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IDENTIFICATION OF *HELICOBACTER SPP.* IN GASTRIC MUCOSA OF CONVENTIONALLY BREEDING PIGS IN PELAGONIA AREA, R. MACEDONIA[7]

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Abstract

The purpose of this study is to identify Helicobacter species with 2 different morphology (*Helicobacter*-like organisms and *Gastrospirillum*-like organisms) in gastric imprint of pig stomach. A total of 120 gastric specimens, (60 from intensive and 60 from extensive pig production) were analyzed. The presence of *Helicobacter* species was detected in 9 of total 60 (15%) pigs from intensive production and 6.67% (4/60) belong to „*Helicobacter pylori* morphotype", while 8.33% (5/60) belong to „*Gastrospirillum*-morphotype". Helicobacter species in pigs from extensive production was found in 14 of total 60 (23.33%). „*Helicobacter-pylori* morphotype" were present in 5 of total 60 samples (8.33%), while bacteria with spiral appearance (*Gastrospirillum*-morphotype) were identified in 9 of total 60 samples (15%). Also, semi-quantitative evaluation of bacterial density was performed. The gastric imprint is useful, fast, simple and inexpensive method, widely used for confirmation of presence of *Helicobacter* species.

[7] original scientific paper

Key words: pigs, *Helicobacter* species, gastric mucosa, imprint cytology.

INTRODUCTION

The presence of spiral bacteria in the stomach of animals was first described by Rappin J. (1881) and Bizzozero G. (1893). In 1990, spiral bacteria were also observed in pig stomachs (Queiroz D.M., et al., 1990). *H. pylori* is considered as a human pathogen, while *H. heilmannii* colonizes the stomach of pigs, dogs and cats, while rarely in humans. It has been suggested that contact with pigs increases the risk of infection with *H. heilmannii* in humans (Meining A, et al., 1998). Krakowka S. and coworkers (2005a), for the first time successfully has isolated two small, curved bacteria from the stomach of conventional breeding pigs. Both isolates are morphologically very similar to H. pylori isolated in humans, but different from spiral *H. heilmannii*. *Helicobacter pylori* is largely localized in the mucous layer that coats the stomach or near mucous-producing cells, whereas *H. suis* is localized close to or within the parietal cells of the fundic glands (Zhang G, et al., 2016). Similar findings are also observed in humans (Joo M, et al., 2007).

Helicobacter pylori-like bacteria characterised as high pathogenic, has been associated with ulceration of the oesophageal or glandular portion of the pig stomach, severe gastritis and formation of lymphoid follicles (Krakowka S, et al., 2005a). In humans, *H. pylori* infection is also correlated with the inflammatory response and associated with gastritis and ulcus formation (Paull G, Yardly J.H, 1989). Wyatt J.I. et al. (1990) reported that severe glandular atrophy associated with intestinal metaplasia is very common in *H. pylori* infection in humans. On the contrast, infection with *Helicobacter heilmannii,* which has been shown to have low pathogenicity (Krakowka S, et al., 2005) was accompanied by only mild gastritis and no ulceration in pigs (Krakowka S, et al., 2005a). A study conducted by Singhal A.V. and Sepulveda A.R. (2005) showed that precense of *Helicobacter heilmannii* in human was associated with mild chronic gastritis and prominent lymphoid aggregates.

The presence of *Helicobacter* spp. can be proven very effectively by cytological findings, direct gastric tissue smear or biopsy. Cytological analysis of the gastric mucosa imprint is acceptable sensitive test for establishing the presence of *Helicobacter* spp. (Knezević-Štromar I. et al., 2008). Among numerous staining procedures for the identification of *Helicobacter* spp., the more widely used staining techniques are: May-Grunwald-Giemsa (Knezević-Štromar I. et al., 2008), Loeffler-methylene blue (Misra S.P. et al., 1998), and Diff-Quik method (Al-Ali J. et al., 2010).

MATERIAL AND METHODS

In this study were used stomach samples from 120 apparently healthy, fattening pigs (60 from intensive and 60 from extensive production system). Pigs from intensive production are from private farming household „Dzesi"-Bratindol, Pelagonia area, while pigs from extensive production are from various rural farming households, near Bitola. All pigs belong to Large White and Landrace breed, of both sexes, from 6-7 months of age, weighing about 100 kg. The stomachs were transported from the abattoir on ice box and processed within 3 h after collection in order to avoid loss of viability of bacteria due to delay by transportation. The stomachs were excised along the greater curvature, and the contents were discarded. The stomachs were then washed gently in tap water, taking care to remove only food particles. Stomach mucus was smeared directly on clean glass slides, air dried and. stained with Loeffler-methylene blue, according to Misra V. et al. (1994**)**. Bacterial cell morphology (spiral or 1-2 coiled) was analyzed by light microscope. Semi-quantitative evaluation of bacterial density was conduct as: 0 - absence of bacteria, 1 - sporadic presence of bacteria, only in some of the visible fields, 2 - a small number of bacteria (2-3) in each of the examined fields, 3 - a large number of bacteria (more than 10) in most of the examined fields.

