Association of Tightly Spiraled Bacterial Infection and Gastritis in Pigs

Jong-Hwan PARK, Beom-Jun LEE1, Yong-Soon LEE1 and Jae-Hak PARK*

Departments of Laboratory Animal Medicine and 1Veterinary Public Health, College of Veterinary Medicine and School of Agricultural Biotechnology, Seoul National University, Suwon 441-744, Korea

(Received 14 December 1999/Accepted 13 March 2000)

ABSTRACT. Tightly spiral bacteria were observed only in the pyloric mucosa of 4 (8.0%) of 50 swine stomachs, mainly in the surface of epithelia, the gastric pits and the lumen of gastric glands. The presence of the spiral bacteria was significantly associated with chronic pyloric gastritis (p<0.05). Mean gastritis score of the bacteria-positive pyloric mucosa was 3.25 ± 0.25, whereas that of the bacteria-negative pyloric mucosa was 2.37 ± 0.12. Parakeratosis and hyperkeratosis were spontaneously seen in the cardiac mucosa, regardless of the bacterial infection. Mean gastritis score of the bacteria-positive cardiac mucosa was 3.27 ± 0.32, whereas that of the bacteria-negative cardiac mucosa was 2.84 ± 0.13. There was no significant difference between the bacteria-positive and negative cardiac mucosa (p=0.05). Inflammatory response in the fundic mucosa was rare (gastritis score=0.75 ± 0.08). The tightly spiraled bacteria were not cultured with various culture media. These results suggest that the presence of tightly spiraled bacteria is associated with only the pyloric gastritis in pigs.

KEY WORDS: chronic gastritis, gastric mucosa, swine, tightly spiraled bacteria.

“Gastrospirillum suis”, a tightly spiral bacterium, colonizes the gastric mucosa of swine [1, 4, 7, 8]. By 16S rRNA sequencing, the bacterium is known to belong to the genus Helicobacter and is 99.5% similar to “G. hominis” type I [3, 6]. A possible link between such bacteria and gastric diseases of pig has been evoked [1, 12]. Such diseases contribute an important problem in veterinary medicine leading to significant economic consequences, with up to 2.5% of mortality in pigs due to gastrointestinal hemorrhage [14]. Ulceration of the gastric pars esophagus is also a common problem in intensive pig production [1, 12]. Several factors have been suspected in the pathogenesis of these lesions, but the etiology still remains unknown.

In the present report, we investigated naturally occurring swine gastritis in Korea. In addition, the gastritis scores in the cardiac, fundic, and pyloric mucosa of swine stomach were calculated and analyzed for an association of the gastritis with the tightly spiraled bacterial infection.

MATERIALS AND METHODS

Sample preparation: Stomach samples were obtained from 50 apparently healthy pigs (about 5 months old) weighing about 100–110 kg after slaughter at a slaughterhouse in Suwon, Korea. The stomach samples were transported to the laboratory in individual ice box (4°C) and cut open along the greater curvature from the pylorus to the diverticulum, and the contents were discarded. The stomachs were then washed gently in tap water, taking care to remove food particles only. The mucosal surfaces were examined and assessed for macroscopic lesions.

