Detection of Gastric *Helicobacter*-like Microorganisms in Feral Raccoon Dogs (*Nyctereutes Procyonoides*) and Domestic Dogs (*Canis Familiaris*)
Kuņģa helikobaktēriju noteikšana savvaļas jenotsuņiem (*Nyctereutes procyonoides*) un mājas suņiem (*Canis familiaris*)

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Abstract. This study was done to evaluate the prevalence of *Helicobacter*-like microorganisms in the stomach of feral raccoon dogs (*Nyctereutes procyonoides*) from the Chonbuk province at the southwest part of Korea and of domestic dogs (*Canis familiaris*) from Latvia. Mucosal samples were taken from several places of cardiac, fundic, and pyloric gland region of the stomach to detect *Helicobacter spp.* by employing urease test, brush cytology, and light histological investigation. All sampled raccoon dogs and four domestic dogs from the five sampled animals showed positive urease test and presence of tightly spiraled *Helicobacter*-like microorganisms. A positive urease test was observed in 75.7% of all examined samples of raccoon dogs and in 67.0% of all samples of domestic dogs. *Helicobacter*-like microorganisms were detected in 98.5% of all examined samples of raccoon dogs and in 69.4% of samples of domestic dogs by brush cytology, and in 91.2% of all examined samples of raccoon dogs and in 68.2% of samples of domestic dogs by histological examination. Compared to gastric regions in feral raccoon dogs, the employed detection methods showed that almost all samples (100%) were positive for *Helicobacter spp.* in the cardiac gland region. Whereas in domestic dogs, all detection methods demonstrated 80% of positive samples in cardiac and 80% of positive samples in fundic gland region of the stomach. Urease test results were controlled after 10, 30, and 60 minutes. The amount of positive samples of raccoon and domestic dogs increased with the checking time, so it is important to check results of test after 60 minutes of reaction.
Key words: *Helicobacter spp.*, dogs, stomach, mucosa.

Introduction

Many *Helicobacter* species have been identified in the gastrointestinal tract of domestic and wild animals, and humans. Some *Helicobacter* species have been formally recognized and have often been associated with the condition of gastric and/or enterohepatic disease including gastroenteritis, gastric ulcers, hepatitis, and cancer (Fox, Lee, 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* form in humans with gastritis and gastric ulcer in 1984 (Marshall, Warren, 1984), *Helicobacter spp.* (*Helicobacter*-like microorganisms) have been detected in several animal species, such as dogs, cats, pigs, cheetahs, ferrets, polar bears, sea lions, monkeys, and rodents (Bronsdon et al., 1991; Lee et al., 1992; Eaton et al., 1993; Fox et al., 1995; Eaton et al.; 1996; Jalava et al., 1997; Neiger et al.; 1998; Hwang et al., 2002; Oxley et al., 2004).

Literature shows that *Helicobacter* species are mostly microaerophillic, gram-negative, spiral-shaped bacteria with multiple terminal flagellae and high-level urease activity, which allows them to survive in an acidic environment (Eaton et al., 1996). The sources and routes of transmission have been established following numerous studies, which suggested oral–fecal and oral–oral transmission of *Helicobacter*-like microorganims (Bussac, 1999).

Up to now many diagnostic methods have been developed to detect *Helicobacter pylori* infection: some invasive (such as rapid urease test, brush cytology, histology, electronmicroscopy, culture, and polymerase chain reaction) and others non-invasive (such as serology, urea breath test, and, more recently,

H.pylori antigen determination in feces) (Happonen et al., 1996).

The aim of this study was to detect *Helicobacter*-like microorganisms in feral raccoon (*Nyctereutes procyonoides*) and domestic dogs (*Canis familiaris*).

