The aims of our study were to determine the prevalence of helicobacters in a population of Slovenian dogs, to characterise the intensity of infection and to find out if the epidemiological parameters i. e. age, feeding regimen, gender, breed, location and indoor/outdoor living conditions have any influence on infection and the intensity of infection.

A total of 185 randomly chosen dogs from all parts of Slovenia, at ages from 9 days to 15 years, of both genders and 44 different breeds, without any gastrointestinal disorders, were included in our study. Helicobacters were detected in stomachs of 92.4% of dogs. We determined a mild infection in 17.3% of dogs, a moderate infection in 48.1% and a strong infection in 27% of dogs.

Studying epidemiological parameters and their effects on infection we concluded that age and feeding regimen affect the infection and the intensity of infection, whereas gender, breed, location and indoor/outdoor living conditions do not.

Key words: dogs, epidemiology, gastric helicobacteriosis, Slovenia

Introduction / Uvod

Helicobacters are spiral, motile, microaerophilic, gram-negative, non-sporulating (Neiger and Simpson, 2000) bacteria, which can be found in the gastrointestinal and hepatobiliary system of various domestic and wild animal species and humans (Andersen, 2001).
On the basis of the determination of their 16S rRNA and 23S rRNA sequence, DNA hybridization and electron microscopic appearance, they have been classified into several *Helicobacter* species (Happonen et al., 2000; Jalava et al., 2001).

To date, six helicobacters were isolated from the stomach of dogs: *H. felis* (Cattoli et al., 1999), *H. bizzozeronii* (Cattoli et al., 1999; Vajner et al., 2000), *H. salomonis* (Hänninen et al., 1998), "*Flexispira rappini*" (Jalava et al., 1998), *H. bilis* (Eaton, 1996) in "*H. heilmannii*" (Simpson et al., 1999).

Infection can be detected using invasive tests - histopathology, brush cytology, rapid urease test, culture, PCR, electron microscopy, and noninvasive tests – urea breath, blood tests, and serology (Neiger and Simpson, 2000).

Helicobacter infection is highly prevalent in dogs. In the world there are 67 to 100% infected dogs without any sign of vomiting (Neiger and Simpson, 2000), 100% infected shelter and laboratory dogs (Peyrol et al., 1998) and 61 to 86% of dogs with signs of gastrointestinal disorders (Yamasaki et al., 1998). The exact mode of transmission is still unknown. The most credible is oro-oral and fecal-oral spread (Neiger and Simpson, 2000), but the fact that *H. pylori* was discovered also in milk, water and flies should not be overlooked (Shineng and Stutzenberger, 2000).

Living conditions in which dogs are in very close contact may play an important role in helicobacter infection, because shelter and laboratory dogs have a much higher prevalence of infection than other dogs (Peyrol et al., 1998). Age can also be an important factor, because puppies may be less often invaded than adults, but it is still uncertain if this and some other epidemiological parameters may affect the infection (Neiger and Simpson, 2000).

In our study we necropsied 185 randomly chosen dogs without gastrointestinal disorders, from all parts of the country and we determined the prevalence of gastric helicobacters in the Slovenian dog population. We also characterised the intensity of infection and evaluated the influence of age, feeding regimen, gender, breed, location and housing conditions on infection and the intensity of infection with gastric helicobacters.

### Materials and methods / Materijal i metode rada

**Animals / Životinje**

Our research included 185 randomly chosen dogs of 44 different breeds, without gastrointestinal disorders, from all parts of Slovenia which died or had been euthanised. Both genders were included at the age from 9 days to 15 years. They lived either indoors or outdoors and were fed with commercially prepared food or home made food or were still suckling.
Necropsy and histological examination /
Makroskopska i histološka ispitivanja

All dogs were necropsied, stomachs were removed, opened along the smaller curvature and rinsed in tap water in order to remove the food. Samples from fundus were taken for histopathological examination and put in 10% buffered formalin for 24 hours. Formalin fixed samples were routinely embedded in paraffin and cut in 6-µm sections. They were stained with the toluidine-blue staining method and examined with a light microscope for the presence of helicobacters and the intensity of the infection, which was defined by the bacteria count at high magnification (x 1000): mild – less than 5 helicobacters per section; moderate – 5 to 20 helicobacters per section; severe – more than 20 helicobacters per section.

