastric biopsies has had a 100% specificity and a 97% sensibility. The high specificity of the urease quick test in humans can be explained by the fact that the gastric mucosa is in most cases colonized only by *Helicobacter pylori*.

In dogs and cats, compared to the cytopathologic, the urease test we have noticed an excellent sensibility but the specificity varied between 55.55% at the antrum until 96.96% at the cardia. This fact can be explained by the presence at body and antrum level of other urease-producing microorganisms.

*H. pylori* infection is difficult to identify from gastric biopsies by histopathological examination because of its very small dimensions (2-4/0.5 µm). For that reason it requires special staining, such as Giemsa staining and/or immunohistochemistry (IHC) with antibodies anti *H. pylori*. In dogs and cats, histopathology with common stains, including the Giemsa technique has identified a number of bacteria polymorphic from the point of view of the size and the number of twists, which leads us to state that the diagnosis is H. spp. and not H. pylori.

IHC has revealed in all 19 human gastric biopsies studied the presence of *H. pylori* bacterium on the surface of the foveolar epithelium and in the gastric glands. The sensitivity of the IHC in this case compared with the urease respiratory test has been in this case of 100%. In dogs, IHC has revealed the presence of *H. pylori* at 6/30 (20%) and in cats at 4/10 (40%) of the specimens; all these cases have been confirmed by PCR.

In humans, due to the fact that the gastric mucosa is colonized almost in every case solely by *H. pylori*, it can successfully be used a primary polyclonal antibody anti *H. pylori*. In animals, mainly in dogs and cats, due to the presence of a highy number of *Helicobacter* spp. at the gastric level, for the immunohistochemical technique it is advised the primary monoclonal antibodies for *Helicobacter pylori* to be used. These antibodies have the purpose of avoiding the nonspecific reactions given by other species of *Helicobacter*, such as *H. felis, H. heilmannii, H. canis* and *H. bizzozeronii*. 
Thereby for the certainty diagnosis of the *Helicobacter pylori* infection we have intended to use a much more superior technique, such as PCR. From the 4 bacteria cultures isolated from the human patients we have obtained positive results for *H. pylori* in every case. The same cultures were negative for *H. felis* and *H. heilmannii*.

Using the same primers like in humans we have identified helicobacterial gastric infections with *H. pylori*, *H. felis* and *H. heilmannii* in dogs and cats.

An important virulence factor it is considered to be the product of the vacA gene, the vacuolated citotoxin, which causes the vacuolation of the target cells’ cytoplasm both in vivo and in vitro. Although all the strains have this gene, only 50-60% are capable to cause the vacuolation of the epithelial cells’ cytoplasm. Another virulence factor is the CagA protein (protein associated with the citotoxicity), which is codified by the cagA gene, found in 60-70% from the *H. pylori* strains. The bacterial samples which had the cagA s2 allele amplified have been positive at three out of four patients. We have also obtained an amplification strip at 300 bp at one sample in which we used the vacA primer of 259 pb (possibly non-specific product). Unlike the humans, in dogs and cats the two vacA and cagA genes could not have been identified from the gastric biopsies.

Besides the specific particularities found in gastric infection with *Helicobacter* spp. in humans, dogs and cats, in this study we have revealed a series of similarities between etiology, pathogenesis, common gastropathies associated with the infection and the diagnosis methods used to detect these bacteria in the stomach.

Based on the highlighted aspects in this study regarding the common infection with *H. pylori* in humans, dogs and cats we can state that these pets could represent potential infection sources in helicobacterial infection in human and vice versa.

Chapter II.7, entitled “*Experimental gastric infection with Helicobacter pylori in Guinea pigs -pathogenetic aspects and treatment*”, represents the last part of independent research of the PhD thesis.

The purpose of this study was to induce a gastric infecton with *Helicobacter pylori cagA* and *vacA posive* in Guinea pigs and to show the histological, haematological and biochemical changes that occur during the eradication therapy of this infection through the administration of a triple medication (Omeprazol, Rifaximine, Amoxiciline) and probiotics (Enterolactis).