The main tasks of the research were:

1) to detect *Helicobacter*-like microorganisms in mucosal samples of stomach employing three different diagnostic methods: urease test, brush cytology, and histological examination;
2) to compare and evaluate the most susceptible diagnostic methods for detecting *Helicobacter*-like microorganisms in the gastric mucosal samples of the examined dogs;
3) to establish prevalence of *Helicobacter*-like microorganisms in different parts of the stomach: cardiac, fundic, and pyloric gland region;
4) to study and compare the prevalence of *Helicobacter*-like microorganisms in feral raccoon dogs from the Republic of Korea and in domestic dogs from Latvia.

Materials and Methods

Mucosal samples of the stomach were taken from eight raccoon dogs from Korea Animal Protection Society and from five dogs after the death of the animals (under agreement of the owners) in the Center of Veterinary Education, Latvia.

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, for detection of *Helicobacter spp*., 136 gastric mucosal samples of feral raccoon dogs and 85 gastric mucosal samples of domestic dogs were examined during the study. For brush cytology, urease test, and light histological investigation, mucus and mucosal samples were obtained from each sample site within two hours after the death of the animals.

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% of unbuffered urea in distilled water and phenol red indicator (pH 6.3). The results were recorded in 10 min., 30 min., and 60 min. after each sample was placed in reagents. A color change from pale yellow to bright pink was considered positive to *Helicobacter*-like microorganisms.

Collecting 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 a clean slide. The preparations were then air-dried and stained by *Diff-Quick* staining method. *Helicobacter*-like microorganisms 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 auto tissue processor *Tissue-Tek II*, embedded in paraffin, sectioned in 5 μm thick sections with microtome *SLEE Mainz Cut 4055*, and stained by *Diff-Quick* method. *Helicobacter*-like microorganisms were detected in gastric mucosa with light microscope at 1000 magnification.

Statistical analyses of the results were performed by SPSS 11.5 program. Differences between the results produced by urease test, brush cytology and histological examination were processed by the nonparametric *k-related Cochrans`s Q test*. Occurrence of *Helicobacter spp.* in the mucosal samples of different parts of stomach in raccoon and domestic dogs was analyzed with *Chi-square test* of independence (Paura, Arhipova, 2002; Arhipova, Bāliņa, 2003).

Results and Discussion

All examined raccoon dogs and four of the five examined domestic dogs showed positive results by all the methods used for detection of *Helicobacter spp*. The results of the study for occurrence of *Helicobacter*-like microorganisms in raccoon dogs are summarized in Table 1. As to raccoon dogs, statistical analysis showed significant differences between the results obtained by various diagnostic methods ($p<0.01$). Urease activity was observed in 75.7% (in 103 samples) of the examined 136 gastric samples after 60 min. of incubation. The highest detection rate of *Helicobacter*-like organisms was detected by brush cytology. Spiral-shaped organisms were observed in 98.5% (on 134 brushes) of the examined 136 brushes of gastric mucosa. During the histological examination, *Helicobacter*-like microorganisms were observed in the mucosa of stomach in 91.2% (in 124 samples) of the examined 136 samples (Table 1).

The results of the study for occurrence of *Helicobacter spp.* in domestic dogs are summarized in Table 2. Unlike the results of detection of *Helicobacter*-like microorganisms in raccoon dogs, statistical analyses did not show significant differences between the used diagnostic methods ($p>0.05$) in domestic dogs. Urease activity was observed in 67.0% (in 57 samples) of the examined 85 gastric samples after 60 min. of incubation. Spiral-shaped organisms were observed in 69.4% (on 59 brushes) of the 85 brushes of gastric mucosa by brush cytology, and in 68.2% (in 58 samples) of the examined 85 samples of gastric mucosa by histological examination (Table 2).

