

# *Helicobacter*-like organisms in the stomach of pigs with and without gastric ulceration

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## Summary

**Objectives:** To determine whether *Helicobacter* organisms are present in commercially reared pigs in North America and whether the presence of these bacteria is associated with gastric ulcers.

**Methods:** Stomachs of pigs from four farms with diverse management systems were examined. The presence of *Helicobacter* was determined using an in-situ urease mapping technique and performing microscopic examination using Warthin-Starry silver stain. Culture was attempted using methods appropriate for *Campylobacter* species and *Helicobacter pylori*. The presence of lesions in the pars oesophagea based on macroscopic examination was recorded.

**Results:** Spiral-shaped bacteria morphologically similar to *H. heilmannii* were detected in the stomachs of pigs from three of the four herds. The highest prevalence of positive stomachs (87%) occurred in a farrow-to-finish herd with a continuous-flow grower-finisher barn with floor feeding. The presence of *Helicobacter*-like organisms was not related to the presence of gastric ulcers.

**Implications:** Programs to eliminate *Helicobacter* have been shown to be highly successful in curing gastric ulceration in humans, but on the basis of this study, an antibiotic approach is unlikely to be of benefit in solving gastric ulcer problems in swine. The prevalence of *Helicobacter* in stomachs of pigs at slaughter may be related to husbandry practices and biosecurity. It may be possible to produce pigs free of *Helicobacter*, and this might be important from a public health standpoint. However, at present, it appears that *H. heilmannii* is not a serious health risk to humans.

**Keywords:** gastric microbiology, pars oesophagea, swine, urease testing, *Helicobacter heilmannii*

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A naturally occurring, spiral-shaped bacterium (morphologically distinct from *Helicobacter pylori*) has been identified in the gastric mucosa of commercially reared pigs.<sup>1</sup> Initially, this microorganism was named *Gastrospirillum suis*, but comparison of 16S rDNA sequence of the porcine bacterium and a morphologically similar bacterium which infects a small percentage of humans has revealed that these two organisms are likely the same species and a member of the Family *Helicobacter*.<sup>2</sup> The name *Helicobacter heilmannii* is now generally accepted for this gastric bacterium, which has been found to infect not only pigs and humans, but dogs and cats as well.<sup>3</sup>

An association between the presence of *H. heilmannii* and ulceration of the pars oesophagea has been observed in several studies.<sup>4–6</sup> However, experimental infection of gnotobiotic pigs with *H. heilmannii* has failed to produce lesions in the pars oesophagea.<sup>7</sup>

The presence of *H. heilmannii* in commercially reared swine in North America is unknown. The bacterium can not be detected using conventional hematoxylin-eosin staining and culture has been unsuccessful. The purpose of this trial was to determine whether *Helicobacter*-like organisms could be readily detected in North American swine, and whether the presence of these bacteria was associated with gastric ulceration.

## Materials and methods

A total of 199 pigs were examined. Sampling was not random but represented an entire marketing group for Herds 1, 2, and 3. In Herd 4, pigs were selected in a consecutive manner at slaughter.

## Herds supplying pigs

Herd 1 was the University of Guelph Swine Research Centre herd, a 300-sow, farrow-to-finish operation in which all-in–all-out (AIAO) management was used at each phase of production. Grower-finisher rooms were thoroughly cleaned between fills. The flooring is partially slatted. Pigs were fed ad libitum a finely ground pelleted ration that was corn-soybean based. The grain portion of the diet had an average particle size of about 700 µm. This herd was originally populated by caesarian-derived pigs and is maintained as a specific-pathogen-free (SPF) farm; i.e., rigid biosecurity is practiced and the herd is closed to new additions.

Herd 2 was a University of Guelph-owned grower-finisher facility operated AIAO by site. The flooring was solid concrete without bedding.

Pigs were fed ad libitum and the same feed was used as in Herd 1. The pigs were obtained from a commercial nursery operation and housed at this unit from 20 kg (55 lb) until market weight.

Herd 3 was a 150-sow, farrow-to-finish, privately owned farm in southwestern Ontario. The grower-finisher pigs were housed in a continuous-flow barn with solid cement floors. Pigs were fed a coarse-ground mash feed composed of high-moisture corn, soybean supplement, and a vitamin-mineral premix. Particle size of the feed was not analyzed but whole kernels of corn were evident. The feed was prepared on the farm. Pigs were fed twice daily by dispersing feed on the floor of each pen.

