

LOCATION OF HELICOBACTER IN THE GASTRIC MUCOSA OF DOMESTIC DOGS (*CANIS FAMILIARIS*)

Dace Bērziņa, Edīte Birģele

Latvia University of Agriculture

dace.berzina@llu.lv, edite.birģele@llu.lv

Abstract

This study was done to evaluate the prevalence and colonization density of helicobacters in the gastric mucosa of domestic dogs (*Canis familiaris*). Mucosal samples were taken from several places of cardiac, fundic and pyloric gland region of the stomach to detect helicobacters with urease test, brush cytology, and light histological examination. Fourteen dogs of fifteen sampled animals showed positive urease test and the presence of tightly spiraled helicobacters. Positive urease test was observed in 80.4% of all examined samples. Helicobacters were detected in 82.7% of all examined samples by brush cytology and 80.8% of all examined samples by histological examination. Compared to gastric regions, in all employed detection methods showed significantly higher prevalence of helicobacters in cardiac and fundic gland region than in the less effected pyloric gland region of the stomach. Histological examination showed the location and colonization density of the bacteria at the superficial epithelium, gastric pits, and deep glandular epithelium of the gastric mucosa in different regions of the stomach. Colonization density of bacteria was evaluated in 4 groups: as absent, mild, moderate, and severe colonization density of helicobacters. Occurrence of helicobacters in the cardiac and fundic gland region showed mild and moderate colonization density of helicobacters at the superficial epithelium and at the gastric pits of the gastric mucosa. Colonization density of spiral-shaped bacteria between the deep glandular epithelium was more pronounced in the pyloric gland region than in the cardiac and fundic gland regions of the gastric mucosa in the domestic dogs.

Key words: helicobacters, dogs, gastric, superficial epithelium, pits, glands.

Introduction

Many *Helicobacter* species colonize the stomachs and intestines of humans and several domestic and wild species of animals (Neiger et al., 1998). Some helicobacter species have been formally recognized and have often been associated with condition of gastric and/or enterohepatic disease including gastroenteritis, gastric ulcers, hepatitis, and cancer (Fox et al., 1997; Solnick, Schauer, 2001). However, not all *Helicobacter* species are considered pathogenic and instead may form part of the host's indigenous gastrointestinal microflora (Simmons et al., 2000).

Since the first isolation of *Helicobacter pylori* from humans with gastritis and gastric ulcer in 1984 (Marshall and Warrens, 1984), helicobacters have been detected in several animal species, such as dogs, cats, pigs, cheetahs, ferrets, polar bears, sea lions, monkeys, and rodents (Bronson et al., 1991; Lee et al., 1992; Eaton et al., 1993, 1996; Fox and Lee, 1995; Jalava et al., 1997; Neiger et al., 1998;

Hwang et al., 2002; Oxley et al., 2004). Our previous study shows that helicobacters are detected also in the gastric mucosa of feral raccoon dogs (Bērziņa, Birģele, 2006). Several

Up to now many diagnostic methods have been developed to detect *Helicobacter* species, some invasive, such as rapid urea

cytology, histology, electronmicroscopy, culture, polymerase chain reaction, and others non-invasive, such as serology, urea breath test (Happonen et al., 1996) and recently *H. pylori* antigen is also determined in feces (Happonen et al., 1998). It is reported that appearance of helicobacters and histopathological changes in the mucosa of stomach can be detected with several histological staining methods, such as hematoxylin and eosin, Giemsa-Wright and Warthin-Starry stains (Hermanns et al., 1995; Happonen et al., 1996; Neiger et al., 1998). Previous investigations show that helicobacters are detected at superficial epithelium, gastric pits and deeper in the glandular epithelium of the gastric mucosa (Hermanns et al., 1995; Happonen et al., 1996; Otto et al., 1994). Histological examination also allows evaluation of the mucosal status and shows high sensitivity and specificity. This is also pointed out in other studies (Megraud, 1996); however, it is comparatively expensive and labor intensive (Chu et al., 1997).

The aim of our study was to detect the prevalence and colonization density of helicobacters in the gastric mucosa of domestic dogs (*Canis familiaris*).

The main tasks of this work:

- 1) to detect helicobacters in the mucosal samples of the stomach with three different diagnostic methods – urease test, brush cytology, and histological examination;
- 2) to establish the prevalence of helicobacters in different parts of the stomach – cardiac, fundic, and pyloric gland region;
- 3) to evaluate helicobacters at different parts of gastric mucosa: superficial epithelium, gastric pits, and deeper glandular epithelium.