For this study, there have been used a number of 20 Guinea pigs (*Cavia porcellus*), 10 females and 10 males, each approximately 3 months of age at the beginning of the experiment.
The *H. pylori* strains have been isolated from four different bacterial cultures obtained from human gastric biopsies of four different patients. After obtaining these cultures, they were typified through the PCR technique, all four being cagA/vacA+. Suspensions with a 10 UFC/ml(1MF) density have been used for the infection.

The infection with *H. pylori* cagA/vacA+ has been induced to 15 of the Guinea pigs, oraly, with the esophago-gastric tube. 1 ml of the bacterial suspension has been administrated three days in a row. Before inducing the infection, during the three days of bacterial adiminstration and a day before the infection was induced, 1mg/kg of Omeprazol was administrated. After 14 weeks, faeces were taken individually from each infected Guinea pig, for the confirmation of the infection through the PCR technique, using specific primers for *Helicobacter pylori*.

After confirming the infection with *Helicobacter pylori*, we created an eradication plan, using a series of medical substances (Amoxiciline, Rifaximine, Omeprazol) and probiotics: to one group infected with *Hp* we have administrated probiotics with 5 days before and during the antibiotics treatment, and to the the other group only probiotics during the antibiotics therapy. After the treatment, blood samples were taken from the Guinea pigs for biochemical and haematological investigations, after which they were put to sleep in order to take gastric mucosa samples for further histopathological, citopatological, microbiological, immuno histochemical and PCR technique investigations, for evaluating the gastric lesions and confirming the eradication of the *Helicobacter pylori* infection.

The information gathered has been statistically processed with the IST test (Independent sample test) for independent samples, considering $p$ as statistically important values (statistically important value) less than 0.05. The IST test examines whether there are important differences between the two compared groups concerning the average of the analysed dependent variables. The experimental model has been used with the FMV Cluj-Napoca bioethics committee permission.

The gastric infection with *H. pylori* has been successfully induced to all animals and it was confirmed after 14 days postinfection through the PCR technique.

Haematologically, a growing number of the white blood cells, lymphocytes and neutrophils has been seen in the infected Guinea pigs compared to the witness groups, but without an important statistic value($p<0.005$). At the same time, it was noticed a reduction of these parameters after both treatment protocols, but only in neutrophils these parameters had a statistical importance($p<0.05$) between the infected group and the one treated with antibiotics, with a previous premedication with probiotics. There have also been a short number of platelets (as a possible side effect of Rifamixine) in treated Guinea pigs compared to those who were
untreated \((p<0.01)\) between the untreated \(Hp\) group and the one treated with probiotic premedication) and between the two treated groups there have been no significant statistical differences \((p<0.05)\).

Biochemically, a series of specific markers have been monitored for gastric and especially hepatic diseases, which occurred after the \(H.pylori\) infection, as well as after the antibiotic treatment. A growth of GOT and GPT was noticed in the infected and the untreated group, compared to the witness group \((Hp-)\), but only the GPT enzyme has had a statistical importance, where \(p<0.05\). After the treatment, the GPT value decreases and remains in the physiological limits in both differently treated groups, its value is lower but without statistical importance in the probiotics premedicated Guinea pigs group. GOT, on the other hand, has increased its value in the antibiotics treated group and without probiotics premedication, compared to the probiotics premedication treated group \((p<0.05)\). The same thing happens with the alkaline phosphatase.

At the post-mortem examination, multiple gastric diffuse erosions and hyperplasia of the gastric mucosa have been found in the infected and untreated Guinea pigs. The evaluation of the \(H.pylori\) bacteria isolated from the gastric mucosa fragments of the infected and untreated group has been made through microbiological, citopatological, rapid urease test, histopatological with common and Giemsa stains, immunohistochemical tests and PCR technique. Through the microbiological examination the \(H.pylori\) has been identified after 7 days of incubation at 2/5 of the animals in the infected and untreated group. The urease activity has been present at 3 Guinea pigs from the infected and untreated group and the histopathological test didn’t show the presence of \(H.pylori\) bacteria. The immunohistochemical tests with anti-Hp Ac showed the infection of two Guinea pigs from the infected and untreated group, and was negative for the other groups.