Herd 4 was a large corporate farm in the Midwestern region of the United States. Pigs were housed in totally slatted grower-finisher barns, which were filled at one time and thoroughly washed between fills. Feed was a finely ground pelleted ration based on corn and soybean meal and fed ad libitum. Previous feed particle size analysis indicated that the average particle size of the grain portion of the feed was less than 700 µm. All pigs in this operation were taken off feed prior to shipping to market. Feed was withheld for about 24 hours prior to slaughter.

## Stomachs

Stomachs were obtained immediately after the pigs were killed and eviscerated. Pigs from Herds 1, 2, and 3 were shipped the short distance (less than 1 hour) to the University of Guelph abattoir and slaughtered within 4 hours of their arrival. Pigs from Herd 4 were slaughtered at a private slaughterhouse owned by the same corporation that owned Herd 4. At the time of slaughter, it was recorded whether the pigs had been allowed to eat within the preceding 12-hour period. In Herds 1, 2, and 3, some pigs had been allowed to eat until they were shipped to the abattoir and slaughtered within 4 hours after their arrival at the slaughter house. Other pigs from Herds 1, 2, and 3 had feed withheld for approximately 24 hours before they were shipped.

Each stomach was incised along the greater curvature from the diverticulum to the pylorus. Ingesta was removed and the mucosal surface gently washed with tap water. The stomach was evaluated for the presence or absence of lesions in the pars oesophagea:

- a normal, smooth, glistening surface or a roughened surface with evidence of parakeratosis was classified as a 0;
- the presence of erosions or extensive ulcerations was graded as a score of 1.

## Testing for *Helicobacter*

Biopsies about 3 × 3 cm from the antral region were collected and transported to the laboratory in 2 mL sterile saline. The tissue samples, upon arriving at the laboratory, were manually macerated and a small amount of this suspension was used to inoculate chocolate agar plates. Inoculated plates were maintained at 37°C in a microaerophilic atmosphere for 10 days. All colonies were further investigated using Gram staining. In addition, attempts were made to culture bacteria on media

designed to support growth of *Campylobacter*,<sup>8</sup> as well as Belo-Horizonte media, which is the standard bacteriological culture media of *H. pylori*.<sup>9</sup>

An in-situ urease assay was performed according to the methods of Grasso, et al.<sup>10</sup> Briefly, the open stomachs were placed in an aluminum tray with the mucosal surface exposed. A thin layer of gel-like medium consisting of 2% urea, 0.0012% phenol red, 0.3% agar, 0.01% yeast extract, 0.0091% monopotassium phosphate, and 0.00995% disodium phosphate (pH 6.7–7.0) was spread over the entire mucosal surface. When bacteria such as *Helicobacter* split urea, ammonia is produced which causes pH to rise and liberates the phenol red indicator. All samples were visually monitored for 1.5 hours to detect areas of color change from yellow-orange to deep pink.

A single tissue biopsy was taken from an area of color change, or in the cases where no color change was noted, biopsies were taken at the junction of the antrum/oxynitic region. In all cases, a biopsy was taken from the pars oesophagea. Tissue was fixed in formalin and processed for histological examination. Section replicates were stained with hematoxylin and eosin (HE), and Warthin-Starry (WS) histochemical stains.<sup>11</sup> The person evaluating the slides for the presence or absence of spiral-shaped bacteria was blinded to the origin of the sample.

## Statistical analysis

The association between the presence of *Helicobacter*-like organisms on histological examination and the presence of erosive lesions of the pars oesophagea was determined using the Breslow-Day  $\chi^2$  test. The agreement between histological examination and urease mapping to detect the presence of *Helicobacter* was determined by calculating Kappa.

## Results

*Helicobacter*-like organisms were observed to be present by microscopic examination in the stomachs of pigs from three of the four herds. These bacteria were morphologically similar to *H. heilmannii* in that they were tightly spiraled (about five turns), and about 3–7 µm in length (Figure 1). Spiral-shaped bacteria were not found in the biopsies from the pars oesophagea, but only in the glandular region. *Helicobacter*-like organisms were readily observed using WS staining but not with HE staining. No *Helicobacter*-like organisms were isolated on any culture attempts.



The prevalence of *Helicobacter*-like organisms varied from herd to herd and were not associated with the presence of erosive lesions of the pars oesophagea (Table 1). Of the 199 stomachs examined, histological evidence of *Helicobacter*-like organisms was noted in:

- zero of 65 (0%) from Herd 1,
- 31 of 57 (54.3%) from Herd 2,
- 28 of 32 (87.5%) from Herd 3, and
- six of 45 (13.3%) from Herd 4.

