Histopathological study of porcine gastric mucosa with and without a spiral bacterium ("*Gastrospirillum suis*")

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Summary. Tightly spiralled bacteria ("*Gastrospirillum suis*") were seen in the pyloric mucosa of the stomach of 13 (10·8%) of 120 pigs that appeared clinically healthy at slaughter and in the fundic mucosa of three (5·0%) out of 60 pigs. The spiral organism could not be cultured from any pig. Chronic gastritis was observed in the pyloric mucosa of 53 (44·2%) of 120 pigs and in the fundic mucosa of 7 (11·7%) of 60 pigs. The 13 pigs with spiral bacteria in the pyloric region comprised one animal (7·7%) with normal pyloric mucosa, two (15·4%) with "borderline gastritis", and 10 (76·9%) with chronic gastritis—in one instance accompanied by signs of activity (numerous polymorphonuclear cells). The three pigs with spiral bacteria in the fundic mucosa comprised two animals with a normal fundic region and one with "borderline gastritis". The presence of the spiral bacterium was significantly associated with pyloric gastritis ($p=0\cdot013$) and with numbers of lymphoid follicles ($p=0\cdot014$).

Introduction

Interest in gastric microbiology and histopathology has increased since 1983, when Warren¹ and Marshall² described a spiral bacterium, now named *Helicobacter pylori*, in the stomachs of patients with gastritis and peptic ulcer. This bacterium is now recognised as the most important causal agent of antral gastritis in man.³

Although naturally occurring spiral bacteria have been seen consistently in the stomach of some other mammals,^{4–9} they have not been associated with pathological changes,^{10,11} except in ferrets.¹² In animals, spiral bacteria tend to infect the gastric body and are commonly seen deep in the gastric glands and within parietal cells.^{4,5,9,11}

Recently, we described a tightly spiralled bacterium, provisionally named "*Gastrospirillum suis*", colonising the pyloric and fundic mucosa of the pig stomach.^{13,14}

In the present report, we describe a histological study of the pyloric and fundic mucosa of pig stomachs colonised by "*G. suis*" or free from the organism.

Materials and methods

Animals

Two series of male and female pork-weight pigs (mean weight 110 kg), slaughtered at about 6 months

of age, were studied. In the first series, fragments of gastric tissue from the pyloric region of 60 pigs were collected. In the second, fragments from both the pyloric and fundic mucosa were obtained from a further 60 pigs.

The stomachs were opened along the greater curvature and thoroughly washed in tap water. Macroscopic lesions, when present, were noted; chronic gastritis was suspected when surface oedema and redness were present in the mucosa, with or without erosions and areas showing haemorrhage.

Tissue samples of the pyloric mucosa were taken from the lesser curvature, 4 cm from the *torus piloricus*. Samples of the fundic mucosa were obtained from the greater curvature of the gastric body.

Histological examination

Fragments of the pyloric and fundic mucosa were fixed in Bouin's fluid for 18–24 h, dehydrated in an alcohol-xylene series, and embedded in paraffin wax. From each block, sections 4 µm thick were prepared and stained with haematoxylin and eosin (H&E) for histological examination, or with carbol fuchsin¹⁵ for the detection of spiral bacteria.

Gastric mucosa was regarded as normal when there was a complete lack of mononuclear and polymorphonuclear cells in the lamina propria throughout its entire thickness and no changes were observed in the glands and superficial epithelium. Chronic gastritis was indicated by the presence of mononuclear cells with a few polymorphonuclear cells. The presence of

many polymorphonuclear cells was taken to indicate activity.

The term "borderline gastritis" was used to describe cases in which there was infiltration of discrete mononuclear cells in the lamina propria without other changes. This infiltration was diffusely or focally distributed in both the basal and superficial regions of the mucosa. "Borderline gastritis" was included in the category of "normal or near normal mucosa" as described previously,¹⁶ the infiltration of mononuclear cells being too slight to be considered to indicate chronic gastritis. The presence of lymphoid follicles with prominent germinal centres was recorded.

Microbiological examination

In the first series, fragments from the pyloric mucosa of each stomach were kept in thioglycollate broth at 4°C for no longer than 2 h before being cultured in anaerobic, aerobic and micro-aerophilic conditions.¹⁷ Plates containing Belo Horizonte medium,¹⁷ Skirrow's medium,¹⁸ Butzler's medium,¹⁸ and Brucella agar supplemented with sheep blood 10% were used.

In the second series, specimens of pyloric and fundic mucosa were cultured similarly but on three selective media consisting of Brucella agar plus sheep blood 10% and one of the following three supplements: (i) amphotericin B 2 mg/L; (ii) amphotericin B 2 mg/L, vancomycin 6 mg/L and trimethoprim 5 mg/L; or (iii) amphotericin B 2 mg/L, vancomycin 6 mg/L, nalidixic acid 20 mg/L and starch 1 g/L. Separate sets of plates were incubated at 25°C, 37°C and 42°C and examined daily for up to 7 days.

Statistical analysis was done by the Fisher and Mantel-Haenszel χ^2 tests. The results were considered to be significant when $p < 0.05$.

Results

The results of the two series are described together.

In six pigs with macroscopic changes suggestive of pyloric chronic gastritis, histological features of gastritis were found. However, some pigs had histological evidence of gastritis of the pylorus in the absence of macroscopic signs.