Materials and Methods

Mucosal samples of the stomach were taken from fifteen domestic dogs immediately after the death of animal (under agreement of the owner) in the Center of Veterinary Education, Latvia within one year period.

Mucosal samples were obtained from strictly determined seventeen sites of the stomach: four sites of cardiac gland region, eight sites of fundic gland region, and five sites of pyloric gland region. In total, during this study there were examined 255 gastric mucosal samples of domestic dogs for detection of helicobacters. Mucus and mucosal samples for brush cytology, urease test, and light histological examination were obtained from each

sample site within two hours after the death of animals (Happonen et al., 1996).

Small gastric mucosal samples were cut with scissors and placed in 24 well plates for urease test. The samples were incubated in 1 ml of reagent containing 10% unbuffered urea in distilled water and phenol red indicator (pH 6.3). The results were recorded in 10min, 30min, and 60min after each sample was placed in reagents (Bērziņa, Birģele, 2006). A color change from pale yellow to bright pink was considered positive to helicobacters.

Collection of mucus for brush cytology was performed using sterilized small cotton brushes. The brush was rolled over the gastric mucosa at the sample site and subsequently rolled on the clean slide. The preparations were then air dried and stained with *Diff-Quick* staining method (Happonen et al., 1996). Helicobacters were observed with light microscope *Leica* oil immersion lens at 1000 magnification.

Gastric mucosal samples for histological examination were fixed in 10% neutral buffered formalin, routinely processed in Tissue Auto processor *Tissue-Tek II*, embedded in paraffin, sectioned in 4 µm thick sections with microtome *SLEE Mainz Cut 4055* and stained with *Diff-Quick* method (Happonen et al., 1996). Helicobacters were detected at the gastric mucosa with light microscope at 1000 magnification.

Histological examination of each sample included evaluation of location and colonization density of helicobacters at the superficial epithelium, gastric pits, and deeper glandular epithelium of the cardiac, fundic and pyloric gland regions of the stomach. The presence of helicobacters was evaluated in accordance to 4 grades: as absent with no bacteria seen in the visual field, as mild with 1-10 bacteria seen in the visual field, as moderate with 10-50 bacteria seen in the visual field, and as severe with more than 50 bacteria seen in the visual field (Happonen et al., 1998; Neiger et al., 1998).

Statistical analyses of results were performed by SPSS 11.5 program. Differences between the results produced by urease test, brush cytology and histological examination were treated by nonparametric *K-related Cochran's Q test*. Occurrence and colonization density of helicobacters at the different parts of mucosal epithelium in the investigated gastric gland regions were analyzed with *Chi-Square test* of independence (Paura, Arhipova, 2002; Arhipova, Bāliņa, 2003).

Results and Discussion

Fourteen of fifteen examined domestic dogs showed positive results by all used detection methods for helicobacters. Statistical analyses did not show significant difference between all of our used diagnostic methods ($p > 0.05$) in domestic dogs. Urease activity was observed in 205 (80.4%) of examined 255 gastric samples after 60 min of incubation. Spiral-shaped organisms were observed in 211 (82.7%) of 255 brushes of gastric mucosa by brush cytology and in 206 (80.8%) of examined 255 samples of gastric mucosa by histological examination.

Brush cytology is recommended for practical use as it is relatively simple, cheap, and rapid method for screening infection with helicobacters. Histological examination is also sensitive, but it is comparatively expensive and requires special laboratory skills and equipment. It is used to evaluate location and colonization density of helicobacters and to detect histopathological changes in the mucosa of the stomach.

The results of urease test in domestic dogs are demonstrated in Figure 1. It is seen that urease activity after 10 minutes of incubation was detected in 43.3% of samples within the cardiac gland region, in 50.0% samples within the fundic gland region, and in 28.0% of samples within the pyloric gland region. Whereas urease activity after 30 minutes of incubation was detected in 83.3% of samples within the cardiac gland region, in 91.7% of samples within

the fundic gland region, and in 50.7% of samples within the pyloric gland region. So, detection rate after 30 minutes of incubation increased approximately twice ($p < 0.05$) in all examined gland regions. Eventually, urease activity after 60 minutes of incubation was detected in 86.6% of samples within the cardiac gland region, in 94.2% of samples within the fundic gland region, and in 53.3% of samples within the pyloric gland region. This suggests that a longer incubation period increased the detection rate of helicobacters. These results are quite close to our studies of *Helicobacter spp.* in the gastric mucosa of feral raccoon dogs (Bērziņa, Birģele, 2006).