Overall, 95 of the 199 (47.7%) stomachs were scored as 1 (i.e., had gross evidence of erosive lesions). *Helicobacter*-like organisms were found in 18 of the 95 (18.9%) stomachs with erosions and in 47 of the 104 (45.2%) stomachs without lesions. Examination of the stomachs from the three herds where *Helicobacter*-like organisms were observed revealed that there is almost an equal likelihood that *Helicobacter*-like organisms were found in stomachs with erosive lesions (28%) as stomachs without lesions (32%) ( $\chi^2 = 0.49$ ,  $P > 0.1$ ). There was a substantial agreement between the in situ urease test and the histological observation of *Helicobacter*-like organisms (Kappa = 0.68).

Pigs that were fasted prior to slaughter did not have a greater likelihood of having erosive lesions of the pars oesophagea. Lesions were noted in 45 of 94 (47.9%) fasted pigs and in 50 of 105 (47.6%) of nonfasted pigs. In Herd 1, there were no stomachs with *Helicobacter* present, and in Herd 4, there were no pigs that had eaten within 12 hours of slaughter, so the relationship between fasting and the presence of *Helicobacter*-like organisms was only examined in Herd 2 and 3. In these two herds, 35 pigs were fasted and 54 were not fasted. The presence of *Helicobacter*-like organisms was greater in the nonfasted group (75.9%) compared to the fasted group (51.4%), ( $\chi^2 = 4.65$ ,  $P < .05$ ).

## Discussion

The prevalence of *Helicobacter*-like organisms in the stomachs of commercially reared pigs in North America may vary a great deal from herd to herd. In this limited trial of four farms, there was tremendous variation among farms in the proportion of *Helicobacter*-positive stomachs. However, as only one point prevalence estimate per farm was obtained, this might reflect a considerable temporal variation around the mean, or it might indicate a consistent herd-to-herd variation. Further studies following these herds over a period of time are required to prove which scenario is correct. As well, a random survey

of a large number of farms is required in order to determine the prevalence of *Helicobacter*-like organisms in the North American swine industry. The fact that three of four of the herds examined in this study were positive suggests that the organisms are likely widespread in the pig population, but further research is needed to confirm this hypothesis.

Among the four farms in this trial, the wide variation in facility design and management practices probably accounted for the differences in bacteria detection rate, although this is only speculation at this point. The herd with the highest prevalence of *Helicobacter*-like organisms (Herd 3) used solid flooring, continuous-flow animal movement, and floor feeding. These factors would all contribute to a very high rate of fecal-oral spread. On the other hand, Herd 1—a closed herd with strict biosecurity and a high health status—appeared to be free of *Helicobacter*-like organisms. This suggests that the same biosecurity measures used to maintain freedom from other major swine pathogens may be successful in preventing the introduction of *H. beilmannii*. This might prove to be an important finding. A recent survey of human patients with *H. beilmannii* gastritis has indicated that pig contact is a risk factor.<sup>12</sup> Although *H. beilmannii* is not regarded as a serious public health risk, freedom from even minor zoonotic organisms may become an important marketing issue in the near future.

Abattoir surveys from other countries indicate the prevalence of *H. beilmannii* to be anywhere from about 10% of pigs examined<sup>5,13</sup> to about 60%–80%.<sup>4,6,14</sup> These studies have tended to be small and have examined a convenience sample rather than a sample that reflects the population. In addition, some diagnostic techniques have been shown to be more sensitive than others in detecting the presence of *Helicobacter*.<sup>4</sup> The mouse inoculation technique has been shown to be a better method of finding positive animals than histology or urease testing.<sup>4</sup> It is possible that the findings of the present study are an underestimate of the prevalence of *Helicobacter*. The use of in-situ urease mapping appeared to be helpful in detecting positive animals and localizing the urease-producing bacteria. It has been suggested that *H. beilmannii* colonize in clusters and therefore a biopsy sample may not reflect the true status of the animal if it is taken from the wrong location. The urease test should improve the accuracy of histological examination,<sup>10</sup> and might be used as a screening procedure to determine herd status. Both false positives and false negatives using the urease test were observed in this trial. Presumably if there are only a small number of bacteria, they may not produce sufficient urease to cause a color change. On the other hand, other bacteria can produce

urease and therefore produce a color change in the absence of *Helicobacter*. It is possible that *Helicobacter* are present to produce a color change but the tissue examined by histology did not contain bacteria. This type of error could be rectified by multiple sampling. In human medicine, *H. pylori* is so widespread that two biopsies are considered sufficient to detect *Helicobacter* infection in > 95% of cases.<sup>15</sup>