Brush cytology of raccoon dogs demonstrated the highest detection rate of *Helicobacter*-like organisms. These results are similar to those of the

Table 1

The amount of *Helicobacter*-positive samples in raccoon dogs by different detection methods

Number of raccoon dog	*Helicobacter*-positive samples		
	Urease test	Brush cytology	Histological examination
1	11	17	15
2	17	17	17
3	17	17	17
4	12	16	16
5	11	16	16
6	9	17	12
7	9	17	14
8	17	17	17
Total amount of positive samples	103	134	124
Total amount of all examined samples	136	136	136
Percentage of positive samples	75.7%	98.5%	91.2%

Table 2

The amount of *Helicobacter*-positive samples in domestic dogs by different detection methods

Number of domestic dog	*Helicobacter*-positive samples		
	Urease test	Brush cytology	Histological examination
1	12	12	12
2	16	16	16
3	12	16	13
4	17	17	17
5	0	0	0
Total amount of positive samples	57	59	58
Total amount of all examined samples	85	85	85
Percentage of positive samples	67.0%	69.4%	68.2%

previously recorded studies into detection methods for *Helicobacter*-like microorganisms (Happonen et al., 1996). Brush cytology offers comparably high sensitivity along with urease test and is recommended for practical use, as it is a relatively simple, cheap, and rapid method for screening *Helicobacter* infection. Histological examination is also sensitive, but it is comparatively expensive and requires special laboratory skills and equipments.

The results of urease test in raccoon dogs are demonstrated in Figure 1: urease activity after 10 minutes of incubation was detected in 68.8% of samples within the cardiac gland region, in 34.4% of samples within the fundic gland region, and in 10.1% of samples within the pyloric gland region. Whereas urease activity after 30 minutes of incubation was detected in 96.9% of samples within the cardiac gland region, in 79.95% of samples within the fundic gland region, and in 30.0% of samples within the pyloric gland region. Eventually, urease activity after 60 minutes of incubation was detected in 100% of samples within the cardiac gland region, in 85.9% of samples within the fundic gland region, and in 40.0% of samples within the pyloric gland region. Thus, a

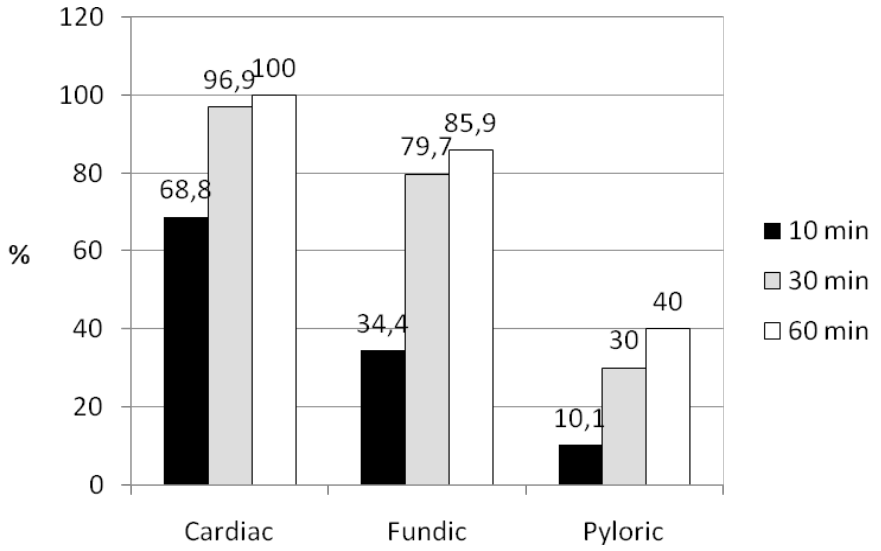

Fig. 1. Percentage of *Helicobacter*-positive mucosal samples of different sites by urease test in raccoon dogs.

