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## HISTOLOGICAL ASSESSMENT OF BACTERIAL DENSITY AND INFLAMMATORY CELL INFILTRATE IN PIGS WITH *HELICOBACTER SPP.*-ASSOCIATED CHRONIC GASTRITIS<sup>1</sup>

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### Abstract

The aim of this study was to determine the association between *Helicobacter spp.*: *Helicobacter pylori*-like organisms (*HLO*) and *Gastrospirillum*-like organisms (*GLO*) density and severity of chronic inflammatory infiltrate in the pyloric mucosa of pig's stomach. A total of 120 fragments of pyloric mucosa were included in the study. It was seen that *HLO* was found mostly in grade 2 of density, while *GLO* in grade 1 of density. Chronic inflammatory infiltrate was estimated mostly as grade 2 in *HLO* positive samples and grade 1 in *GLO* positive samples. Regardless of the bacterial density, we found that grade of chronic inflammatory infiltrate was higher in *HLO* positive samples than *GLO* positive samples.

**Keywords:** pigs, *Helicobacter spp.*-gastritis, pyloric mucosa, chronic inflammatory infiltrate.

### INTRODUCTION

*Helicobacters* are Gram-negative, microaerophilic bacteria that have been isolated from the gastric mucosa of several animal species, including pigs. Two *Helicobacter* species differ morphologically from each other: *Helicobacter*

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<sup>1</sup> original scientific paper

*pylori*-like organisms (*HLO*) are curved or S-shaped, while *Gastrospirillum*-like organisms (*GLO*) have spiraled, corkscrew appearance. While many studies have reported presence of spirally shaped bacteria with *GLO* morphology in the stomach of pigs (Mendes et al. 1990; Queiroz et al. 1991; Barbosa et al., 1995), there is a paucity of literature about the existence of curved-shaped *Helicobacter pylori*-like organisms. Once or twice curved, swine-origin *Helicobacter pylori*-like organisms, isolated from gastric mucosa of conventional breeding pigs were initially described by Krakowka et al. (2005). The inflammatory response due to *Helicobacter pylori* infection depends on the bacterial virulence factors, the intensity of bacteria colonization and the host immune response (Ardakani, Mohammadzadeh, 2006). Infection with *Helicobacter pylori* is correlated with histological characteristics of active and chronic gastritis, which is more prominent in the antrum than in the corpus (Dixon et al., 1996). Chronic gastritis in pigs caused by *Gastrospirillum*-like organisms is significantly milder compared to *Helicobacter pylori*-associated gastritis. Pigs infected with *Gastrospirillum*-like organisms did not develop severe, but only mild pyloric gastritis (Mendes et al., 1991, Queiroz et al., 1996, Roosendaal et al., 2000). Histological changes of chronic gastritis include lymphocytes, plasma cell infiltration and other inflammatory cells in lamina propria (Alireza A, et al., 2008). In both pigs and humans, inflammatory cell types are distinctly different: neutrophils compose the bulk of the cellular infiltrate in *Helicobacter pylori*-infected humans, while in pigs the primary inflammatory cells are the lymphocytes (Bertram et al., 1991). The Sydney system is a good scoring system for classification and grading of gastritis that was devised by a group of experts at the 9<sup>th</sup> World Congress of Gastroenterology in Sydney, Australia in 1990. In 1994 in Houston, Texas, experts designed the updated Sydney system, which uses none, mild, moderate and severe grades of chronic gastritis (Dixon et al., 1996).

## MATERIAL AND METHODS

Stomachs from 120 apparently healthy pigs (64 male and 56 female), 6-7 months old, Landrace breed, collected postmortem from abattoir, were opened longitudinally along great curvature and washed in tap water. Biopsy samples from the pyloric mucosa were fixed in 10% neutral buffer formalin and embedded in paraffin. Sections about 5 µm thick were prepared and stained with

haematoxylin and eosin (H&E) for routine histopathological examination and modified Giemsa stain for the detection of *Helicobacter spp.* The density of *Helicobacter spp.* was estimated according to the updated Sydney system (Stolte and Meining, 2001) as: 0 – none, 1 (mild) - scattered organisms covering <1/3 of the surface, 2 (moderate) - intermediate numbers, 3 (severe) - organisms clusters >2/3 of the surface. Chronic inflammatory infiltrate was graded according to Park et al. (2000) 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 the presence of lymphoid follicles
- 4: Very dense infiltration of lymphocytes and plasma cells and presence of lymphoid follicles

