PRELIMINARY DATA ON *HELCOBIACTER SP.* AND *CANDIDATUS HELCOBIACTER SUIS* INFECTION RATE IN PORCINE GASTRIC MUCOSA

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Abstract

In our study carried out on 29 porcine stomachs we found, based on PCR, that all the tested stomachs were *Helicobacter* genus positive and that 13 (45%) of the tested stomachs were *Candidatus Helicobacter suis* positive. With regard to particular porcine gastric mucosa site samples, it was noted that 26 samples of the *pars esophagea*, 23 samples of the fundus, and 25 samples of the pylorus were *Helicobacter* genus positive and that 4 samples of the *pars esophagea*, 8 samples of the fundus, and 12 samples of the pylorus were *Candidatus Helicobacter suis* positive. Since we could designate to a *Candidatus Helicobacter suis* only part of the *Helicobacter* genus positive samples we conclude that more detailed studies, involving 16S rRNA gene sequencing, are necessary in order to recognize *Helicobacter* species colonizing swine stomach.

Key words: swine, gastric mucosa, *Helicobacter*, PCR.

In 1983 Warren and Marshall (23) discovered and then cultivated spiral bacteria, presently known as *Helicobacter pylori*, that colonized human gastric mucosa. Lately, they proved its association with chronic gastritis and ulcer formation. Since then, over 20 new *Helicobacter* species were described with still more possible candidates to be established. Gastric *Helicobacter* infection has been described in many domestic animals, namely cats (18), dogs (10, 11), mice (8), hamsters (7), cattle (6), swine (5) and associated with gastritis, gastric and duodenal ulcers, and gastric cancer.

*Gastrospirillum suis* was the first gastric bacteria described in swine (15). Lately, on the basis of 16S rRNA gene sequence analysis, it was qualified as a member of *Helicobacter* genus and accordingly renamed *Candidatus Helicobacter suis*. Interestingly, it was also stated that 16S rRNA gene sequence of this particular swine pathogen is in 99.5% homologous to sequence of human *Helicobacter heilmannii* type 1, which suggests that it is probably the same bacterium (5) and points at the possible zoonotic potential (12). *Helicobacter heilmannii* was reported in 0.2 to 0.6% of human gastric mucosa samples taken from patients with gastric problems and its presence was always associated with chronic gastric inflammation (1). However, *Candidatus Helicobacter suis* is not the only of *Helicobacter* species that was demonstrated in inflamed porcine gastric mucosa. *Helicobacter heilmannii* type 2, *Helicobacter bilis*, *Helicobacter pullorum* are the other known to colonize porcine gastric mucosa (3, 19). According to histopathology the prevalence of *Helicobacter* infection in swine was described in different studies as ranging from 9.4% to 62.5% (2). In more sensitive PCR studies the prevalence was markedly higher with figures from 63.8% to 86.6% (4, 21).

Gastric ulcers are common in swine and are responsible for serious economic losses to swine industry. Ulcerative or pre-ulcerative lesions, at different stage, were noted from 5% to 100% of slaughtered pigs. Gastric ulcers in swine are responsible for lack of appetite, decreased weight gain, anaemia and sudden death. It is considered that up to 2.5% of growing/finishing pigs die from alimentary tract bleeding due to gastric ulcers before they reach market weight (24). Several factors are discussed as a cause of gastric ulcers in swine, among them environmental stress and small size of feed particles have the recognized share. Studies of Barbosa et al. (2) and Queiroz et al. (16) disclosed the possible role of *Helicobacter* infection in pathogenesis of gastric ulcers in swine. More detailed information on *Helicobacter* in swine is available in a recent review article by Sapierzyński and Fabisiak (20).

To our knowledge, there are no data on *Helicobacter* prevalence in gastric mucosa of pigs
Material and Methods

Samples. Twenty nine stomachs were obtained from clinical healthy pigs, about 6-month-old and weighing approximately 100-120 kg, after slaughter at abattoir located in the central Poland. The stomachs were opened immediately after slaughter along the greater curvature and the gastric content was gently discarded by washing. From each stomach cuttings were taken from the gastric mucosa area of the pars esophagae, fundus and pylorus.

DNA extraction. DNA from each gastric sample was extracted using a Genomic DNA Prep Plus® kit (A&A Biotechnology, Poland) in accordance with the manufacturer’s instructions.

