

## Correlation between Different *Helicobacter* Morphotypes and Histological Changes in Pig Gastric Mucosa

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### ABSTRACT

**Background:** Two distinct morphologic types of bacteria which belong to the *Helicobacter* species, have been described in pigs: once or twice curved *Helicobacter pylori*-like bacteria (HLO) and the multicoiled, *Gastrospirillum*-like bacteria (GLO). The objective of this study was to determine the incidence of *Helicobacter* spp. using modified Giemsa stain and to define the relationship between presence of *Helicobacter* spp. and histopathological changes of gastric mucosa in pigs.

**Materials, Methods & Results:** A total of 120 pig stomachs (60 from intensive and 60 from extensive breeding) were enrolled in this study and 240 fragments of fundic and pyloric mucosa were taken for histopathological examination. By modified Giemsa staining of gastric mucosa, *Helicobacter*-like organisms were confirmed in 4/60 (6.67%) of pigs in intensive and 5/60 (8.33%) of pigs in extensive breeding. The incidence of tightly spiral shaped *Gastrospirillum*-like organisms in pigs of intensive and extensive breeding were 5/60 (8.33%) and 9/60 (15%), respectively. The severity of gastritis was scored to the Sydney System with some modifications. There was no significant difference between HLO-positive and HLO-negative fundic mucosa in pigs of both breeding systems. In contrary, there was significant difference between HLO-positive and HLO-negative pyloric mucosa of pigs in intensive ( $P < 0.001$ ) and in extensive breeding ( $P < 0.05$ ). In intensive breeding, there was significant difference between GLO-positive and GLO-negative fundic mucosa ( $P < 0.05$ ), while there was no significant difference between GLO-positive and GLO-negative pyloric mucosa.

**Discussion:** The prevalence of *Helicobacter* spp. bacteria were in coherence with literature data. The higher prevalence of both morphological type of bacteria, were considered in pigs in extensive breeding. The hygienic conditions and management factors in pigs farm are the possible impact for higher bacterial transmission. The association of high prevalence of *H. pylori* and poor hygienic condition was shown by epidemiological studies conducted on humans. In both, humans and pigs, the presence of *H. pylori* correlates with an inflammatory response, but there are differences in inflammatory cell population. In *H. pylori* infected humans, neutrophils composed the bulk of cellular infiltrate, while in pigs, the primary inflammatory cell was the lymphocyte, which is in accordance to results published by others authors. Thus it indicates that different hosts exhibit a different pathohistological response to the *Helicobacter* spp. infection. In human as well as in veterinary pathology, the fact of the different pathogenicity of various *Helicobacter* species is well known. In all HLO-positive pyloric mucosa, moderate to severe focal or diffuse infiltration of mononuclear cells and lymphoid follicles with germinal centers, were observed. A similar conclusion was drawn from results of an experimental infection study in pigs. There was significance between HLO-positive and HLO-negative pyloric mucosa in both, intensive and extensive breeding. In the contrast, GLO were not associated with the presence of severe gastritis, but only with mild to moderate superficial infiltration of lymphocytes and plasma cells in both, fundic and pyloric mucosa. There was no significant difference between GLO-positive and GLO-negative pyloric mucosa of pigs in both breeding systems. Despite the low gastritis score of fundic mucosa in pigs in intensive breeding, there was a significance difference between in GLO-positive and GLO-negative fundic mucosa. It is believed that the possible reason of such results is the meals with low fiber content and low particles size. These results suggest that the presence of HLO, but not of GLO is associated with the pyloric gastritis in pigs.

**Keywords:** *Helicobacter*, gastritis, histopathology, pigs.

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## INTRODUCTION

Bacteria that colonize the stomach, including curved or S-shaped *Helicobacter*-like organisms (HLO) and spiral-shaped *Gastrospirillum*-like organisms (GLO) are isolated from humans [11,26] and several non-human species, including pigs [1,13,23]. Infection with *H. pylori* is correlated with histological characteristics of active and chronic gastritis. Chronic gastritis is characterized by increased infiltration of the lamina propria with mononuclear leukocytes [24], and polymorphonuclear neutrophils, which indicates an active component [4]. In both humans and pigs, the infection with *H. pylori* elicited inflammatory response, but there are differences between inflammatory cell populations. In *H. pylori* infected humans, neutrophils composed the bulk of cellular infiltrate, while in pigs, the primary inflammatory cell was the lymphocyte [3]. The fact of different pathogenicity of different *Helicobacter* species is well known in veterinary, as well as in human medicine [20]. *Helicobacter pylori*-like bacteria, characterized as high pathogenic, provoke severe gastritis and lymphoid follicles formation. In contrary, infection with *Helicobacter heilmannii*, which has been shown to have low pathogenicity, was accompanied by only mild gastritis [14].