## RESULTS

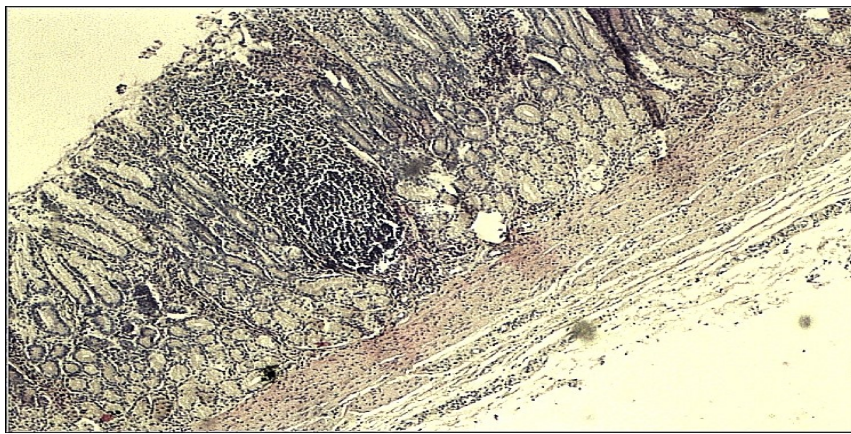
A maximum number of biopsy samples (4 out of 9 i.e. 44.44%) was found to have grade 2 of *HLO* density. Second highest frequency (3 out of 9 i.e. 33.33%) was observed in grade 1 of *HLO* density. Grade 3 of *HLO* density was found in 2 out of 9 (22.22%) biopsy samples. Table 1 presents different grades of chronic inflammatory infiltrate in biopsy samples and their association with grades of *HLO* density.

Grade 2 of chronic inflammatory infiltrate was found in 88.89% of biopsy samples (8 out of 9). The inflammatory infiltrates extended from the surface epithelial cells to the lamina muscularis mucosae. (Fig. 1). Only 1 out of 9 biopsy samples (11.11%) was found to have grade 3 of chronic inflammatory infiltrate (Fig. 2). None of the biopsy samples exhibited grade 1 of chronic inflammatory infiltrate.

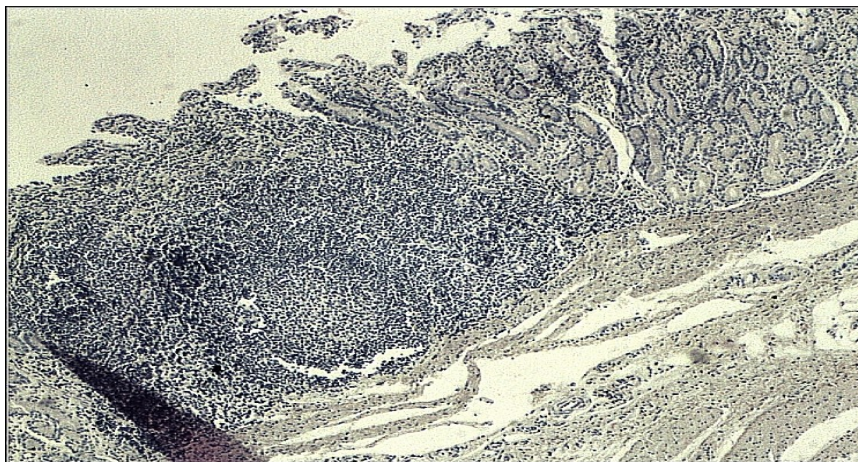
**Table 1.** Grades of chronic inflammatory infiltrate in pyloric mucosa and their association with *HLO* density.

<b>Grades of <i>HLO</i></b>	<b>Grades of chronic inflammatory infiltrate</b>			<b>Total (n)%</b>
	<b>Grade 1</b>	<b>Grade 2</b>	<b>Grade 3</b>	

density	(n) %	(n) %	(n) %	(n) %
Grade 1	0	3 (33.33%)	0	3 (33.33%)
Grade 2	0	4 (44.44%)	0	4 (44.44%)
Grade 3	0	1 (11.11%)	1 (11.11%)	2 (22.22%)
Total (n)%	0	8 (88.89%)	1 (11.11%)	9 (100%)



**Figure 1.** Grade 2 of chronic inflammatory infiltrate in lamina propria of pyloric mucosa with aggregates of mononuclear inflammatory cells (H&E, x40)



**Figure 2.** Grade 3 of chronic inflammatory infiltrate in lamina propria of pyloric mucosa with lymphoid follicle formation (H&E, x40)

A maximum number of biopsy samples (14 out of 14 i.e. 100%) was observed to have grade 1 of *GLO* density. The grades of chronic inflammatory infiltrate in pyloric mucosa and their association with grade of *GLO* density are presented in Table 2.

Overall 71.43% of biopsy samples (10 out of 14) were found to have grade 1 of chronic inflammatory infiltrate, mainly in the upper third of lamina propria (Fig. 3). Grade 2 of chronic inflammatory infiltrate was found in 4 out of 14 (28.57%) biopsy samples. None of the biopsy samples exhibited grade 3 of chronic inflammatory infiltrate.