Epidemiological and statistical analysis /
Epidemiološka i statistička analiza

For the determination of effects of epidemiological parameters on infection and the intensity of infection all dogs were divided into two groups according to gender: 114 males and 71 females; into two groups according to age: 6 puppies up to 2 months of age, which still lived with bitches, and 179 dogs older than 2 months; into two groups according to breed: 25 German Shepherds, and 93 mixed breeds (67 of them were pure breed but the number of dogs of a single breed was too low to perform a correct statistical analysis); into three groups according to nutrition regimen: 4 suckling puppies, 59 dogs fed with commercially prepared food (two of them were 2 month old puppies, which were also suckling bitches’ milk), and 122 dogs, fed with home prepared food; into six groups according to region: 52 dogs from Ljubljana and its surroundings (the central part of Slovenia with a continental climate), 18 dogs from the Primorje region (the southwestern part of the country with a Mediterranean climate), 32 dogs from the Gorenjska region (the northern part of the country with a cold alpine climate), 47 dogs from the Dolenjska region (the southern part of the country with a continental climate), 28 dogs from the Styria region (the eastern part of the country with a continental climate) and 8 dogs from the Prekmurje region (the north-eastern part of the country with a continental climate); and finally into two groups according to housing conditions: 58 indoor living dogs and 127 outdoor living dogs.

We determined the significance of differences in the prevalence and the intensity of infection between groups for each epidemiological parameter using Pearson’s chi-squared test and measuring the degrees of freedom (DF).

Results / Rezultati

Helicobacters were detected in stomachs of 92.4% of dogs. A mild infection was determined in 17.3% of dogs, a moderate in 48.1% of dogs and a severe infection in 27% of dogs.
A total of 93% (106 dogs) of male dogs and 91.5% (65 dogs) of female dogs were infected. In 54.4% of males and 38% of females we detected a mild infection, in 16.7% of males and 18.3% of females a moderate, and in 21.9% of males and 35.2% of females a severe infection. The differences in infection (chi-squared=0.722, DF=1) and the intensity of infection (chi-squared=0.14, DF=3) between genders were not statistically significant.

The rate of infection was 33.3 % (2 dogs) in dogs up to 2 months of age, and 94.4 % (169 dogs) in dogs older than 2 months. Both infected puppies, which were 2 months old, showed a mild infection, all four younger puppies (two of 9 days, one of 14 days, and one of 1 month) were uninfected. The intensity of infection in older dogs was as follows: 48.6% had a mild infection, 17.9% had a moderate and 27.9% had a severe infection. Comparison between the age groups and the infection (chi-squared=0.001, DF=3) and the intensity of the infection (chi-squared=0.001, DF=3) showed statistical significance (Table 1).

Table 1. The intensity of infection with Helicobacters according to age of dogs / Tabela 1. Intenzitet helikobakter infekcije u zavisnosti od starosti pasa

<table>
<thead>
<tr>
<th>Age / Starost</th>
<th>Intensity of infection / Intenzitet infekcije</th>
<th>Total / Ukupno</th>
</tr>
</thead>
<tbody>
<tr>
<td>Up to 2 months / Do 2 meseca</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Older than 2 months / Stariji od 2 meseca</td>
<td>10</td>
<td>87</td>
</tr>
<tr>
<td>Total / Ukupno</td>
<td>14</td>
<td>89</td>
</tr>
</tbody>
</table>

We determined a 92% (23 dogs) infection rate of German Shepherds - 13% had a mild infection, 24% a moderate, and 16% a severe infection; and a 93.5% (87 dogs) infection rate of mixed breeds – 42% had a mild infection, 17.2% a moderate and 31.2% a severe infection. Comparison of the prevalence of infection in German Shepherds (chi-squared=0.93, DF=1) and mixed breeds (chi-squared=0.56, DF=1) versus other dogs showed no statistical significance.