Through the PCR technique, an amplification of the DNA bacterial sequences has been obtained for \(H.pylori\) in 4/5 Guinea pigs from group A (infected and untreated). There were no amplified sequences in the negative witness group and in B and C groups (infected and differently treated), which explains the eradication of the Hp infection after the treatment.

Histologically, there have been no gastric lesions in the Guinea pigs negative witness group. In comparison with this group, there have been noticed chronic active gastritis in the Guinea pigs from group A (infected and untreated), and the intensity of the inflammatory process was weak to moderate.

In group B (infected with \(Hp\) treated without probiotic premedication) there has been no gastric inflammation evidence. In group C (infected with \(Hp\) and treated with probiotic premedication and antibiotics) there have been some discrete gastric chronic and active lesions.
Statistically there have been no significant differences between the experimental groups (p<0.005) concerning the intensity of the gastric lesions.

Concerning the number of apoptosis and cellular divisions, there have been a greater number of these two in the infected groups, compared to the negative witness ones (p=0.028).

The presented study showed that the oral infection of Guinea with *Hp* isolated from humans determines chronical active gastric injuries and induces apoptosis and cellular proliferations that can diminish once the infection is eradicated. Also, we elaborated a new protocol of eradication of the infection by introducing a new type of antibiotic, Rifaximine and a probiotic premedication, which led to the diminishing of the side effects of antibiotics.

**General conclusions**

1. This study showed that the gastric infection with *H. pylori* evolves frequently in cats and dogs, most of the time as a chronic and asymptomatic infection.

2. In dogs, the associated injuries were represented by chronic gastritis and gastric polyps, and in cats acute erosive and cankerous gastritis.

3. The study has also shown in both cats and dogs an important corelation between the number of the *H.pylori* and the intensity of the gastric injuries, especially in the pylorus region.

4. Through the IHC and PCR techniques, the different species of *Helicobacter* (*H.pylori, H. heilmanii* and *H.felis*) with pathogenetic and zoonotic importance were shown.

5. The antral gastric polyps were present in a relatively great number of dogs (n=15), contrary to what the specialty literature has shown, most of these polyps having a hyperplastic nature.

6. The polyposis gastric injuries, through the epithelial metaplasia/displasia sources, may represent areas for gastric cancer development in dogs; the identification of a benign adenomatous polyp confirms it.

7. The presence of the *Hp* infection in the inflammatory, hyperplastic and adenomatous polyps is another argument for the etiology of these injuries in dogs, an unpresented aspect in the specialty literature.

8. The presence of cronical gastritis due to *Hp* in the adjacent gastric polyps region tells even more that the gastric polyps can develop due to a chronic tissue injury produced by GHLO, with an atypical regeneration of the gastric mucosa.
9. The urease test, the citopathological test and the histopathological with common and Giemsa stains can easily be used to diagnose the gastric infection with *Helicobacter* spp. in cats and dogs.

10. Through the immunohistochemical and molecular diagnostic methods, the infection with *Helicobacter pylori* in dogs was shown, aspect which is still questioned today by the specialty literature.

11. This study has shown many commons aspects between humans, cats and dogs regarding the etiopathogenesis of the gastric infection with * Hp*, as well as the diagnosis methods.

12. Considering the aspects shown in this study, concerning the common infection with *H. pylori* in humans, cats and dogs, we can state that these pets represent potentially sources of infection with *Hp* for humans and the other way around.

13. Introducing a new antibiotic, Rifaximine, together with the probiotics premedication in the treatment of the * Hp* infection scheme can become, in the future, a new therapeutic protocol for humans, cats and dogs.