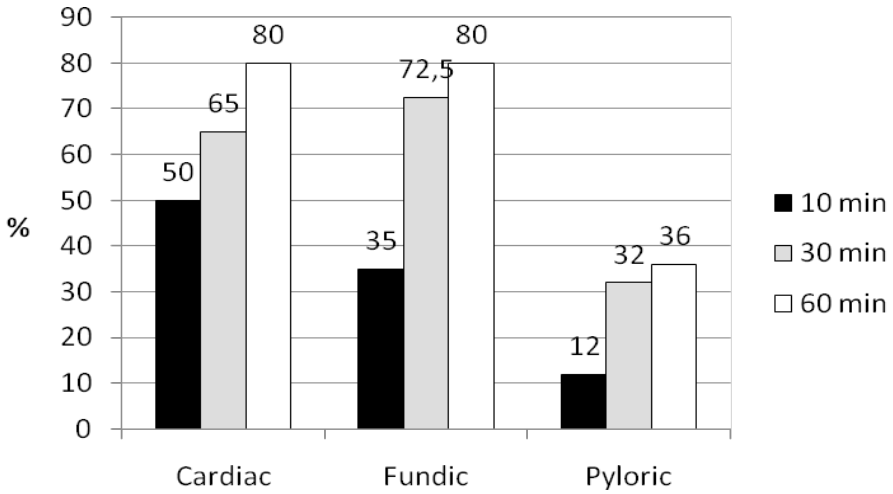

Fig. 2. Percentage of *Helicobacter*-positive mucosal samples of different sites by urease test in domestic dogs.

longer incubation period increased the detection rate of *Helicobacter*-like microorganisms.

Study of domestic dogs showed similar results (Figure 2)**.** Urease activity after 10 minutes of incubation was detected in 50.0% of samples within the cardiac gland region, in 35.0% of samples within the fundic gland region, and in 12.0% of samples within the pyloric gland region. Whereas urease activity after 30 minutes of incubation was detected in 65.0% of samples within the cardiac gland region, in 72.5% of samples within the fundic gland region, and in 32.0% of samples within the pyloric gland region. So, detection rate after 30 minutes of incubation increased approximately twice in the fundic gland region. Eventually, the results after 60 minutes of incubation showed the highest urease activity in 80.0% of samples within the cardiac gland region, in 80.0% of samples within the fundic gland region, and in 36.0% of samples within the pyloric gland region.

In comparison, the *Helicobacter spp.*-positive results in different parts of stomach and the positive rate of cardiac and fundic gland region in both raccoon and domestic dogs were substantially higher ($p<0.01$) than in pyloric gland region of stomach. These results are quite close to other studies into *Helicobacter*-like microorganisms in domestic dogs, where the highest *Helicobacter spp.* detection rate has been observed in the corpus (fundic gland region) of the stomach (Happonen et al., 1996).

The different detection rates of *Helicobacter*-like microorganisms in raccoon and domestic dogs by brush cytology are presented in Figure 3. Statistically

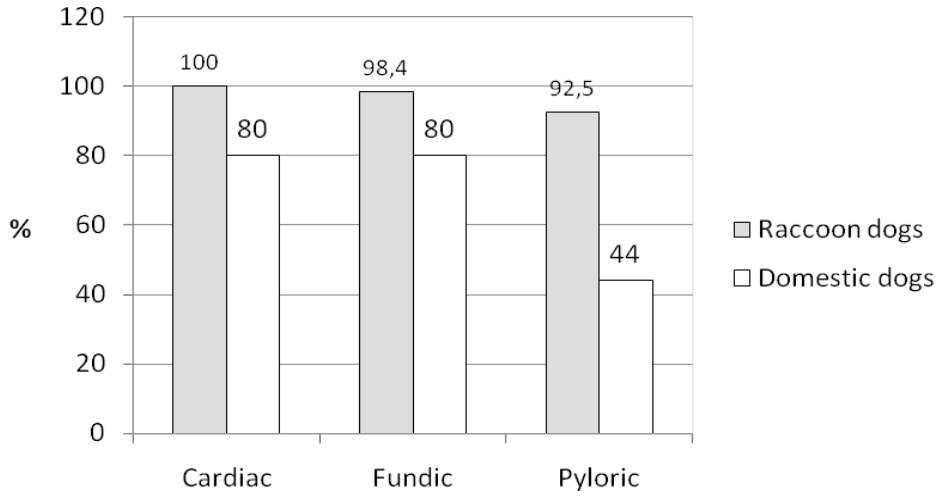

Fig. 3. Percentage of *Helicobacter*-positive mucosal samples of different sites by brush cytology in raccoon and domestic dogs.