Helicobacter genus PCR amplification. In a Helicobacter genus PCR amplification nested PCR was used. Primers, based on a 16S rRNA gene sequence, were designed according to the previous work (22). The first PCR was performed applying primers HelF (5’ CGTGGAGATGAAGGTITTA 3’) and HelR1 (5’ TACACCAAGAATTCCACCTA 3’) in a total volume of 25 µl of reaction mixture containing 1X Taq buffer with (NH₄)₂SO₄, 200 µM of each deoxynucleoside triphosphate, 2 mM MgCl₂, 0.6 µM of each primer, 0.5 U of Taq polymerase and 50 pg of DNA template. All reagents for PCR were purchased from Fermentas, Lithuania. PCR was comprising 10 min of preincubation at 94°C, followed by 30 cycles of 30 s of DNA denaturation at 94°C, 30 s of primer annealing at 58°C, 30 s of DNA elongation at 72°C. Final extension was performed for 7 min at 72°C. A volume of 2.5 µl of the obtained PCR product was used as a template in the second PCR which was performed under the same condition as the first one and primers HelF and HelR2 (5’ AATTCCACCTACCTCTCCTCCC 3’) were used.

Candidatus Helicobacter suis PCR amplification. Primers, based on a 16S rRNA gene sequence, were designed according to the previous work (5). Primer names and sequence were as follows: HelSuis1 (5’ TTGGGAGGCTTTCTTTCCA 3’B) and HelSuis2 (5’ TTGGGAGGCTTTCTTTCCA 3’) (23). Amplification reaction was performed in a total volume of 25 µl containing 1X Taq buffer with (NH₄)₂SO₄, 200 µM of each deoxynucleoside triphosphate, 2 mM MgCl₂, 0.4 µM of each primer, 0.5 U of Taq polymerase and 50 pg of DNA template. PCR was comprising 9 min of preincubation at 95°C, followed by 40 cycles of 30 s of DNA denaturation at 94°C, 30 s of primer annealing at 60°C, 45 s of DNA elongation at 72°C. Final extension was performed for 5 min at 72°C.

The products of DNA amplification were separated through electrophoresis on 1.5% agarose gel in the presence of ethidium bromide. To designate the product of amplification GeneRuller™ 100 bp DNA Ladder Plus (Fermentas, Lithuania) was used.

Results

Macroscopic evaluation of the stomach mucosa revealed evident signs of pre-ulcerative or ulcerative lesions in 3 out of 29 stomachs. In the first stomach, multiple ulcerative lesions in the fundus were visible, in the second one, two epithelial erosions, one located in the fundus and the second one in the antrum, 2.5 cm in diameter each were noticed, and in the third, post-ulcerative scar tissue-like lesion of fundus mucosa, 1 cm in diameter was found. Apart from these with ulcerative lesions, in other 5 out of 29 stomachs slight to severe reddening of the mucosal surface was observed. Severe reddening located in the fundus of the stomach was found in 3 stomachs.

Band of 270 bp for Helicobacter genus PCR amplification (Fig. 1) and 433 bp for Candidatus Helicobacter suis PCR amplification (Fig. 2) were accepted as a positive result of the test. Images were taken with VersaDoc Model 1000 Imaging System and developed with Quantity One 4.4.0 (BioRad).

All 29 stomachs tested were found positive in Helicobacter genus PCR amplification. When focusing on the results of the reaction on particular sites samples, it was noted that 26 samples of the pars esophagae, 23 samples of the fundus, and 25 samples of the pylorus were positive. In 20 stomachs, samples of mucosa from all three included sites were found positive.

Thirteen of the tested stomachs were found positive in Candidatus Helicobacter suis PCR amplification. With regard to the results of the reaction on particular sites samples, it was noticed that 4 samples of the pars esophagae, 8 samples of the fundus, and 12 samples of the pylorus were positive. In only 2 stomach mucosa samples from all three included sites were found positive (Table 1).

Findings from macroscopic lesions evaluation were compared to Candidatus Helicobacter suis PCR results. Two of the stomachs with ulcerative lesions were also found to be positive in Candidatus Helicobacter suis PCR but 1 stomach with multiple ulcerative lesions was found negative. Reddening of the gastric mucosa surface was present in Candidatus Helicobacter suis PCR positive as well as in negative stomachs.