Compared to *Helicobacter spp.* positive results in different parts of the stomach, positive rate of cardiac and fundic gland region in domestic dogs was significantly higher ($p < 0.05$) than in pyloric gland region of the stomach. These results are quite close to other studies of *Helicobacter* like microorganisms in domestic dogs where the highest detection rate of *Helicobacter spp.* has been found in fundic gland region of the stomach (Happonen et al., 1996). Whereas, our study shows that detection rates of helicobacters in domestic dogs are different in comparison with the prevalence of helicobacters in the gastric mucosa of feral raccoon dogs. There were no significant differences ($p > 0.05$) in occurrence of *Helicobacter spp.* in the samples of different gastric parts in the feral raccoon dogs (Bērziņa, Birģele, 2006).

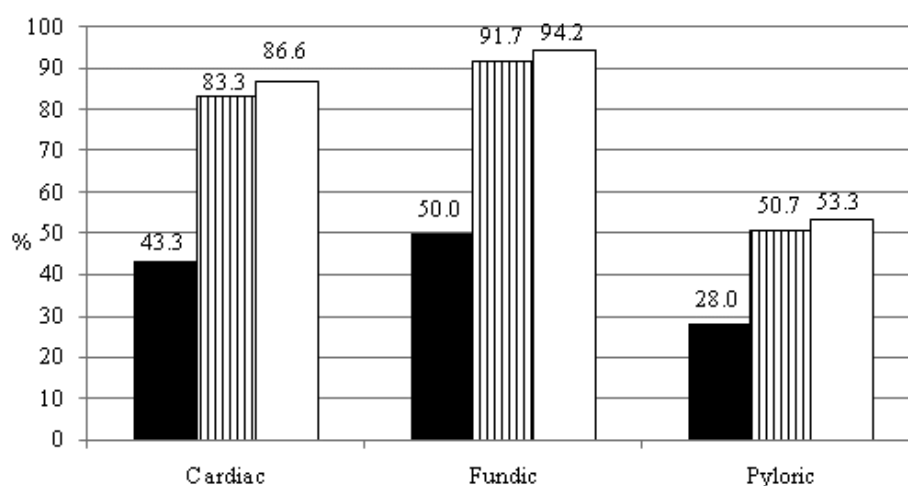


Figure 1. Percentage of *Helicobacter* positive mucosal samples from different sites by urease test (■ 10 min □ 30 min □ 60 min).

The detection rates of helicobacters in domestic dogs by brush cytology are presented in Figure 2. We established that there is no significant difference ($p>0.05$) in occurrence of helicobacters in the cardiac and the fundic gland region of the stomach: the spiral-shaped bacteria were observed in 91.7% of samples from cardiac gland region and in 94.2% of samples in the fundic gland region. It should be emphasized that brush cytology, equally to urease test, also showed that detection rate of helicobacters in the pyloric gland region is approximately twice less ($p<0.05$) than in the cardiac and fundic gland region. The brush cytology detected only 57.3% of positive samples in the pyloric gland region (Figure 2). These results are quite similar to other studies

were gastric *Helicobacter* like microorganisms were mainly found in the fundic gland region of domestic dogs (Happonen et al., 1996).

Detection of helicobacters by histological examination showed similar detection rates as urease test and brush cytology (Figure 2). Spiral-shaped bacteria were observed at the gastric mucosa of domestic dogs in 88.3% of samples from the cardiac, and in 93.3% of samples from the fundic and in 54.7% of samples from the pyloric gland region.

Histological examination also showed that detection rate of helicobacters in the cardiac and fundic gland region was significantly higher ($p<0.05$) than in the pyloric gland region of the stomach.