RESULTS

Out of a total of 60 pigs in intensive system, the presence of Helicobacter spp. was found at 9 (15%). According to morphological

characteristics, 6.67% (4/60) were 1-2 coiled and belong to „*Helicobacter pylori* morphotype", while 8.33% (5/60) were multicoined with a different number of spiral curves which belong to „*Gastrospirillum*-morphotype". The presence of Helicobacter spp. in stomach mucosa of pigs in extensive system was 23.33% (14/60). Bacteria of *HLO* morphology were present in 5 of total 60 samples (8.33%), while bacteria with spiral appearance were identified in 9 of total 60 samples (15%) (Table 1). Using a semi-quantitative evaluation of *Helicobacter-like organisms (HLO)* in pigs in intensive system, we established that of total 60 pigs, in 4 pigs were confirmed *HLO* bacteria, of which: 1 pig with *HLO* 3, 2 pigs with *HLO* 2 and 1 pig with *HLO* 1. In pigs in extensive system, we confirm that out of 60 pigs, in 5 pigs were confirmed *HLO* bacteria, of which: 2 pigs with density of *HLO* 3, 2 pigs with *HLO* density 2 and 1 pig with *HLO* density 1. (Table 2, Figure 1a,b and c). Semi-quantitative evaluation of *Gastrospirillia-like organisms (GLO)* in pigs in intensive production system approved that of total 60 pigs, in 5 were confirmed *GLO* bacteria and in all 5 cases the density of the bacterium was *GLO* 1. In pigs in extensive production system, we establish presence of bacteria with *GLO* morphology in 9 of 60 pigs, all of them with bacterial density 1. (Table 3, Figure 2a and b).

Table 1. Prevalence of *Helicobacter* species

Type of bacteria	Number of pigs in intensive system	Percent (%)	Number of pigs in extensive system	Percent (%)
Helicobacter spp.	9	15	14	23.33
HLO	4	6.67	5	8.33
GLO	5	8.33	9	15
Total	60	100	60	100

Table 2. Semi-quantitive evaluation of *HLO*

Semi-quantitative evaluation of „HLO”	Number of pig in intensive system	Number of pigs in extensive system
0	56	55
1	1	1
2	2	2
3	1	2
Total	60	60

Table 3. Semi-quantitive evaluation of *GLO*

Semi-quantitative evaluation of „GLO”	Number of pig in intensive system	Number of pigs in extensive system
0	55	51
1	5	9
2	0	0
3	0	0
Total	60	60