Histopathological and microbiological examination: Fragments of the pars esophaga, cardia, fundus and pylorus were fixed in 10% formalin for over 24 hr, dehydrated in alcohol-xylene series, and embedded in paraffin wax. From each block, sections 2-µm thick were prepared, and stained with hematoxylin and eosin (H&E) for histological examination. The sections were also stained with modified Steiner’s silver for the detection of spiral bacteria. Briefly, after deparaffinization, the slides were soaked in 1% uranyl nitrate for 15 min and then 1% silver nitrate for 90 min at 60°C. After washing three times with distilled water, the slides were soaked twice in 95% and 100%, respectively, followed by 2.5% gum mastic solution for 5 min at room temperature. After drying the slides in air, they were soaked in a reducing solution (10 ml of 1% uranyl nitrate for 15 min and then 1% silver nitrate for 90 min at 60°C). After washing three times with distilled water, the slides were soaked twice in 95% and 100%, respectively, followed by 2.5% gum mastic solution for 5 min at room temperature. After drying the slides in air, they were soaked in a reducing solution (10 ml of 2.5% gum mastic solution, 25 ml of 2% hydroquinone, 5 ml of 100% alcohol, and 0.2 ml of 1% silver nitrate) for 25 min at 45°C and observed by light microscopy. According to the microbiological examination, the stomachs were divided into two groups, bacteria-positive group and bacteria-negative group. The degree of gastritis in the cardiac, fundic and pyloric mucosa was evaluated by the scoring criteria shown in Table 1.

Bacterial culture: Fragments of the fundic and pyloric mucosa were cut to very small pieces with a sterilized knife. The tissue pieces were inoculated into chocolate agar (BBL®, U.S.A.) plate containing 5% horse serum, GC modified chocolate agar (DIFCO, U.S.A.) plate containing 10% horse serum and antibiotics (ampicillin B, trimethoprim, polymyxin B, and vancomycin), or brain heart infusion agar (BBL®, U.S.A.) plate. The plates were incubated in an incubator with GasPak (GasPakPlus®, BBL®, U.S.A.) for 4–7 days.

Statistical analysis: An ANOVA program for statistical analysis was performed on data for the gastritis score and the significant difference between the bacteria-positive and nega-
tive mucosa was analyzed by the student’s t test at the level of p<0.05.

RESULTS

Macroscopically, proliferation of gastric fold and severe congestion were observed in the fundic region of 5 samples (10%), and focal necrosis of the gastric fold was also observed in the fundic region of 2 samples (4%). Congestion of gland pits and hyperkeratosis of the pars esophagea were seen in 6 samples (12%) and 2 samples (4%), respectively.

With Steiner’s silver stains, tightly spiraled bacteria were observed only in the pyloric mucosa of 4 (8%) out of a total of 50 stomachs, but not in the pars esophagus, the cardiac mucosa, and the fundic mucosa. The spiral bacteria were mainly seen in the mucus, the gastric pits, and the lumen of gastric glands (Fig. 1a, b). Microorganisms were not seen in lymphoid follicles, macrophages, or the laminar propria. In addition, the cultivation of the tightly spiral bacteria was not successful with various culture media.

In the pars esophagus, hyperkeratosis and parakeratosis were spontaneously seen in the mucosa layer of the pars oesophagea, regardless of bacterial infection (Fig. 2a). The epithelial hyperkeratosis and the epithelial parakeratosis were observed in 11 samples (22%) and in 21 samples (42%), respectively (Table 2). However, no glanulomatous lesion and ulcer were seen.

In the cardia, a moderate to marked infiltration of lymphocytes, plasma cells, and eosinophils was observed in 47 (94%) of 50 samples (Fig. 2b, Table 2) and the average gastritis score from a total of 50 samples was 2.86 ± 0.09 (Table 3). The average gastritis score of the bacteria-positive cardiac mucosa form a total of 4 samples was 3.27 ± 0.32, whereas that of the bacteria-negative cardiac mucosa from a total 46 samples was 2.84 ± 0.13 (Table 3). Epithelial hyperplasia was also seen in 3 samples (6%).

In the fundus, the mild inflammatory response, showing a slight to moderate infiltration of lymphocytes and plasma cells, was mainly observed in the gastric pits (Fig. 2c). Epithelial degeneration in 8 samples (16%) and cystic dilatation in 2 samples (4%) were also observed (Table 2), and the gastritis score from a total of 50 samples was 0.75 ± 0.08 (Table 3).