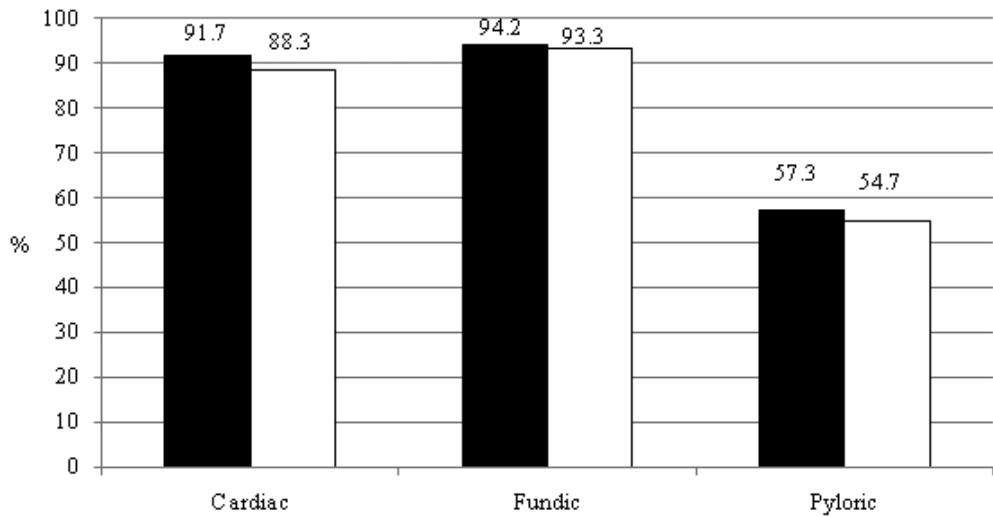


Figure 2. Percentage of *Helicobacter* positive mucosal samples from different sites by brush cytology and histological examination (■ Brush cytology □ Histological examination).

Concerning the study of colonization density of helicobacters at the different parts of gastric mucosa, firstly we will discuss the results of the cardiac gland region (Figure 3). It was established that the amount of samples with mild colonization density of helicobacters was significantly higher ($p<0.05$) at the superficial epithelium and the gastric pits of the mucosa. Mild colonization density of spiral-shaped microorganisms was observed at the superficial epithelium in 67.9% of samples, and at the gastric pits in 69.8% of samples in the cardiac gland region. Moderate colonization density was observed at the superficial epithelium and the gastric pits in 11.3% of samples. Severe colonization density was only in

9.4% of samples at the superficial epithelium and in 9.4% of samples at the gastric pits of the gastric mucosa. There were no bacteria at the superficial epithelium in 11.3% of samples, and at the gastric pits in 9.4% of samples. Between the deep glandular epithelium there were no helicobacters in most (86.6%) of samples. Whereas mild, moderate and severe colonization density of helicobacters between the deep glandular epithelium was only in 9.4% of samples in each part of mucosa in the fundic gland region, which means that helicobacters are less distributed between the deep glandular epithelium in both cardiac (Figure 3) and fundic (Figure 4) gland regions.

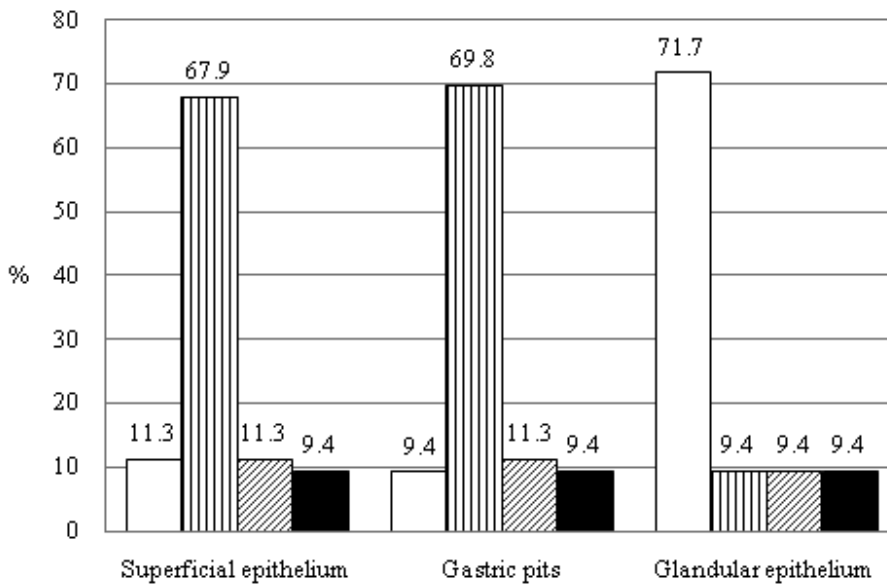


Figure 3. Percentage of samples with different colonization density of helicobacters at different sites of mucosal epithelium in the cardiac gland region (□ Absent ▨ Mild ▤ Moderate ■ Severe).