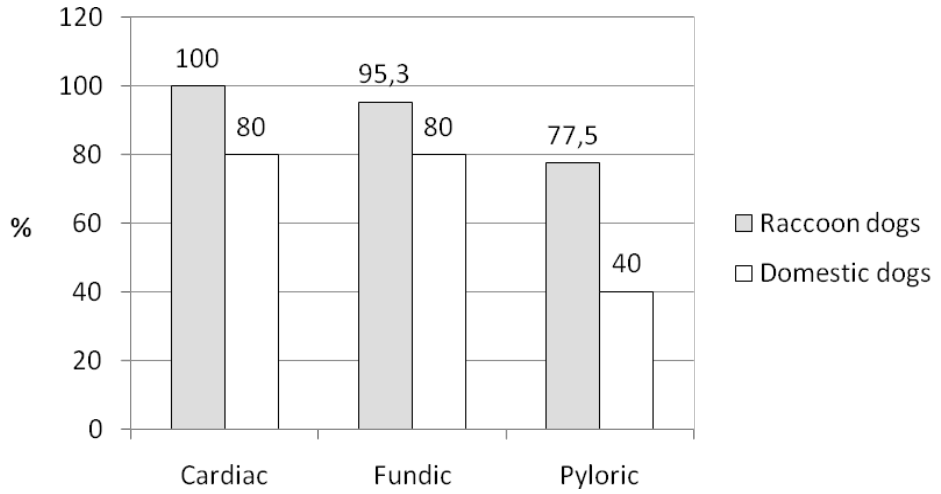

Fig. 4. Percentage of *Helicobacter*-positive mucosal samples of different sites by histological examination in raccoon and domestic dogs.

there are no significant differences ($p>0.05$) between the occurrence of *Helicobacter spp.* in the samples of different gastric regions of raccoon dogs. The spiral-shaped *Helicobacter*-like microorganisms were observed in 100% of samples from cardiac, in 98.4% of samples from fundic, and in 92.5% of samples from pyloric gland region of the stomach. Whereas study of domestic dogs showed that detection rates of *Helicobacter*-like microorganisms by brush cytology are not significantly different ($p>0.05$) between the cardiac and fundic gland region of the stomach. The spiral-shaped *Helicobacter* organisms were observed in 80% of samples from both cardiac and fundic gland region. The difference between occurrence of spiral-shaped microorganisms in the gastric part was most evident ($p<0.05$) in the pyloric gland region where brush cytology showed only 44% of positive samples (Figure 3). These results are quite similar to those of other studies which state that gastric *Helicobacter*-like microorganisms are mainly observed in the corpus (fundic gland region) of the stomach (Happonen et al., 1996).

Detection rates of *Helicobacter*-like microorganisms in raccoon and domestic dogs by histological examination are presented in Figure 4. Statistically there has been found a significant difference ($p<0.05$) of occurrence of *Helicobacter spp*. between gastric parts of both raccoon and domestic dogs. Spiral-shaped *Helicobacter* organisms were observed in gastric mucosa of raccoon dogs in 100% of samples from cardiac, in 95.3% of samples from fundic, and in 77.5% of samples from pyloric gland region of the stomach. Whereas study of domestic dogs showed that *Helicobacter*-like microorganisms are observed in gastric mucosa in 80% of samples from both cardiac and fundic gland region and in 40%

of samples from pyloric gland region of the stomach (Figure 4).