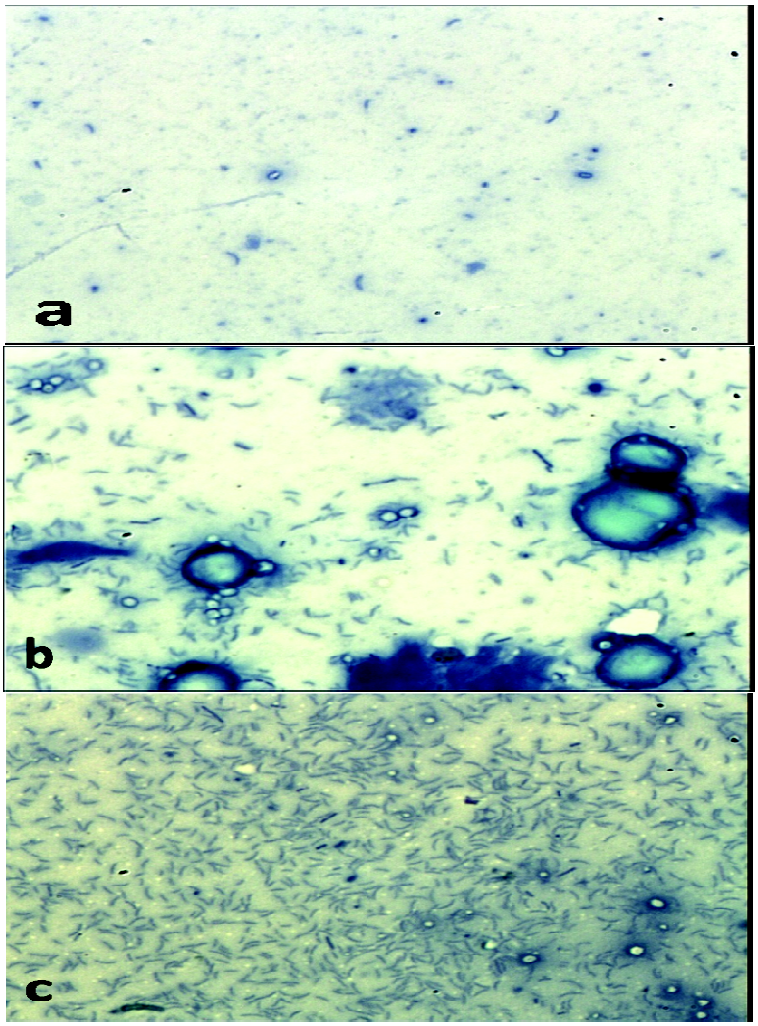

Figure 1. Density of *HLO* in gastric imprint: a) *HLO 1* b) *HLO 2* c) *HLO 3* (Loeffer's -methylene blue, 400X)

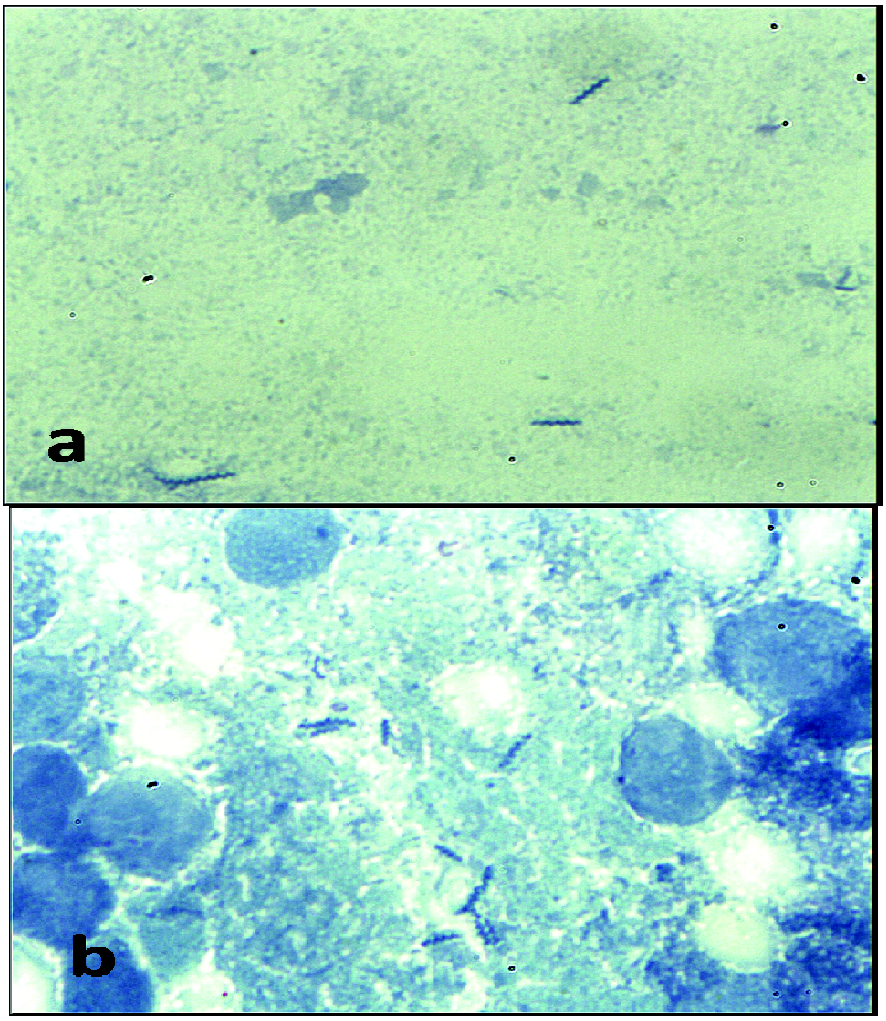

Figure 2a and b. *GLO* with density *GLO 1*: a) solitary b) in clusters of few bacteria (Loeffer's - methylene blue, imersion 1000X).