Colonization density of helicobacters at different sites of gastric mucosa in the fundic gland region is presented in Figure 4. Occurrence of spiral-shaped bacteria was evidently higher ($p < 0.05$) at the superficial epithelium and the gastric pits of the mucosa. Moderate colonization density of helicobacters was observed at the superficial epithelium in 74.1% of samples and at the gastric pits in 83.9% of samples. At the superficial epithelium there were 14.3% of samples with mild, and 4.5% samples with severe colonization density

of helicobacters, but 7.1% of samples with no bacteria in the fundic gland region. At the glandular epithelium of mucosa there was a significantly higher ($p < 0.05$) amount (86.6%) of samples with no bacteria. Whereas mild, moderate and severe colonization density of helicobacters between the deep glandular epithelium was only in 4.5% of samples in the fundic gland region. Thus, the amount of samples with no bacteria between the deep glandular epithelium was significantly higher ($p < 0.05$) in both cardiac and fundic gland regions.

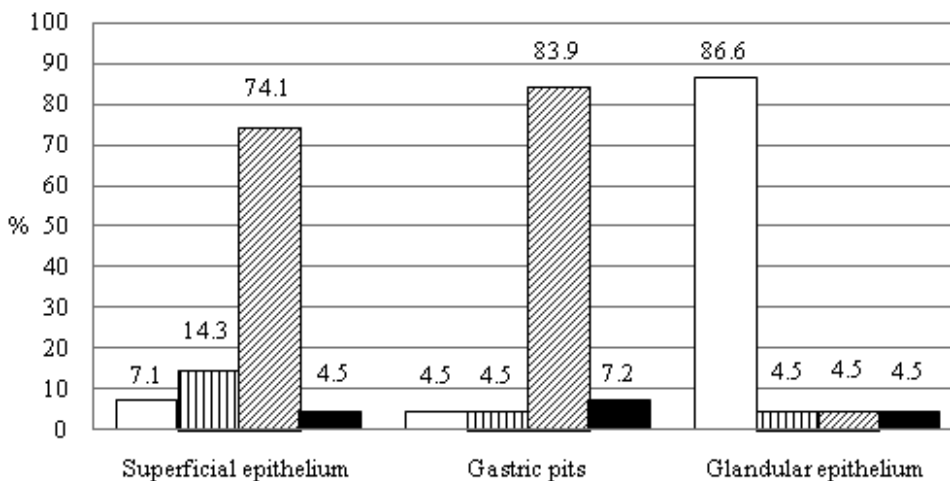


Figure 4. Percentage of samples with different colonization density of helicobacters at different sites of mucosal epithelium in the fundic gland region (□ Absent ▨ Mild ▤ Moderate ■ Severe).

Colonization density of helicobacters at different sites of gastric mucosa in the pyloric gland region is presented in Figure 5. There was no significant difference ($p>0.05$) in colonization density of helicobacters between different parts of gastric mucosa in the pyloric gland region. At the superficial epithelium there were 41.5% of samples with mild, 34.1% of samples with moderate, and 12.2% of samples with severe colonization density of helicobacters, as well as 12.2% of samples with no bacteria in the mucosa. At the gastric pits there were 48.8% of samples with moderate, 26.8% of samples with mild, and 12.2% of samples with severe colonization density of helicobacters, as well

as 12.2% of samples with no bacteria in the mucosa. Between the deep glandular epithelium there were 41.5% of samples with moderate, 21.9% of samples with mild, and 17.1% of samples with severe colonization density of helicobacters, but 19.5% of samples with no bacteria in the mucosa. Thus, the amount of samples with moderate colonization density of helicobacters between the deep glandular epithelium in the pyloric gland region was significantly higher ($p<0.05$) than in the cardiac and fundic gland region. This study is going to be continued to investigate the association between location, colonization density of helicobacters in the different parts of stomach.