The infection rate of dogs fed with home prepared food was 95.9% (117 dogs) and 91.5% (52 adult dogs and both suckling puppies of 2 months of age, fed also with commercially prepared dog food) for dogs fed with commercially prepared dog food. All suckling puppies were uninfected. The difference between different feeding regimens and the infection (chi-squared=0.001, DF=2) and the intensity of infection (chi-squared=0.001, DF=6) was statistically significant (Table 2).
Table 2. The intensity of the infection with Helicobacters according to nutrition regimen of dogs

<table>
<thead>
<tr>
<th>Food / Hrana</th>
<th>Intensity of infection / Intenzitet infekcije</th>
<th>Total / Ukupno</th>
</tr>
</thead>
<tbody>
<tr>
<td>Home prepared food / Domaća ishrana</td>
<td>5 56 21 40</td>
<td>122</td>
</tr>
<tr>
<td>Bitches’ milk / Kujino mleko</td>
<td>4 0 0 0</td>
<td>4</td>
</tr>
<tr>
<td>Commercially prepared food / Kupovna hrana</td>
<td>5 33 11 10</td>
<td>59</td>
</tr>
<tr>
<td>Total / Ukupno</td>
<td>14 89 32 50</td>
<td>185</td>
</tr>
</tbody>
</table>

The prevalence of infection was the highest in the Primorje region, where all examined dogs were infected, followed by the Dolenjska region with 95.7% of infected dogs, in the third and the fourth place were Gorenjska with 90.6% and Ljubljana and its surroundings with 90.4% of infected dogs, the Styria region was in the fifth place with 89.3% of infected dogs and the lowest prevalence of infection was in the Prekmurje region, where 87.5% of all examined dogs were infected. In the Primorje region, 44.4% of dogs had a mild infection, 33.3% had a moderate, and 22.2% had a severe infection. In the Dolenjska region 38.3% had a mild infection, 8.5% had a moderate, and 48.9% had a severe infection. In the Gorenjska region 56.3% of dogs had a mild infection, 12.5% had a moderate, and 21.9% had a severe infection. In Ljubljana and its surroundings 50% of dogs had a mild infection, 19.2% had a moderate, and 21.2% had a severe infection. In the Styria region 53.6% of dogs had a mild infection, 28.6% had a moderate, and 7.1% had a severe infection. In the Prekmurje region 50% of dogs had a mild infection and 37.5% had a severe infection. There was no moderate infection detected in any dogs in this region. The difference in infection between all six Slovenian regions was not statistically significant (chi-squared=0.45, DF=5). A statistically significant difference was calculated comparing regions and the intensity of infection (chi-squared=0.01, DF=15).

Discussion / Diskusija

The infection rate in dogs living outdoors was 94.5% (120 dogs) and in dogs living indoors 87.9% (58 dogs). A total of 48.8% of outdoor living dogs and 46.6% of indoor living dogs had a mild infection, 15% of outdoor living and 22.4% of indoor living dogs had a moderate, and 30.7% of outdoor living and 19% of indoor living dogs had a severe infection. The difference between the housing conditions compared to the infection (chi-squared=0.13, DF=1) and the intensity of infection (chi-squared=0.13, DF=3) was not statistically significant.
The prevalence of infection in asymptomatic Slovenian dogs (92.4%) is higher than the prevalence in the same category of dogs from the United States (67-77%) (Eaton et al., 1996; Strauss-Ayali et al., 1999), Italy (79%) (Scanziani et al., 1997), Germany (82%) (Hermanns et al., 1995) and Japan (86%) (Yamasalo et al., 1998), equal to that in dogs from Netherlands (Cattoli et al., 1999) and lower comparing to the prevalence of infection in dogs from Finland and Turkey (100%) Happonen et al., 1998, Haziroglu et al., 1995).

