

ВЗАЄМОДІЯ ВІРУС-ХАЗЯЇН, ЯК МОЖЛИВІСТЬ ЕРАДИКАЦІЇ ВІРУСНОЇ ДІАРЕЇ ВРХ

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Вірусна діарея великої рогатої худоби є, можливо, однією з найбільш поширених інфекцій великої рогатої худоби, але заходи щодо боротьби з цією інфекцією стали доступними порівняно недавно. У цій статті коротко описується як взаємодія вірусу з його хазяїном впливає на програми ліквідації ВД ВРХ, а також представлені підходи щодо викоринення вірусної діареї ВРХ, що були обрані швейцарською програмою ліквідації ВД ВРХ.

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POSITIVE FINDING FOR SPIRAL BACTERIA SIMILAR TO SPECIES FROM *HELICOBACTER* GENUS FOUND IN PORCINE ULCERATIVE LESIONS

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**Introduction.** The presence of hyperkeratosis and ulcers in the region of pars oesophagea of pigs' stomachs has been registered around the world. As a follow-up signs anorexia, chronic anaemia, acute gastric haemorrhage and loss of body mass were reported. Etiological and pathogenetic mechanisms of this syndrome and its economic importance have still not been clearly defined [1]. Although Friendship reports that this infection leads to economic losses [2]. In the past, food and stressogenic factors were noted as etiological factors. It was only recently that infective etiology has been defined [1]. Although the spiral bacteria in gastric mucosa was found with mammals at the end of the nineteenth century, intensive research into this started as late as 1983 year, when Warren and Marshall described the presence of *Helicobacter pylori* in gastric mucosa with people suffering from gastritis and peptic ulcer. Whilst seeking potential reservoirs of *Helicobacter pylori*, Queiroz et al. first identified the presence of spiral, urease-positive bacteria in the samples of gastric mucosa with 10.8% of the examined pigs [3]. These agents were initially marked as *Gastrospirillum suis*, and later on, based on the analysis of 16S rDNA sequence analysis as *Helicobacter heilmannii*. De Groote suggests the name *Candidatus Helicobacter suis* [4]. O'Rourke et al. defined two types of *Helicobacter heilmannii* based on the presence of the 16S rDNA genome sequence and urease gene [5]. *Helicobacter heilmannii* type 1 is morphologically and genetically identical to the bacteria formerly marked as *Helicobacter suis* [6]. This agent is capable of infecting both people and pigs, although its presence has been identified with dogs and cats, too [7]. It is known that with people it can lead to gastritis, peptic ulcer, stomach adenocarcinoma and MALT lymphoma, as well as that through  $\gamma$ -glutamyl transpeptidase it leads to cell necrosis of gastric mucosa with people, just as *Helicobacter pylori* does [8]. Schott proposed factors that enable the infection of people with agents from the group *Helicobacter heilmannii sensu lato* [9]. De Groote reports that *Helicobacter suis* is most frequently present with people in the group of non-*Helicobacter pylori* [10]. Melnichouk et al. report prevalence of the pig infection of 87% [11]. Friendship believes that little data is available about the prevalence of this infection with pigs and reports values of 9.4% and 10.8% [12]. Park et al. have reported values of prevalence of *Helicobacter* sp. infection with pigs of 95.5% confirmed using the PCR method. The same authors report that the values of prevalence range from 8.0% to 77.0%, depending on a geographical region and the applied method of diagnostics. They also say that the values of prevalence are higher when using a PCR test [13]. Different tests are applied for the diagnostics of this porcine infection. Today, a PCR test has been developed to prove the presence of *Helicobacter suis* genome in porcine gastric mucosa samples [1]. Until recently it was considered that this agent cannot be cultivated in conditions *in vitro*, but in 2008. year, Baele reported a successful isolation and characterisation of *Helicobacter suis* sp. nov. from the pig stomach tissue [6]. To present the main agent, the most frequent staining techniques are haematoxylin/eosin, Warthin-Starry and Giemsa. Urease test is of special significance [12]. The same author believes that no certain etiological connection has been confirmed between the results of spiral bacteria in porcine gastric mucosa and ulcerative lesions [2]. Apart from *Helicobacter suis* and other spiral bacteria like *Arcobacter* strain can be present in pigs' stomachs, although they are morphologically different [14]. Although until today, no clearly established etiology of ulcer disease with pigs has been clearly established, the zoonosomal potential of *Helicobacter suis* is known, as well as the fact that pigs are an important source of human infection. The aim of this paper is to point to positive findings of spiral bacteria similar to species from *Helicobacter* genus in ulcerative lesions with pigs.