**Table 2.** Grades of chronic inflammatory infiltrate in pyloric mucosa and their association with *GLO* density

Grades of <i>GLO</i> density	Grades of chronic inflammatory infiltrate			Total (n)%
	Grade 1 (n)%	Grade 2 (n)%	Grade 3 (n)%	
Grade 1	10 (71.43%)	4 (28.57%)	0	14 (100%)
Grade 2	0	0	0	0
Grade 3	0	0	0	0
Total (n)%	10 (71.43%)	4 (28.57%)	0	14 (100%)





**Figure 3.** Grade 1 of chronic inflammatory infiltrate in lamina propria of pyloric mucosa with superficial infiltration of lymphocytes and plasma cells (H&E, x40)

### DISCUSSION

The pig's stomach can be naturally colonized with two morphological types of bacteria that belong to *Helicobacter species* (curved shaped *Helicobacter pylori*-like and spiral-shaped *Gastrospirillum*-like) (Queiroz et al., 1991, Mendes et al., 1994, Grasso et al., 1996, Krakowka et al., 2005, Pejchinovska et al., 2017). *Gastrospirillum*-like organisms have also been observed in human gastric mucosa, but sporadically (Heilmann, Borchard 1991). To our knowledge, only a limited literature exists on semi-quantitative evaluation of *Helicobacter spp.* (*HLO* and *GLO*) in pigs. In the present study, we determined the density of *HLO* and *GLO* bacteria. The highest percentage of *HLO* positive samples have grade 1 and grade 2 of bacterial density. These findings are similar to the results of other studies in humans, who had reported mild or moderate grade of *Helicobacter pylori* colonization (Manxhuka-Kerliu et al., 2009, Basir et al., 2017, Sayin 2019). Our results have shown that all *GLO* positive samples have grade 1 of bacterial density. The results are discordant with Barbosa et al. (1995) and Park et al. (2004) who reported the highest percentage of the marked grade of *GLO* density. These discrepancies might be due to differences in laboratory techniques for demonstration of *Helicobacter*

*spp.*, different sampling practices or different study designs. Gastric inflammation is an invariable finding in patients infected with *Helicobacter pylori* and presents the host's immune response to the organism. The chronic inflammatory infiltrates consisting of lymphocyte and plasma cells in *Helicobacter pylori* chronic gastritis is due to antigen-specific cellular and humoral immune mechanisms (Bodger and Crabtree 1998). In the current study, it was found that the highest percentage of *HLO* positive biopsy samples, have grade 2 of chronic inflammatory infiltrate. Our findings correspond with Eaton et al. (1990) and Poutahidis et al. (2001), which demonstrated the moderate to severe focal or diffuse lymphocytic gastritis in the pyloric mucosa of experimentally infected conventional pigs. A similar conclusion was drawn from the results of *Helicobacter pylori* infection in humans (Khalid et al., 2015, Sayin 2019). In the present study, all *GLO* positive samples, have grade 1 of chronic inflammatory infiltrate. These findings are in accordance to Mendes et al. (1991) and Queiroz et al. (1996) who reported that mild pyloric gastritis was present in all *GLO*-positive pigs. In humans, *Helicobacter heilmannii* s.l.-infected cases presented mild active chronic gastritis in the antrum and mild inactive gastritis in the fundus (Bento-Miranda, Figueiredo, 2014). In our study, grade of chronic inflammatory infiltrate was higher in *HLO* positive biopsy samples than *GLO* positive biopsy samples, which is in agreement with Krakowka et al. (2005a). An analogous conclusion was reached by Joo et al. (2007) who found that in humans, mononuclear cell infiltration was significantly lower in *Helicobacter heilmannii*-associated gastritis than in *Helicobacter pylori*-associated gastritis.

## CONCLUSION

Although various non-invasive procedures for the detection of *Helicobacter* species are available, a gastric biopsy still remains the gold standard for adequately determination of the *Helicobacter spp.* density and the severity of gastric inflammation. After the semi-quantitative determination of *Helicobacter spp.* and histopathological evaluation of *Helicobacter spp.*-associated chronic gastritis, it is concluded that despite the bacterial density, chronic inflammatory infiltrate grade was higher in *Helicobacter pylori*-like associated gastritis than in *Gastrospirillum*-like associated gastritis. However, it is widely known that *Helicobacter spp.*-induced gastritis reduced feed efficiency and depressed growth rate in pigs. Therefore, histopathological assessment of gastric mucosa is essential for accurate diagnosis of *Helicobacter spp.* infection and requires further attention.

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