Discussion

Helicobacter gastric infection was shown in different domestic animal species, among them in swine. From the first description of spiral microorganism colonizing porcine gastric mucosa (13) most of the research was directed to problems of Helicobacter prevalence in swine and to correlation between Helicobacter presence in porcine gastric mucosa and ulcerative lesions in the mucosa.
Fig. 1. Gel electrophoresis of *Helicobacter* genus PCR amplification. Lanes 1 to 9: samples tested; lane M: GeneRuler™ 100 bp DNA Ladder Plus; lane K+: positive control sample; lane K-: negative control sample.

Fig. 2. Gel electrophoresis of *Candidatus Helicobacter suis* PCR amplification. Lanes 1 to 5: samples tested; lane M: GeneRuler™ 100 bp DNA Ladder Plus, lane K+: positive control sample, lane K-: negative control sample.

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Early studies based on histopathology (15) found 10.8% of the tested swine colonized by Helicobacter. In more recent French study (21) carried out on 60 sows Helicobacter infection rate was much higher and reached 63%. Also the research of Cantet et al. (3) involved a group of 60 animals from 6 different farms (10 pigs from each farm) and found, by means of PCR, that 86.6% of the animals were colonized by Helicobacter. The authors also noted, on the basis of sequencing, that in every case it was Helicobacter heilmannii type 1 (Candidatus Helicobacter suis). In addition they concluded that in swine Helicobacter species are more homogenous than in dogs and cats.

Roosendaal et al. (19) focused their research on samples of porcine gastric mucosa from the pars esophagea and antrum. Molecular analysis of 43 samples revealed that in 42 of them Helicobacter heilmannii type 1 (Candidatus Helicobacter suis) was detected and Helicobacter bils was present only in one sample. In accordance to earlier studies cited here, Park et al. (17) who tested 40 samples of porcine pyloric mucosa found by PCR 95% Helicobacter infection rate (38/40). The analysis of sequenced PCR products showed 99.57% homology to Candidatus Helicobacter suis.

In our research we found all 29 stomachs tested Helicobacter sp. positive. Thirteen of the stomachs were found Candidatus Helicobacter suis positive (45%). Although we noted Helicobacter sp. infection rate as high as other cited authors, the Candidatus Helicobacter suis infection rate was visibly lower in our study than in most others. On the other hand, it is noteworthy that in Venezuelan study (9), carried out on 40 pigs, Candidatus Helicobacter suis infection rate was identical as in our study.

We found that in positive stomachs only 20 for Helicobacter sp. and 2 for Candidatus Helicobacter suis had all three samples, taken from the pars esophagea, fundus, and pylorus, positive. In some studies only samples from the pars esophagea and antrum (19) or pylorus (17) were included. In our study, 26 for Helicobacter sp. and 4 for Candidatus Helicobacter suis samples of the pars esophagea and 25 for Helicobacter sp. and 12 for Candidatus Helicobacter suis samples of the pylorus, were found positive. Compilation of the results presented in this study indicates that choice of samples only from particular site of porcine gastric mucosa might be misleading when the aim is the actual Helicobacter infection rate.

Correlation between Helicobacter presence in porcine gastric mucosa and mucosa ulcerative lesions was studied in several experiments (3, 14, 16, 19). In a research of Cantet et al. (3) all pigs with ulcerative lesions were Helicobacter infected. It allowed concluding, similarly to other mentioned researchers, that Helicobacter is associated with the presence of ulcerative lesions in the pars esophagea.

It is hard to give any explicit conclusions from our study since, based only on macroscopic evaluation, we found ulcerative lesions only in 3 stomachs out of 29 tested. However, it is important to note that in contrast to studies concerning ulcers of the pars esophagea we found ulcerative lesions in mucosa of the fundus. Additionally, 2 stomachs with ulcerative lesions were Candidatus Helicobacter suis positive and one was negative.

Some authors suggest that differences in Helicobacter infection rate may be caused by such factors as breeding and environment. We proved that also choice of samples site may influence the noted infection rate. Our results indicate need for more detailed study based on 16S rRNA sequence analysis in order to designate species composition of Helicobacter colonizing porcine mucosa since we recognized less than a half of samples tested in a Candidatus Helicobacter suis PCR amplification. Results of PCR compared to macroscopic evaluation point out the need of further microscopic study in order to get better knowledge of Helicobacter colonized mucosa status and interaction between mucosa and colonizing Helicobacter in swine. These results tend to support that further studies need to be designed on a larger group of animals in order to give more explaining results.

References