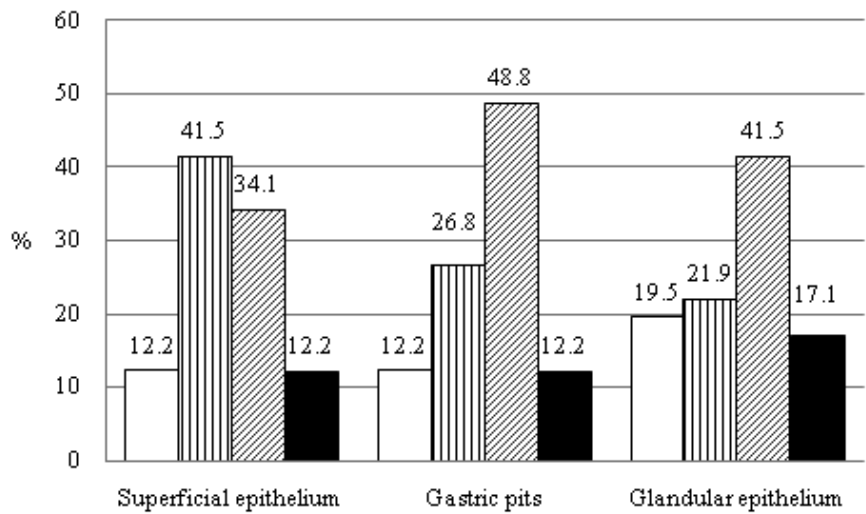


Figure 5. Percentage of samples with different colonization density of helicobacters at different sites of mucosal epithelium in the pyloric gland region (□ Absent ▤ Mild ▨ Moderate ■ Severe).

Conclusions

1.

All used detection methods showed that in comparison to gastric parts, the cardiac and fundic gland region is the most affected site by helicobacters in domestic dogs.
2.

The location of helicobacters in the cardiac and fundic gland region showed mild and moderate colonization density of helicobacters at the superficial epithelium and at the gastric pits of the gastric mucosa.
3.

The colonization density of spiral-shaped bacteria between the deep glandular epithelium is more pronounced in the pyloric gland region than in the cardiac and the fundic gland regions of the gastric mucosa in domestic dogs.