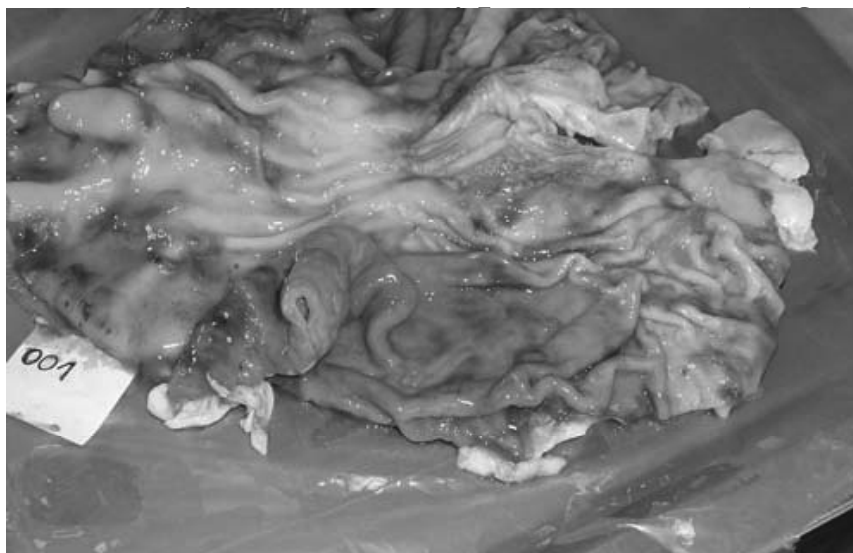
**Material and methods.** Stomachs of 60 pigs were collected right after they had been slaughtered in a slaughterhouse in South Bačka Region of Vojvodina, Serbia during 2010 year. After cutting in and opening stomachs along the greater curvature with sterilised instruments, the contents were emptied and mucosa was washed with sterile saline solution. Following this, pathomorphologic examination was done in order to identify oedema, erythema, haemorrhage, exudates, flat erosions, elevated erosions, ulcers, hyperplasia of rugal folds, and rugal folds atrophy, nodularity, and hyperkeratosis. Simultaneously, smears were swabbed from the surface of gastric mucosa in the regions pars fundica ventriculi and pars pyloric ventriculi, as well as the tissue samples. The laboratory examined microbiologically and pathohistologically the biological materials. From the smears from the gastric mucosa surfaces, slides were made, which were stained with Giemsa and Gram stains, and then microscopically scrutinised. With the tissue samples a quick urease test was done (Urease Hp Test), following the directions from the test producer (Cambridge Life Sciences Ltd, UK). For the pathohistological examination, the tissue samples were prepared and stained with haematoxylin and eosin, following a standard protocol.

**Results.** The pathomorphologic examination of mucosa, ulcer was identified in 20% of the examined stomachs (Figure 1).



**Figure 1.** Ulcer on porcine gastric mucosa

Apart from the ulcer, the most frequently noted pathomorphologic abnormalities were aedema, erythema and hyperkeratosis (Figure 2).



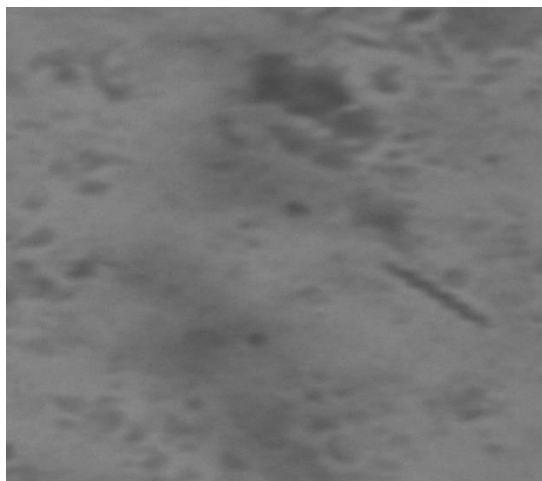
**Figure 2.** Erythema and hyperkeratosis on porcine gastric mucosa

The results of the pathomorphologic examination are shown in table 1.

**Table 1 – Results of pathomorphologic examination of porcine gastric mucosa**

<i>Number of positive</i>	<i>Finding</i>
5	Aedema
7	Erythema
1	Haemorrhage
2	Exudates
1	Flat erosions
0	Elevated erosions
12	Ulcers
3	Hyperplasia of rugal folds
0	Atrophy of rugal folds
0	Nodularity
4	Hyperkeratosis

The microbiological examination of the smears from six stomachs stained with Gram stain, that had noted ulcers, a presence of a couple of spiral microorganisms was confirmed. They were gram-negative, with 3 to 8 exaggerated curvatures 0.8 µm wide and 3-6 µm long (Figure 3).