The present study was performed with two related aims. The first was to demonstrate the incidence of *Helicobacter pylori*-like bacteria (HLO) and *Gastrospirillum*-like bacteria (GLO) in gastric mucosa of pigs. The second goal was to assess possible correlation between different type of *Helicobacter* spp. (HLO and GLO) and histopathological change of gastric mucosa in pigs.

## MATERIALS AND METHODS

### *Animals and tissue sampling*

Stomach samples were obtained from 120 apparently healthy pigs (60 from intensive and 60 from extensive breeding), 6-7 months old, weighing about 100 kg and Landrace breed. The stomachs were randomly selected at the slaughterhouse after the carcasses were eviscerated. The stomachs were transported to the laboratory in ice-box (4°C) and processed within 3 hours after collection. The stomach then were excised along the great curvature, from the diverticulum to pylorus, the contents were discarded and washed gently in tap water, tend to remove only food particles.

### *Histopathological examination*

Biopsy samples were taken from the fundic and pyloric mucosa and fixed in 10% neutral formalin for over 24 hours, dehydrated in graded alcohol-xylen series and embedded in paraffin wax. From each block a series of 5 µm-thick sections were prepared, using a microtome (Leica®)<sup>1</sup>. All tissue sections were stained with hematoxylin (Mayers healum solution®)<sup>2</sup> and eosin (Eosyn Y solution®)<sup>2</sup> for histopathologic examination and modified Giemsa stains (Giemsa stain solution®)<sup>2</sup> for detection of bacteria [8]. The severity of gastritis was scored to the updated Sydney System [6] with some modifications, according to Park *et al.* [19] as: 0 - no infiltration of inflammatory cells, 1-mild infiltration of lymphocytes and plasma cells, 2 - moderate dense infiltration of lymphocytes and plasma cells, but no lymphoid follicles, 3 - moderate dense infiltration of lymphocytes and plasma cells and presence of lymphoid follicles, 4 - very dense infiltration of lymphocytes and plasma cells and presence of lymphoid follicles. Both diffuse infiltration with inflammatory cells and the presence of lymphoid aggregates and lymphoid follicles in the mucosa were taken into consideration. All samples were assessed using the light microscope (Olympus CX31RBSF®)<sup>3</sup>.

According to microscopic examination, the stomachs were divided into two groups: *Helicobacter* spp.-positive: HLO-positive and GLO-positive group and *Helicobacter* spp.-negative: HLO-negative and GLO-negative group. Based on the scoring of the diffuse infiltration with inflammatory cells and the presence of lymphoid aggregates and lymphoid follicles, an overall gastritis score was obtained. Therefore, the average score of fundic and pyloric mucosa was calculated for each group: HLO-positive and HLO-negative, as well as GLO-positive and GLO-negative group.

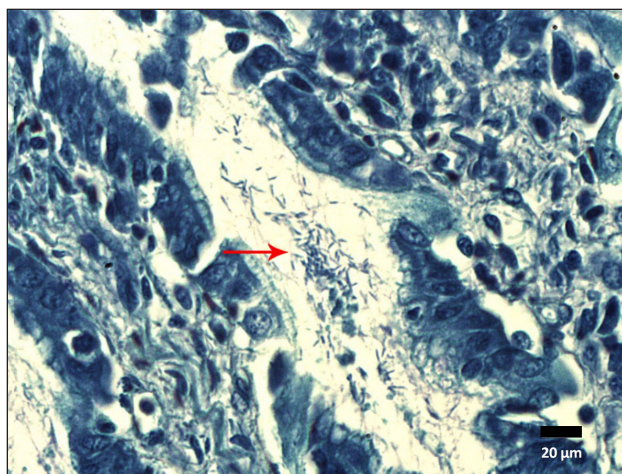
### *Statistical analyzes*

The statistical analysis was performed by StatSoft Statistica (Version 6.0)<sup>4</sup>. The significant differences between HLO-positive and HLO-negative, as well as GLO-positive and GLO-negative fundic and pyloric mucosa was analyzed by two tailed Students *t*-test. Values of *P* < 0.05 were considered significant. All data were shown as mean ± standard deviation (SD).