On the basis of criteria established counting helicobacters at 1000 magnification we determined a mild infection in 17.3% of dogs, a moderate infection in 48.1% of dogs and a strong infection in 27% of dogs. Using the same criteria, Peyrol et al. (Peyrol et al., 1998) described a mild to moderate infection in 42% and a severe infection in 58% of asymptomatic laboratory beagles. The percent of severely infected dogs in the study of Peyrol’s et al. study is a little bit higher than in our dogs. This is probably due to the fact that the beagles used in Peyrol’s study lived in close contact. It is described in literature that shelter and laboratory dogs, which live in close contact are more often and perhaps severely infected with helicobacters than pets are (Peyrol et al., 1998; Vajner et al., 2000).

For the determination of effects of gender, age, breed, nutrition, region and housing conditions on infection and the intensity of infection with helicobacters we calculated the prevalence and the intensity of infection and statistically evaluated the results.

A total of 93% of male dogs and 91.5% of female dogs were infected. The difference in infection and the intensity of infection between genders was not statistically significant. We have demonstrated that gender does not affect the infection and the intensity of infection. Our conclusion is in accordance with other authors' results (Happonen et al., 1996; Hermanns et al., 1995; Peyrol et al., 1998).

The rate of infection was 33.3% in dogs up to 2 months of age, still living with the bitch, and 94.4% in dogs older than 2 months. Comparison between the age groups and the infection and the intensity of infection showed a statistical significance. We have demonstrated that age has an influence on the infection and the intensity of infection. Our result is not in accordance with the results of other authors, who established that infection with helicobacters is not influenced by the age of dogs (Happonen et al., 1998, Happonen et al., 1996, Hermanns et al., 1995; Scanziani et al., 1997; Yamasaki et al., 1998). The discordance rises from the fact that our research included a group of puppies aged up to two months - two puppies of 9 days of age, a puppy of 14 days and a puppy of 1 month, and they were all uninfected, and two infected two month old puppies, and all other studies included only dogs older than two months. We can presume that all suckling puppies have either strong maternal immunity or that helicobacters need a longer period, perhaps several weeks, to harbour the stomach and become sufficiently numerous to cause the infection. Our last hypothesis can be confirmed by Rossi et al. (Rossi et al., 1999), who described the infection in dogs, which was confirmed by culture, eight weeks after experimental infection with \( H. \)
pylori. Simpson at al. (Simpson et al., 1999) confirmed infection with H. felis using histopathology 12 weeks after experimental infection. Also, very interesting is the study of Hänninen et al. (Hänninen et al., 1998) who established that puppies from a litter experimentally infected with H. bizzozeronii transmitted the infection to uninfected animals only after three months.

We determined a 92% infection rate of German Shepherds and a 93.5% infection rate of mixed breeds. Comparison between German Shepherds and mixed breeds showed no statistical significance. We demonstrated that breed does not affect the infection and the intensity of infection. Other authors came to the same conclusion (Happonen et al., 1996; Hermanns et al., 1995).

The infection rate of dogs fed with mixed (home prepared) food was 95.9% and 91.5% for dogs fed with commercially prepared dog food. There was no statistical significance between the infection and the intensity of infection comparing dogs fed with mixed food and dogs fed with commercially prepared food. The difference in infection and the intensity of infection was statistically significant only if comparing suckling puppies with dogs fed with mixed or commercially prepared food. All four puppies, which were only sucking bitches’ milk, were uninfected, and both suckling puppies, which were also fed with commercially prepared food, were infected. We demonstrated that nutrition affects infection and the intensity of infection. It is possible that milk, which raises the pH value of gastric juice from 1 to 5, creates conditions which are less suitable for helicobacters, which prefer a very acid milieu.