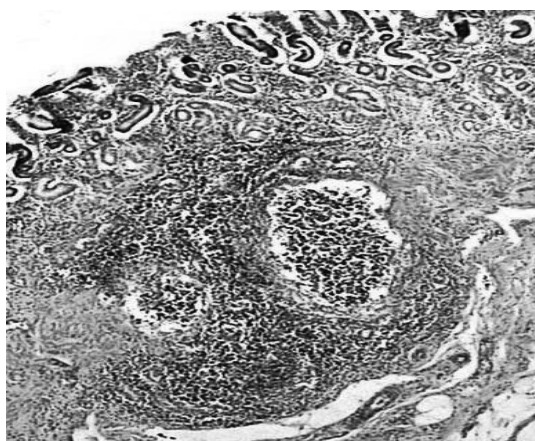


**Figure 3.** Positive finding with spiral bacteria in gastric mucosa swab, stained with Gram stain  
These tissue samples were positive to urease (Figure 4).



**Figure 4.** Positive urease test (colour purple marks a positive test)

The pathohistological examination of the tissue samples stained with haematoxylin and eosin, which did have ulcerative lesions and a confirmed presence of spiral bacteria, identified the infiltration of inflammatory cells and aggregation of lymphoid follicles (Figure 5).



**Figure 5.** Pathohistological result in tissue sample stained with haematoxylin and eosin

**Discussion.** There are significant differences in the values of prevalence occurrence of gastric ulcer with pigs and infection with *Helicobacter* species. Choi reports the *Helicobacter* species infection prevalence of 63.8% which was established using PCR [15]. Similar high values of prevalence have been reported by Cantet et al. By applying the PCR test they have confirmed the prevalence values of gastric mucosa infected with *Helicobacter* species of 80% and 86.6% [16]. Grasso et al. report a positive correlation of 89.2% between the occurrence of porcine gastritis and positive test to urease [17]. Contrary to these reports, Querioz et al. report the prevalence of infection with spiral bacteria, similar to helicobacter strain bacteria of 10.8% established with the microscopic examination of stained slides [3].

In our research, the pathomorphologic examination of the stomachs, we have registered the prevalence of porcine ulcers of 20%, as well as the prevalence of infection with spiral bacteria similar to *Helicobacter* species of 10% based on the microscopically examined Gram stained slides. The positive finding of spiral bacteria was registered with 50% of stomachs with ulcerative lesions. Queiroz et al. conclude that there is a significant connection between the occurrence of porcine ulcers and positive findings of spiral bacteria [18]. The prevalence values also established in this research work are probably lower than the real ones, considering that they were established according to the examination of stained slides, which corresponds to the conclusions of other authors [15]. The microbiological examination of Gram stained smears from mucosa of six stomachs, with reported visible ulcers, a presence of a couple of spiral gram-negative microorganisms was registered, with 3 to 8 exaggerated curvatures 0.8  $\mu\text{m}$  wide and 3-6  $\mu\text{m}$  long. A similar description of the agents has been given by other authors [4, 17, 19]. All of the tissue samples were positive to urease test, which corresponds to the reports from other authors [1, 6, 17]. In this research, the pathohistological examination of the porcine stomach tissue samples, stained with haematoxylin and eosin, we have identified the infiltration of inflammatory cells and aggregation of lymphoid follicles as well as granulocyte migration with 82% [13]. Pirarat et al. have identified, among other findings, lymphoid aggregates with 74.8% (86/115), as well as the infiltration of inflammatory cells with 38.26% [20]. In their pathohistological examination Queiroz et al. have also registered polymorphonuclear and mononuclear cell infiltration with present neovascularisation [18, 21].

**Conclusion.** This research has confirmed the presence of spiral bacteria similar to *Helicobacter* species in gastric mucosa samples from pigs suffering from ulcer. Since it has been proved that these agents are capable of infecting humans, further research is necessary in order to identify the strains of the agent by applying PCR test. Besides, further epidemiological research is necessary, considering that pigs are a primary source of infection for people.

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#### ВИЯВЛЕННЯ СПІРАЛЬНИХ БАКТЕРІЙ, АНАЛОГІЧНИХ ВИДАМ РОДУ *HELICOBACTER* У ВИРАЗКОВИХ УРАЖЕННЯХ СВИНЕЙ

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*Проведені дослідження підтверджують наявність спіральних бактерій аналогічних бактеріям *Helicobacter* у зразках слизової оболонки шлунка свиней, які мають виразкові ураження. Оскільки було доведено, що ці збудники здатні інфікувати людей, необхідні проводити подальші дослідження з метою виявлення штаму збудника, застосовуючи ПЛР. Крім того, необхідні подальші епідеміологічні дослідження, враховуючи, що свині є основним джерелом інфекції для людей.*