References

1. Arhipova I., Bāliņa S. (2003) *Statistika ekonomikā* (Statistics in economy). Datorzinību centrs, Rīga, 352 p. (In Latvian).
2. Bērziņa D., Birģele E. (2006) Kuņģa *Helicobacter spp.* izplatība jenotsuņiem Korejas Republikas Čonbukas Provincē (Prevalence of gastric *Helicobacter spp.* in feral raccoon dogs in Republic of Korea, Chonbuk province). *Starptautiskā zinātniskā konference "Dzīvnieki. Veselība. higiēna"*. Jelgava. Latvija, pp. 36-42. (In Latvian).
3. Bronsdon M.A., Goodwin C.S., Sly L.I., Chilvers T., Schoenkecht F.D. (1991) *Helicobacter nemestrinae* sp. nov., a spiral bacterium found in the stomach of a pigtailed macaque (*Macaca nemestrina*). *International Journal of Systemic Bacteriology*, 41(1), pp. 148-201.
4. Bussac G. (1999) *Helicobacter pylori* and the oral environment. *Practical Periodontics Anesthetics Dentology*, 11(8), pp. 918-922.
5. Chu K.M., Poon R., Tuen H.H., Law S.Y., Branicki F.J., Wong J. (1997) A prospective comparison of locally made rapid urease test and histology for the diagnosis of *Helicobacter pylori* infection. *Gastrointestinal Endoscopy*, 46 (6), pp. 503-509.
6. Eaton K.A., Dewhirst F.E., Paster B.J., Tzellas N., Coleman B.E., Paola J., Sherding R. (1996) Prevalence and varieties of *Helicobacter* species in dogs from random sources and pet dogs: animal and public health implications. *Journal of Clinical Microbiology*, 34(12):3, pp. 165-245.
7. Eaton K.A., Dewhirst F.E., Radin M.J., Fox J.G., Paster B.J., Krakowka S., Morgan D.R. (1993) *Helicobacter acinonyx* sp. nov., isolated from cheetahs with gastritis. *International Journal of Systemic. Bacteriology*, 43(1), pp. 99-205.
8. Fox J.G., Batchelder M., Marini R., Yan L., Handt L., Li X., Shames B., Hayward A., Campbell J., Murphy J.C. (1995) *Helicobacter pylori*-induced gastritis in the domestic cat. *Infectious Immunity*, 63(7), pp. 2674-2755.
9. Fox J.G., Lee A. (1997) The role of *Helicobacter* species in newly recognized gastrointestinal tract diseases of animals. *Laboratory Animal. Science*, 47(3), pp. 222-277.
10. Hanninen M.L., Happonen I., Jalava K. (1998) Transmission of canine gastric *Helicobacter salomonis* infection from dam to offspring and between puppies. *Veterinary Microbiology*, 62(1), pp. 47-58.
11. Happonen I., Linden J., Saari S., Karjalainen M., Hanninen M.L., Jalava K., Westermarck E. (1998) Detection and effects of helicobacters in the healthy dogs and dogs with signs of gastritis. *Journal of the American Veterinary Medical Association*, 213, pp. 1767-1774.
12. Happonen I., Saari S., Castren L., Tyni O., Hanninen M.L., Westermarck E. (1996) Comparison of diagnostic methods for detecting gastric *Helicobacter*-like organisms in dogs and cats. *Journal of Comparative Pathology*, 115(2), pp. 117-144.
13. Hermanns W., Kregel K., Breuer W., Lechner J. (1995) *Helicobacter* like organisms: histopathological examination of gastric biopsies from dogs and cats. *Journal of Comparative Pathology*, 112, pp. 307-318.
14. Hwang C.Y., Han H.R., Youn H.Y. (2002) Prevalence and clinical characterization of gastric *Helicobacter* species infection of dogs and cats in Korea. *Journal of Veterinary Science*, 3(2), pp. 123-156.
15. Jalava K., Kaartinen M., Utriainen M., Happonen I., Hanninen M.L. (1997) *Helicobacter salmonis* sp. nov., a canine gastric *Helicobacter* sp. related to *Helicobacter felis* and *Helicobacter bizzozeronii*. *International Journal of Systemic Bacteriology*, 47(4), pp. 975- 982.
16. Jalava K., Vandamme P.A.R., Happonen I., Sukura A., Hanninen M.L. (1998) Isolation and identification of *Helicobacter* spp. from canine and feline gastric mucosa. *Applied Environmental Microbiology*, pp. 3998-4006.
17. Lee A., Krakowka S., Fox J.G., Otto G., Eaton K.A., Murphy J.C. (1992) Role of *Helicobacter felis* in chronic canine gastritis. *Veterinary Pathology*, 29(6), pp. 487-581.
18. Marshall B.J., Warren J.R. (1984) Unidentified curved bacilli in the stomach of patients with gastritis and peptic ulceration. *Lancet*, 16,1(8390), pp. 1311-1316.

19. Megraud F. (1996) Advantages and disadvantages of current diagnostic tests for the detection of *Helicobacter pylori*. *Scandinavian Journal of Gastroenterology*, 215, pp. 57-119.
20. Neiger R., Dieterich C., Burnens A., Waldvogel A., Cortesy – Theulaz I., Halter F., Lauterburg B., Schmassmann A. (1998) Detection and prevalence of *Helicobacter* infection in pet cats. *Journal of Clinical Microbiology*, 36, pp. 634-637.
21. Otto G., Hazell S.H., Fox J.G., Howlett C.R., Murphy J.C., O'Rourke J.L., Lee A. (1994) Animal and public health implications of gastric colonization of cats by *Helicobacter*-like organisms. *Journal of Clinical Microbiology*, 32(4), pp. 1043–1049
22. Oxley A.P., Powell M., McKay D.B. (2004) Species of the family *Helicobacteraceae* detected in an Australian sea lion (*Neophoca cinerea*) with chronic gastritis. *Journal of Clinical Microbiology*, 42(8), pp. 3505-3517.
23. Paura L., Arhipova I. (2002) *Neparametriskās metodes. SPSS datorprogramma* (Nonparametrical methods). LKC, Jelgava, 148 p. (In Latvian).
24. Simmons J.h., Riley L.K., Besch-Williford C., Franklin C.L. (2000) *Helicobacter mesocricetorum* sp. nov., a novel helicobacter isolated from the feces of Syrian hamsters. *Journal of Clinical Microbiology*, 38, pp. 1811-1817.
25. Solnick J.V., Schauer D.B. (2001) Emergence of diverse *Helicobacter* species in the pathogenesis of gastric and enterohepatic diseases. *Clinical Microbiology*, 14(1), pp. 59-97.