**Table 1**

Nonassociation of erosive lesions with *Helicobacter*-like organisms

	Herd 1		Herd 2		Herd 3		Herd 4	
	+	-	+	-	+	-	+	-
<i>Helicobacter</i> positive	0	0	8	23	6	22	4	2
<i>Helicobacter</i> negative	53	12	4	22	2	2	18	21

+ erosive lesions present  
- erosive lesions not present

In contrast to several other studies that investigated the association between gastric lesions at slaughter and *Helicobacter*-like organisms,<sup>4-6</sup> we did not observe a relationship between the presence of spiral bacteria in the glandular region of the stomach and ulceration of the pars oesophagea. In this study, the herd with the highest proportion of stomachs with gastroesophageal lesions appeared to be free of *Helicobacter* organisms and the herd with the highest prevalence of *Helicobacter*-positive stomachs had the lowest proportion of ulcers. The difference in the findings between this study and previous reports might be due to the population of pigs examined, or possibly because different detection techniques have been used in the various studies. No other study to date has examined this relationship in North America and it may be that the bacteria, the pigs, or the husbandry conditions are sufficiently different to cause the difference observed between this study and previous studies from Brazil<sup>4,6</sup> and Italy.<sup>5</sup>

Gastric ulceration in swine is generally believed to be caused by multiple factors.<sup>17</sup> It is possible that certain conditions that are strongly associated with gastric ulceration, such as dietary factors, are also associated with the colonization of *Helicobacter*-like organisms in the pig's stomach. Previous studies have not examined these possible confounding factors. In the present study, it did not appear that finely ground feed, which is known to produce ulcers, was linked to the presence of *Helicobacter*-like organisms. Pigs from Herd 3 were fed a coarse feed and tended to have the highest prevalence of *Helicobacter*-like organisms. Withdrawal of feed prior to slaughter has been associated with an increase in the prevalence of ulcers (Straw BE, et al; *Proc IPVS Cong.* 1992; 386. Davies PR, et al; *Proc IPVS Cong.* 1994; 471), but in this study, fewer stomachs were found to contain *Helicobacter*-like organisms in the pigs that were fasted. Therefore, it does not appear that feed withdrawal prior to slaughter has confounded previous studies in a way that would explain the findings of a positive relationship between *Helicobacter* and stomach lesions. The contradictory results between this research trial and others suggest that further studies of a much larger scale are needed to examine this relationship.

Researchers have been eager to compare the gastroesophageal ulcer disease of swine with gastro-duodenal ulcers of humans; however, there are considerable anatomical and physiological differences between the two species. Experimental infection of pigs with *H. heilmannii* and *H. pylori* does not result in ulceration of the pars oesophagea, but instead causes gastritis and lesions in the glandular region of the stomach where the organisms colonize.<sup>7,18</sup> It has been suggested that, in swine, *Helicobacter* stimulates parietal cells to over-secrete acid, either by direct irritation or by causing increased gastrin release.<sup>3</sup> However, in experimental infection of pigs with either *H. heilmannii* or *H. pylori*, an increase in gastric acidity has not been observed.<sup>7</sup> At this time, the mechanism by which *Helicobacter* might cause ulceration of the pars oesophagea is not known. The results of this present study demonstrate that the role of *Helicobacter*-like organisms in the stomach of swine with respect to the creation of gastroesophageal ulcers is unclear, but unlikely to be a major factor.

Surprisingly, in this study, fasting was not observed to be associated with an increase in gastric ulceration. It is possible that the length of

the fasting period was insufficient to cause an increase in stomach lesions. The results of this study are similar to a recent large study (Morrow M, et al. *Report to the NPPC* 1999; 1), which examined the impact of feed withdrawal at slaughter on gastric ulcers and found that pigs fasted up to 24 hours prior to slaughter did not have an increase in ulcer prevalence or severity.

This study has shown that *Helicobacter*-like organisms (presumably *H. heilmannii*) can be readily found in commercial swine in North America using urease mapping and histological examination with WS staining. Their role in gastric ulceration is unclear, but would appear to be relatively minor. *Helicobacter* infection of swine may become more of an issue in the future because of its zoonotic potential. This study suggests the management strategies used to control other swine pathogens might be useful in control of *Helicobacter*.

## Implications

- *Helicobacter*-like organisms can be readily demonstrated by histologic examination of biopsies from the fundic and pyloric regions of stomachs of commercially reared pigs.
- The prevalence of the *Helicobacter*-like organisms varies from herd to herd and appears to be related to management practices and biosecurity procedures. It is likely that the same methods used to control the spread of other gastrointestinal bacteria will be useful in reducing the prevalence of this bacterium.
- The presence of *Helicobacter*-like organisms does not appear to be related to the development of ulceration in the pars oesophagea.

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