Tightly spiralled bacteria were seen on the pyloric mucosa of 13 (10.8%) of 120 pigs and on the fundic mucosa of 3 (5.0%) of 60 animals. All pigs in which spiral bacteria were observed in the fundic mucosa also had these organisms in the pyloric region. The bacteria were more numerous in the pyloric than fundic mucosa and were found in the mucus, in the lumen of the pits, and in the mucosal surface within or beneath the mucus (fig. 1). Micro-organisms were not seen in lymphoid follicles, macrophages or the lamina propria. Neither the tightly spiralled bacteria nor *H. pylori* could be cultured from any pig. Animals were considered bacterium-positive when tightly spiralled micro-organisms were detected in gastric

Fig. 1. Tightly spiralled bacteria in the pyloric mucosa of a pig (carbol-fuchsin staining). Bar, 2.5 μ m.

mucosa, irrespective of whether they were present only in the pyloric region or in both the pyloric and fundic regions.

Histological findings are shown in the table. Chronic gastritis (excluding "borderline gastritis") was observed in the pyloric mucosa of 53 (44.2%) of 120 pigs and in the fundic mucosa of 7 (11.7%) of 60. The 13 pigs with spiral bacteria in the pyloric region comprised one animal (7.7%) with normal pyloric mucosa (fig. 2), two (15.4%) with "borderline gastritis", and 10 (76.9%) with chronic gastritis (fig. 3), one of them with signs of activity (numerous polymorphonuclear cells). Of the three pigs with spiral bacteria in the fundic region, two had normal mucosa and one had "borderline gastritis". Fundic mucosa was normal in the other four bacterium-positive pigs, in which tightly spiralled bacteria were observed only in the pyloric mucosa. Such bacteria were not observed in the seven pigs that had chronic gastritis in the fundic mucosa.

Table. Histopathological findings in the pyloric and fundic mucosa of the stomach of pigs

Histological findings	Number of pigs (bacterium-positive and bacterium-negative) with lesions in the			
	pyloric mucosa		fundic mucosa	
	+	-	+	-
Chronic gastritis with activity	1	8	0	1
without activity	9	35	0	6
Normal/near normal				
BG	2	35	1	23
N	1	29	6	23
Total	13	107	7	53

"Bacterium-positive" means spiralled organisms observed in the pyloric or pyloric and fundic gastric mucosa; BG, "borderline gastritis"; N, normal.

Fig. 2. Normal pyloric mucosa of the pig (H&E). Bar, 32.25 μ m.

Chronic pyloric gastritis (excluding "borderline gastritis") was significantly more common in bacterium-positive than in -negative pigs ($p=0.013$). In contrast, there was no such association in the fundic mucosa ($p=0.399$). Chronic gastritis in both the pyloric and fundic mucosa was observed in only five (8.3%) of 60 pigs, all of which were free from the bacterium.

Large lymphoid follicles with prominent germinal centres (fig. 4) were found in the lamina propria and submucosa of the pyloric region of 29 (24.1%) of 120 pigs. They were associated with normal mucosa in seven pigs, with "borderline gastritis" in a further

Fig. 3. Pyloric mucosa of a pig with chronic gastritis characterised by large numbers of mononuclear cells and a few granulocytes in the upper third of the lamina propria (H&E). Bar, 12.5 μ m.

Fig. 4. Lymphoid follicle in pyloric mucosa of a pig (H&E). Bar, 32.25 μ m.

seven and with chronic gastritis in the remaining 15 animals. There was a significant difference ($p=0.014$) between the occurrence of lymphoid follicles in bacterium-positive pigs (7 of 13; 53.8%) and -negative ones (22 of 107; 20.6%).

Discussion

Naturally occurring gastritis in laboratory and domestic animals has been reported infrequently, and gastric histology of pigs is seldom mentioned in reviews of stomach diseases in animals. Chronic gastritis of the cardiac region and of the *pars oesophagea* is thought to occur sporadically in pigs, but no cause has been found.¹⁹ Spiral-shaped organisms may be demonstrated in the stomachs of many animal species,⁴⁻⁹ but in only one instance—in ferrets colonised by *H. mustelae*—have they been associated with gastritis.¹²

The results reported here showed a significant association between the presence of spiral bacteria and chronic pyloric gastritis in pigs. The histological findings were similar to those reported in man^{16,20} and in gnotobiotic piglets^{21,22} colonised by *H. pylori*. There was also a significant association between the presence of "*G. suis*" and lymphoid follicles. The latter have previously been related, in man, to an immune response to gastric colonisation by *H. pylori*.²³ 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.^{16,20}

Pyloric gastritis was also seen in bacterium-negative pigs. Probably there are many causes of chronic gastritis in swine, e.g., dietary habits²⁴ and parasites such as *Hyostrogylus rubidus*, *Ascarops* spp., *Physoccephalus* spp. and *Simondsia* spp.¹⁹ Candidiasis of the

pars oesophagea may also occur, often in association with pre-ulcerative epithelial hyperplasia and parakeratosis.¹⁹ It is possible that the methods used were not sensitive enough to detect all "*G. suis*"-positive pigs. Recently Dick *et al.*²⁵ used mouse inoculation as a means of maintaining non-cultivable gastric spiral bacteria. Employing this method in another series of pigs, we detected "*G. suis*" in some animals that were negative for this micro-organism by the urease test and carbol fuchsin staining (unpublished results).

It remains to be established whether "*G. suis*" is a pathogen, or whether it colonises previously damaged areas of the gastric mucosa. Further information, for

example on the effect of eradicating the organism by antibiotic treatment, is necessary for a better understanding of the role of "*G. suis*" in the pathogenesis of chronic gastritis in the pig.

It is not known whether histologically detectable gastritis in pigs depresses growth rate or the efficient utilisation of food. If it does, "*G. suis*", which was found in almost 20% of all cases of chronic gastritis in the pigs studied, deserves further attention.

This work was supported by grants from FAPEMIG and FINEP (Brazil). We thank Mr P. P. Silva for proficient technical assistance and Frigorífico São João Ltd for providing the pigs stomachs.

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