The histological examination showed that detection rate of *Helicobacter*-like microorganisms in the cardiac and fundic gland region is significantly higher ($p<0.05$) than in the pyloric gland region of stomach. Consequently, histological examination also allows evaluation of the status of mucosa and shows high sensitivity and specificity, too. This has also been pointed out in other studies (Megraud, 1996); however, histological examination is a comparatively expensive and labor intensive procedure (Chu et al., 1997).

This is the first investigation to detect and compare the presence of *Helicobacter*-like microorganisms in raccoon dogs (*Nyctereutes procyonoides*) of the Republic of Korea and in domestic dogs (*Canis familiaris*) of Latvia. The study of influence of *Helicobacter spp.* on gastric morphofunctional state of these animals is going to be continued.

Conclusions

1. Detection rates of *Helicobacter*-like microorganisms of gastric mucosa by urease tests, brush cytology, and histological examination are statistically different in raccoon dogs ($p<0.01$), but not in domestic dogs.
2. The brush cytology proved to be the most sensitive for detecting *Helicobacter*-like microorganisms in gastric samples of raccoon dogs.
3. For detecting of *Helicobacter* species by urease test it is important to check the results of the test after 60 minutes of reaction.
4. Compared to gastric parts, the cardiac and fundic gland region is the most affected site by *Helicobacter*-like microorganisms of raccoon and domestic dogs.

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Anotācija

Pētījuma mērķis bija noskaidrot kuņģa helikobaktēriju izplatību savvaļas jenotsuņiem (*Nyctereutes procyonoides*) Dienvidkorejas Republikas Čonbukas provincē, kā arī mājas suņiem (*Canis familiaris*) Latvijā. Pētījumiem izmantoti astoņi jenotsuņi un pieci mājas suņi. Gļotādas paraugi tika ņemti no stingri noteiktām kuņģa gļotādas daļām četrās vietās kardiālo, astoņās vietās fundālo un piecās vietās pilorisko dziedzeru zonā. Kopā izmeklēti 136 jenotsuņu un 85 mājas suņu kuņģa gļotādas paraugi. *Helicobacter spp.* noteikšanai tika izmantoti ureāzes tests, gļotādas virsmas noberzumu citoloģija un histoloģiskās izmeklēšanas metodes. Visiem astoņiem izmeklētajiem jenotsuņiem un četriem mājas suņiem (no pieciem) kuņģa gļotādā tika konstatētas *Helicobacter spp*. Jenotsuņiem ureāzes tests pozitīvs bija 75.7% gadījumu, bet mājas suņiem – 67.0% gadījumu. Gļotādas virsmas citoloģiskajā izmeklēšanā *Helicobacter spp*. jenotsuņiem konstatēja 98.5% gadījumu, bet mājas suņiem – 69.4% gadījumu. Histoloģiskajā izmeklēšanā helikobaktērijas jenotsuņiem konstatēja 91.2% gadījumu, bet mājas suņiem – 68.2% gadījumu. Salīdzinot *Helicobacter spp.* lokalizāciju dažādajās kuņģa gļotādas zonās, visplašāk izplatītas tās bija kardiālo un fundālo dziedzeru zonā. Jenotsuņu kuņģu kardiālajā dziedzeru zonā 100% gadījumu tika konstatētas *Helicobacter spp*., bet mājas suņiem gan kardiālo, gan fundālo dziedzeru zonā helikobaktērijas konstatēja 80% gadījumu. Ureāzes testa rezultāti tika pārbaudīti pēc 10, 30 un 60 minūtēm. Pozitīvo paraugu skaits uz *Helicobacter spp*. palielinājās līdz ar reakcijas laika ilgumu, tāpēc ir ļoti svarīgi rezultātus nolasīt pēc 60 minutēm. Šis ir pirmais pētījums par kuņģa helikobaktēriju izplatību savvaļas jenotsuņiem Dienvidkorejas Republikā un mājas suņiem Latvijā.