## RESULTS

### *Detection of Helicobacter-spp. organisms (HLO and GLO)*

According to morphological characteristics, *Helicobacter*-like organisms (HLO) were 1-2 coiled or curved, mainly in the upper half of gastric pits (Figure 1). Microorganisms were not seen in lamina propria or lymphoid follicles. The bacteria were observed in 4 (6.67%) out of a total of 60 stomachs in intensive breeding and 5 (8.33%) out of a total of 60 stomachs



**Figure 1.** Numerous *Helicobacter*-like organisms (arrow) in pig gastric pits (modified Giemsa). [bar= 20 μm].

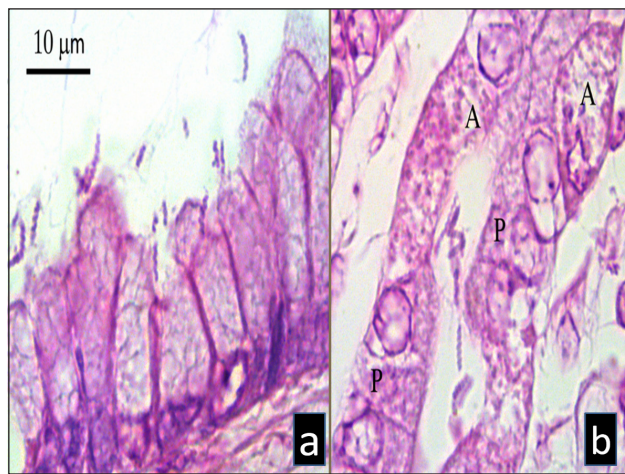
### *Histopathology*

Histopathological evaluation of gastric mucosa of pigs based on presence of different bacteria morphotypes is presented in Table 1. The percentage of *Helicobacter* spp. gastritis (in both, HLO and GLO) was higher in gastric mucosa of pigs in extensive breeding than pigs in intensive breeding. In contrary, the percentage of *Helicobacter*-spp. (HLO/GLO)-negative gastritis was higher in stomach mucosa of pigs in intensive breeding, than pigs in extensive breeding.

### *Histopathologic changes in HLO-positive mucosa*

In the fundic and pyloric mucosa of all infected pigs, were found microscopic lesions specific for *H. pylori* associated gastritis. In the fundic mucosa, the mild inflammatory response was observed as a superficial infiltration of lymphocytes and plasma cells, mainly in upper third of lamina propria, nearby gastric pits. Lymphoid follicles formation close to lamina muscula-

in extensive breeding. The spiral shaped *Gastrosphilum*-like organisms (GLO) were observed in 5 (8.33%) out of a total of 60 stomachs in intensive breeding and 9 (15%) out of a total of 60 stomachs in extensive breeding. The bacterium had a peculiar screw-like appearance, easily differentiated from other curved bacteria. Microorganism was observed in gastric pits or deeply in the lumen of gastric glands (Figure 2). The prevalence of *Helicobacter* spp. bacteria (in both HLO and GLO morphotype) is higher in gastric mucosa of pigs in extensive breeding than pigs in intensive breeding.



**Figure 2.** *Gastrosphilum*-like organisms in the lumen of gastric pit (a) and gland (b), a lack of any leukocytes around, a- Gastric pit, b- GLO deeply in the fundic gland, A- Acidogenic cells, P- Pepsinogenic cells (H&E). [bar= 10 μm].

ris mucosae, were also seen. In intensive breeding, the average gastritis score of the HLO-positive fundic mucosa was  $1.5 \pm 0.58$ , whereas that of the HLO-negative fundic mucosa was  $1.16 \pm 0.37$ . In extensive breeding, the average gastritis score of the HLO-positive fundic mucosa was  $1.4 \pm 0.55$ , whereas that of the HLO-negative fundic mucosa was  $1.13 \pm 0.34$ . There was no significant difference between HLO-positive and HLO-negative fundic mucosa. In the pyloric mucosa, moderate to severe focal or diffuse infiltration trough the lamina propria by lymphocytes and plasma cells was observed. The inflammatory infiltrate extended from the surface epithelial cells to the lamina muscularis mucosae. Lymphocytes tended to accumulate in small aggregates or confluent foci that matured into lymphoid follicles with germinal centers (Figure 3). Except for lymphocytes, the only other inflammatory cells found in significantly increased number were the plasma cells. In intensive breeding, the average gastritis