DISCUSSION

Among the tightly spiral bacteria, *Helicobacter suis* is the main represent that colonize the pig stomach and its prevalence depends on age of the animal, as well as the geographical area (Kopta L.A, et al., 2010). However, the pigs are not the only possible host hosts of *H. suis*. This

bacterium is detected in human gastric biopsies, more frequently often than other *Gastrospirillium*-like species and also is associated with the appearance of gastric diseases such as gastritis, gastric ulcer and gastric cancer (Haesebrouck F, et al., 2009). It is suspected that *H. suis* has zoonotic potential and that it is possible to transfer from pigs to humans. Joosten M. et al. (2013) were found spiral bacteria in the stomach of a veterinarian who was in permanent contact with pigs. Depending on the applied diagnostic methods, the prevalence of spiral bacteria ranges from 8.0 to 77% (Grasso G.M, et al., 1996; De Groote D, et al., 2000; Park J.H, et al., 2000). Different from the method of brush sampling, the imprint method does not extend the time needed for examination, does not affect the cost of examination and does not require specific sampling and additional equipment besides common accessories. Slide imprinting does not damage the specimen, thus it can subsequently be used for analysis by other method (Misra S.P, et al., 1988). The results of our work referred about low prevalence of Helicobacter spp. in both production systems. In group of pigs in intensive production system, the prevalence of *Helicobacter* spp was 15% (9/60), of which 6.67% (4/9) were bacteria with *HLO* morphology, and 8.33% (5/9) were bacteria with *GLO* morphology. In the group of pigs in extensive production system, the prevalence of *Helicobacter* spp. were 23.33% (14/60), of which 8.33% (5/14) were bacteria of *HLO* morphology, and 15% (9/14) were *GLO* bacteria with morphology. This is in accordance to results of Knezević-Štromar I. et al. (2008) who found prevalence of *Helicobacter* spp. in 40 out of 155 (25.81%) samples. On the contrast, Misra S.P. et al. (1998) were found the high prevalence of *Helicobacter pylori* in 97 out of 100 (97%) anthral biopsy of human stomach. Al-Ali J. et al. (2010) have demonstrated the presence of *H. pylori* in 184 of 252 (73.02%) gastric imprints. Our findings correspond to the results of Pirarat N. et al. (2007) who reported about low prevalence of 16.52% (19/115), when Warthin-Starry staining technique was used and 13.91% (16/115) when were used immunohistochemistry methods. Similar findings about low prevalence of Helicobacter spp. were given by Queiroz D.M. et al. (1990) and Grasso G.M. et al. (1996) who found that 10.8% and 9.4% of the pig stomachs colonized by bacteria. The results of our work coincide with the results of

Park H.J. et al. (2000), who referred about prevalence of 8% (4/50) examined pig stomachs. In contrast, there is a high rate of infection with *H. suis*-like bacteria (85.4%) in fattening pigs in Hungary (Szeredi L. et al. 2005). An interesting fact is that when bacterial detection was performed by histological methods using dyes such as: fuscine, Gram, Steiner silver, the prevalence of bacteria was very low (8.0-10.3%) (Grasso G.M, et al., 1996; Park J.H, et al., 2000), but when PCR was used, the prevalence was above 60% (De Groote D. et al. 2000). Several important factors affect the prevalence of infection with Helicobacter spp. such as: the age of the animal, the geographical area (Kopta L.A, et al., 2010), the method of breeding that most studies do not mention (Queiroz, D.M, et al., 1990; Grasso G.M, et al., 1996), stress factors, co-infection with other microorganisms, different patterns of sampling, application of different laboratory methods for the detection, staining methods and sensitivity of the methods. The prevalence of *Helicobacter* spp., (as well as bacteria *HLO* and *GLO* morphology) pigs in extensive system was higher in relation to the pigs raised in intensive system. A possible reason for this discrepancy could be the better hygienic conditions for animal's husbandry, as well as separation of different categories of animals in the intensive breeding system. In pigs reared in an extensive system, different categories of animals are kept together in poor hygienic conditions. Lee J.U. et al. (2006) found that infection in young individuals occurs during breast-feeding, via contaminated saliva or faecal-oral route during cohabitation. Epidemiological studies conducted on humans show the association of high prevalence of *H. pylori* and poor hygienic conditions (Bardhan P.K, 1997). Melnichouk S.L. et al. (1999) point out that farms of SPF animals are free from „*Candidatus H. suis*", which is not the case in conventional farms. These data give rise to the question of the possible impact of housing and management factors in pig farms on the transmission of bacteria.

CONCLUSION

In group of pig in extensive production system, prevalence of *Helicobacter* spp. (in both *HLO* and *GLO*) is higher than pig in intensive

production system. A possible reason of low prevalence of bacteria in pigs in intensive production system may be the better hygienic conditions for animals husbandry and separation of different categories of animals. Unlike, different categories of pigs in extensive production system are kept together in poor hygienic conditions, that could influenced on bacterial transmission and higher prevalence of bacteria. Gastric mucosa imprint cytology is a useful rapid, simple and cheap method for the detection of *Helicobacter* spp., characterized by acceptable sensitivity and specificity. To establish the right place of imprint cytology in detection of *Helicobacter* species, further investigations should be made.

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