In the pylorus, a dense infiltration of lymphocytes and lymphoid follicles in the laminar propria were observed in all (100%) of 4 bacteria-positive samples, whereas 19 (41.3%) of the 46 bacteria-negative samples (Fig. 2d, Table 2). The average gastritis scores in the bacteria-positive samples and the bacteria-negative samples were 3.25 ± 0.25 and 2.37 ± 0.12, respectively (Table 3). There was a significant difference between the bacteria-positive and negative samples (p<0.05).

DISCUSSION

Many spiral bacteria have been demonstrated in the stomachs of many animal species [2, 9, 10]. In this study, tightly spiral bacteria were found only in the pyloric mucosa in pigs, but not in the cardiac and fundic mucosa. In addition, our results reported by the gastritis score showed a significant
association between the presence of spiral bacteria in the pyloric mucosa and chronic pyloric gastritis in pigs. The histological findings were similar to those reported in man and gnotobiotic piglets colonized by *H. pylori* [5, 11]. There was also a significant association between the presence of *Gastrospirillum suis* and lymphoid follicles [8]. In some *G. suis*-positive pigs, the fundic mucosa was normal despite the presence of pyloric gastritis. This is also common in *H. pylori* infection in man [11].

*Gastrospirillum suis* is an uncultured tightly spiraled bacterium that colonizes the gastric mucosa of swine. We also tried to culture the bacteria using various culture media under several conditions. However, the spiralled bacteria were not cultured, which is consistent with other reports [8, 13].

Mendes *et al.* [8] reported that tightly spiraled bacteria were seen in the pyloric mucosa of the stomach of 13 (10.8%) of 120 pigs and in the fundic mucosa of three (5.0%) out of 60 pigs. In this study, the tightly spiraled bacteria were observed only in the pyloric mucosa of the stomach of 4 (8.0%) of 50 pigs that appeared clinically healthy at slaughter. Mendes *et al.* [8] also reported that chronic gastritis was observed more frequently in the pyloric region than fundic region and that the pyloric gastritis was significantly associated with the presence of the spiral bacteria. In this study, the chronic gastritis was observed most frequently in the cardiac region. However, the tightly spiraled bacteria were not seen in the cardiac region. The inflammatory response was not severe in the fundic region.

Barbosa *et al.* [1] reported that there was no association between the *Gastrospirillum suis* and gastritis. Nevertheless, the diagnosis of gastritis in each type of gastric mucosa was based on the different areas out of the four examined. In this study we found a more striking inflammatory reaction in cardiac mucosa, in which bacteria were not found, than in the pyloric or fundic mucosa. However, there was no association between the tightly spiral bacteria infection and the cardiac gastritis. Probably there are many causes of chronic gastritis in swine, e.g., dietary habits and parasites such as *Hystrostrongylus rubidus*, *Ascarops* spp., *Physoscocephalus* spp. and *Simondsia* spp.

Chronic gastritis or gastric ulcer in the cardiac region and in the pars esophagus of the stomach is thought to occur sporad-

Fig. 2. Representative histopathological findings in the pars esophagus (a), cardia (b), fundus (c), and pylorus (d). Hyperkeratosis and parakeratosis were seen in the mucosa of the pars oesophagus. A large lymphoid follicle was observed in the cardiac and pyloric mucosa of a pig infected with tightly spiral bacteria. H&E stain, × 50.
...ness in pigs, but the cause remains unclear [1]. Although several functions are involved in the pathogenesis, the presence of the spiral bacteria is also considered as a possible factor for swine gastric ulcer [12]. They found *Gastrospirillum* spp. in the most regions of the stomach including antral, oxyntic, fundic, cardiac mucosa. In this study, the presence of tightly spiralled bacteria was not associated with the gastric ulcer in the pars esophagus of the stomach.

In conclusion, although an inflammatory response was found most frequently in the cardiac mucosa, there was no association between tightly spiral bacteria infection and cardiac gastritis, whereas there was a close association between the presence of tightly spiral bacteria and pyloric gastritis.

**REFERENCES**