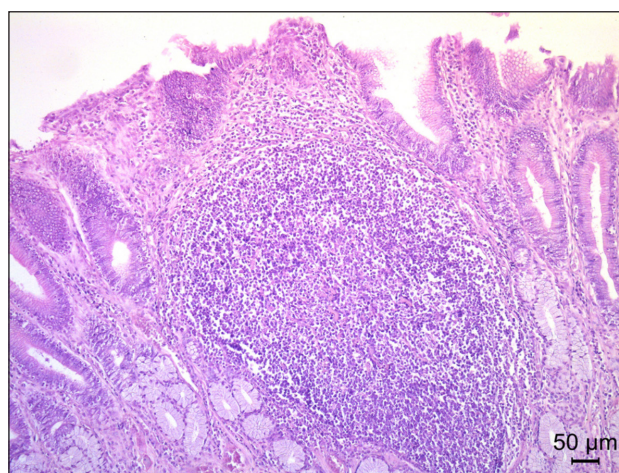
score of the HLO-positive pyloric mucosa was  $2 \pm 0.00$ , whereas that of the HLO-negative pyloric mucosa was  $1.57 \pm 0.50$ . There was a significant difference between HLO-positive and HLO-negative pyloric mucosa ( $P < 0.001$ ). In extensive breeding, the average gastritis

score of the HLO-positive pyloric mucosa was  $2.2 \pm 0.45$ , whereas that of the HLO-negative pyloric mucosa was  $1.38 \pm 0.49$ . There was a significant difference between HLO-positive and HLO-negative pyloric mucosa ( $P < 0.05$ ).

**Table 1.** Histopathological evaluation of gastric mucosa of pigs based on presence of different bacteria morphotypes.

Types of bacterial gastritis	Intensive breeding		Extensive breeding	
	Frequency	Percentage	Frequency	Percentage
HLO-positive gastritis	4	6.67%	5	8.33%
GLO-positive gastritis	5	8.33%	9	15%
<i>Helicobacter</i> spp. (HLO/GLO)-negative gastritis	37	61.67%	24	40%
Normal mucosa without <i>Helicobacter</i> spp.	14	23.33%	22	36.67%
Total	60	100%	60	100%

HLO: *Helicobacter* pylori-like organisms; GLO: *Gastrospirillum*-like organisms.



**Figure 3.** Mild follicular gastritis in the pyloric region (H&E). [bar= 50 μm].

#### *Histopathologic changes in GLO-positive mucosa:*

In the fundic mucosa was observed only mild superficial infiltration of lymphocyte and plasma cells, mainly in upper third of lamina propria, nearby surface epithelium. No lymphoid follicles formation, neither lymphoid aggregates, were seen. In intensive breeding, the average gastritis score of the GLO-positive fundic mucosa was  $1.0 \pm 0.00$ , whereas that of the GLO-negative fundic mucosa was  $1.16 \pm 0.37$ . There was a significant difference between GLO-positive and GLO-negative fundic mucosa ( $P < 0.05$ ). In extensive breeding, the average gastritis score of the GLO-positive fundic mucosa was  $1.0 \pm 0.00$ , whereas that of the GLO-negative fundic mucosa was  $1.13 \pm 0.34$ . There was no significant difference between GLO-positive and GLO-negative fundic mucosa. In the pyloric mucosa,

mild to moderate superficial infiltration of lymphocytes and plasma cells was observed in upper third of lamina propria. Presence of lymphoid follicles in the lower third of lamina propria, were also seen. In intensive breeding, the average gastritis score of the GLO-positive pyloric mucosa was  $1.4 \pm 0.55$ , whereas that of the GLO-negative pyloric mucosa was  $1.57 \pm 0.50$ . In extensive breeding, the average gastritis score of the GLO-positive pyloric mucosa was  $1.22 \pm 0.44$ , whereas that of the GLO-negative pyloric mucosa was  $1.38 \pm 0.49$ . There was no significant difference between GLO-positive and GLO-negative pyloric mucosa in the both group.

#### DISCUSSION

It is well known that two main morphologic types of bacteria (tightly spiraled bacteria- *Gastrospirillum* spp.

and once or twice curved *Helicobacter pylori*-like bacteria) can colonize the stomach of pigs [5,7,13]. Findings from this study of the prevalence of *Helicobacter* spp. bacteria are in coherence with literature data. The prevalence of *Helicobacter* spp. in pig stomachs were reported from 8% to 10.8% [7,19,23]. The higher prevalence of both morphological type of bacteria, were considered in pigs in extensive breeding. Presumably, the hygienic conditions and management factors in pigs farm are the possible impact for higher bacterial transmission. The association of high prevalence of *H. pylori* and poor hygienic condition was shown by epidemiological studies conducted on humans [2]. Also, several factors influenced the infection rate in pigs, such as type of breed [7,23], stress conditions, co-infection with other organisms or even the sensitivity of such detection methods [17].