The prevalence of infection was the highest in the Primorje region, where all examined dogs were infected, followed by the Dolenjska region with 95.7% of infected dogs and Ljubljana and its surroundings with 90.4%, the Styria region was in the fifth place with 89.3% and the lowest prevalence of infection was in the Prekmurje region where 87.5% of all examined dogs were infected. Differences in the prevalence of the infection between regions were small and not statistically significant in spite of quite different climatic conditions in the Slovenian regions: the Primorje region has a Mediterranean climate, the Gorenjska region a cold Alpine climate and all other regions a continental climate. Comparing different regions with the intensity of infection we found out that the difference was statistically significant. This result can be interpreted as false, because in some regions there were not enough dogs to make a precise statistical evaluation. We found out that in the Dolenjska region there was quite a high percentage of dogs (49%) with a severe infection. All these dogs lived in a small troop in very close contact, which is, as many authors believe, perhaps the most important predisposition to become severely infected. We demonstrated that location connected with different climatic conditions does not affect the infection and the intensity of the infection.

The infection rate in dogs living outdoors was 94.5% and in dogs living indoors 87.9%. We can see that indoor living dogs were slightly less infected but the difference between the housing conditions compared to the infection and the
intensity of the infection was not statistically significant. We should not overlook
the fact that all indoor living dogs are occasionally taken for a walk and so they
come in direct contact with other indoor and outdoor living dogs, which can possi-

bly transmit the infection. Therefore we can conclude that housing conditions do
not affect the infection and the intensity of infection. Unfortunately we have found
no data in literature concerning the influence of feeding regimen, location and
housing conditions on infection and the intensity of infection to compare with our

results.

This is the first study of prevalence and intensity of infection with heli-
cobacters in Slovenian dogs and perhaps the first attempt to evaluate the effects
of some epidemiological parameters on the infection and the intensity of infection.
More studies should be performed in order to give more precise answers about
the epidemiological parameters that could have an influence on infection with
helicobacters and the route of transmission of bacteria.

References / Literatura

2. Cattoli G et al. Occurrence and characterization of gastric Helicobacter spp. in naturally
3. Eaton KA et al. Prevalence and varieties of Helicobacter species in dogs from random
sources and pet dogs: animal and public health implications. J Clin Microbiol
1996; 34: 3165-70.
4. Happonen I et al. Detection and effects of helicobacters in healthy dogs and dogs with
5. Happonen I, Linden J, Westermarck E. Effect of triple therapy on eradication of canine
and topographical mapping of gastric helicobacter-like organisms and their asso-
ciation with histological changes in apparently healthy dogs and cats. J Vet
8. Hermanns W, Kregel K, Breuer W, Lechner J. Helicobacter-like organisms: histopa-
thological examination of gastric biopsies from dogs and cats. J Comp Pathol
9. Hänninen M-L, Happonen I, Jalava K. Transmission of canine gastric Helicobacter salo-
monis infection from dam to offspring and between puppies, Vet Microbiol
strain of "Helicobacter heilmannii", a human gastric pathogen, identified as H.
bizzozeronii: evidence for zoonotic potential of helicobacter. Emerg Infect Dis
identification of Helicobacter spp. from canine and feline gastric mucosa. App
Цель нашей работы была утвердить превалирование Helicobacter инфекции в популяциях собак в Словении, характеризовать интенсивность инфекции, и установить ли эпидемиологические параметры, например старость, режим кормления, пол, порода, локация и условия жизни на открытом/в закрытом, имеют влияния на инфекцию и интенсивность инфекции.

Исследования охватили 185 собак, отобранных по принципу случайного образца из всех краёв Словении, старости от 9 дней до 15 лет, оба пола, 44 различные породы, без гастроинтестинальных расстройств. Геликобактерии детектированы в животах 92,4% собак. Нами установлена слабая инфекция у 17,3% собак, умеренно сильная инфекция у 48,1% и сильная инфекция у 27% собак.

Испытая эпидемиологические параметры и их влияние на инфекцию, мы пришли к выводу, что старость и режим кормления влияют на инфекцию и интенсивность инфекции, пока пол, порода, локация и условия жизни на открытом/в закрытом не имеют влияния.

Ключевые слова: собаки, эпидемиология, гастритный геликобактериоз, Словения