Gastritis is the result of a natural infection with *H. pylori* in humans [26] and conventional pigs [13] or an experimental induced infection in gnotobiotic piglets [3,14,15], miniature pigs [12] and conventional piglets [22]. In both, humans and pigs, the presence of *H. pylori* correlates with an inflammatory response, but there are differences in inflammatory cell population. The early stage of *H. pylori* infection in piglets is characterized by neutrophilic infiltrate, which is quickly followed by lymphocytic infiltration [15]. Results of this study indicated that mononuclear cells (lymphocytes and plasma cells) were predominant cell types of inflammatory infiltrate, whereas polymorphonuclear leukocytes (neutrophils) were not present. Previous study also point to lack of neutrophile in inflammatory infiltrate of experimentally induced *H. pylori* infection in conventional piglets [22]. This is in accordance to results published by other authors [1,3,15,22], but contrary to chronic active gastritis associated with *Helicobacter* spp. in humans [9]. Thus it indicates that different hosts exhibit a different pathohistological response to the *Helicobacter* spp. infection [21]. Signs of gastric inflammation, such as lymphocytic and plasmacytic inflammatory cell infiltrates and lymphoid follicle formation, are most prominent in *H. pylori*-inoculated pigs [14]. A similar conclusion was drawn from results of an experimental infection study in: gnotobiotic pigs [15], miniature pigs [12] and conventional pigs [13,22]. Results for histopathological examination revealed a moderate to severe focal or diffuse lymphoplasmacytic inflammation and lymphoid

follicles with germinal centers in all HLO-positive pyloric mucosa, which corresponds to the results of an experimental study [19]. Gastritis in the glandular region was found to be associated with tightly spiraled bacterial infection by several researchers [7,17,19]. In this study no correlation was evident between pyloric gastritis and *Gastrospirillum* spp. infection. Results of this study are consistent with the research of other authors [1,25] which could not find an association between these two parameters. Previously was suggested that *Gastrospirillum* spp. does not seem to elicit gastric inflammatory disease in pigs [1]. However, despite the low gastritis score of fundic mucosa in pigs in intensive breeding, there was a significance difference between in GLO-positive and GLO-negative fundic mucosa. It is believed that the possible reason of such results is the type of animal nutrition. Furthermore, the meals with low fiber content and low particles size lead to an increased risk of damage to gastric mucosa, especially in animals under stress condition [18]. In most of the GLO-positive pyloric mucosa, the lymphoid aggregates and follicles were observed in the basal portion of lamina propria. Lymphoid aggregates and lymphoid follicles formation in the lamina propria are a common finding in pigs infected with *Helicobacter* spp. [16]. Strong correlation between “*G. suis*” and the formation of lymphoid follicles was also reported previously [17].

In humans as well as veterinary pathology, the fact of different pathogenicity of different *Helicobacter* spp. is well known [20]. The results of current study revealed the higher gastritis score and significance between HLO-positive and HLO-negative pyloric mucosa in both, intensive and extensive breeding. In contrary, lower gastritis score and no significant difference between GLO-positive and GLO-negative pyloric mucosa was found in pigs of both breeding systems. These findings indicated that infection of pigs with different *Helicobacter* species can affect gastric mucosa in various manners and various pathogenic effects of different, but related microorganisms are possible. *Helicobacter pylori*-like bacteria characterised as high pathogenic, has been associated with severe gastritis and formation of lymphoid follicles [14]. In contrast, infection with *Helicobacter heilmannii*, which has been shown to have low pathogenicity [10] was accompanied by only mild and limited gastric inflammation [14].

## CONCLUSIONS

The results of present study showed that two distinct morphologic types of bacteria (*Helicobacter pylori* morphotype and *Gastrospirillum* morphotype) which belong to the *Helicobacter* species are existed in pigs. *Helicobacter* spp. in gastric mucosa of pigs which belong to different genus, differ significantly in both, pathogenicity and virulence. Obviously, *Gastrospirillum*-like morphotype does not seem to elicit gastric mucosal inflammatory response, but *Helicobacter pylori*-like morphotype act as contributing factor to development of pathological changes in the pyloric mucosa of pigs. Therefore, histopathological examination remains the gold standard for

diagnosis of gastritis, which will provide useful information that can enhance nutrition and health status of pigs.

## MANUFACTURERS

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