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Gastric ulceration in swine: Overview and infectious etiology control

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The presence of spiral-shaped bacteria were first described in the stomachs of animals by Rappin (1881)[1] and Bizzozero (1893)[2]. Although similar organisms were observed in human stomachs as early as the 1930s[3], little attention was given to these bacteria until the past 20 years. Marshall and Warren (1984)[4] proposed that curve-shaped bacteria (now known as *Helicobacter pylori*) were a common cause of gastric ulceration and chronic gastritis in humans.

This discovery of a bacterial etiology revolutionized the treatment of human peptic ulcer disease and greatly improved the chances of a patient being cured. As a result of these developments in human medicine, attention has been directed towards gastric microbiology in domestic animal species.

In 1990, spiral-shaped bacteria were observed in the stomach of pigs. These organisms were named "*Gastrospirillum suis*" because of their morphological similarity to known bacteria found in other species[5]. "*Gastrospirillum*" are larger than *Helicobacter pylori* (7–10 _m compared to 4 _m in length) and have a tightly coiled appearance of between four to six coils, whereas *H. pylori* are curved or 's'-shaped.

Bacterial characteristics and nomenclature

Based on DNA studies, the group of bacteria classified as "*Gastrospirillum*" are now considered members of the *Helicobacter* genus. All *Helicobacter* are gram-negative and micro-aerophilic. Commonly they have multiple polar flagella that are always sheathed and exhibit urease, catalase, and oxidase activity. Three species with the long and tightly coiled (*gastrospirillum*-like) morphology have been cultured and characterized from gastric samples of cats and dogs: *Helicobacter felis, Helicobacter bizzozeroni,* and *Helicobacter salomonis.* The *Gastrospirillum*-like bacteria observed in the antral pits and at the mucosal surface of the porcine stomach remain unculturable.

According to the guidelines of the International Code of Nomenclature of Bacteria[6], which state the necessity of a broad range of phenotypic and phylogenetic data, the official designation of the "*Gastrospirillum*" organism of pigs is impossible until it is cultured[6]. DeGroote et al (1999)[7] have proposed that the name of this new candidate species be named "*Candidatus Helicobacter suis.*"

"*Gastrospirillum*-like" organisms are occasionally found in human stomachs[8]. At least two different types of these coiled bacteria exist, based on phylogenetic research and have been tentatively named "*Helicobacter heilmannii*" type 1 and type 2[9]. Likewise, two distinct "*Gastrospirillum*-like" bacteria have been observed in pig stomachs[10]. It has been shown that there is a 99.5% 16S rDNA sequence homology between *H. heilmannii* type 1 and "*Candidatus H. suis*," suggesting that these organisms belong to the same species[11]. There are several papers published referring to the swine gastric organisms as *Helicobacter heilmannii*, but until these bacteria are successfully cultured from pigs, this name may not be appropriate and the name *Candidatus H. suis* should probably be used.

Epidemiology

Helicobacter-like organisms have been observed in the stomachs of swine in various countries, including Canada and the United States[12]. The prevalence of these organisms in the general swine population is unknown, but is most likely widespread. Early studies of pigs randomly selected from Brazilian[13] and Italian[14] slaughter plants found approximately 10% of stomachs to be positive. More recent studies have reported that pigs from certain herds have much higher levels with 60–80% of animals testing positive[10,12,15]. Some of the differences in prevalence are likely due to the methods used for bacterial detection, but there also appears to be marked herd-to-herd variation. Possibly management factors such as all-in—all-out pig flow and SPF procedures reduce the prevalence[12]. The most likely method of transmission of *Helicobacter* organisms from pig-to-pig is via fecal-oral spread.

It is possible that *Candidatus H. suis* can be spread to and from other animal species. Cats and dogs carry similar organisms and a recent Italian survey of wild rats reported that 23% of the animals examined showed histological evidence of a spiral bacterium morphologically similar to the *Helicobacter* of swine[16].

Presumably, pigs are a natural reservoir for *Candidatus H. suis* and once infected, the pig remains host to this organism for a prolonged time period, possibly the lifetime of the pig. In humans, *H. pylori* appears to be well adapted to survival in the stomach and despite the development of antibody titres and an inflammatory reaction, colonization of the bacteria persists for many years[17].

Pathogenesis

Virulence factors for *Candidatus H. suis* have not been identified and it is unclear whether this organism is pathogenic in swine. Gnotobiotic pigs experimentally infected with *Candidatus H. suis* produce an inflammatory response similar to experimental infection with *H. pylori* except that the reaction is distributed mainly in the fundus compared to the cardia and antrum regions of *H. pylori*-infected pigs[18,19]. In humans, *Helicobacter* infect and inflame the tissue that becomes ulcerated and therefore this would suggest that direct insult from bacteria leads to ulcerative lesions. *H. pylori* releases urease which leads to the production of ammonia causing irritation to the gastric tissue. In addition, cytotoxins have been identified which are directly associated with the degree of gastritis in gnotobiotic pigs and in humans. *Helicobacter pylori* strains that produce a toxin inducing the formation of vacuoles in tissue culture are 30–40% over-represented in ulcer patients compared to those with gastritis alone[17]. The gene responsible for toxin production has been identified and named vacA. A second gene of *H. pylori* that is associated with pathogenicity has been sequenced and named cagA. About 50% of patients with chronic gastritis alone are infected with cagA strains of *H. pylori*, but almost all patients with duodenal ulcers are infected with cagA strains[17].

In swine, the *Helicobacter* organisms are found in the glandular regions of the stomach, but the vast majority of gastric ulcers involve the *pars oesophageal* area where *Helicobacter* are never found. Theoretically, *Helicobacter* organisms might contribute to ulceration of the *pars oesophagea* in an indirect way, by causing hyper- acid secretion. It has been suggested that *Helicobacter* might create a neutral pH in the layer overlying the gastric epithelium and thus interfere with the normal inhibition of gastrin release via detection of intraluminal acid[20]. However, in a study using pigs experimentally infected with *Candidatus H. suis*, no difference in gastric pH values were found between pre- and post-infection[18].

Diagnosis

The simplest method to confirm the presence of *Helicobacter*-like organisms is to perform histological examination on gastric mucosa using a stain that will allow visualization of the distinctively shaped organisms. Silver stains or Giemsa staining can be used. It is advisable to examine several biopsies from each stomach because colonization of *Helicobacter* is patchy and false negative readings are common. Recently, 16S ribosomal DNA-based PCR assays have been developed for detection of "*Candidatus H. suis*" and have been shown to produce a higher proportion of positive results than histology alone[21].

Breath tests designed to detect urease-producing gastric bacteria have been commonly used in humans and more recently in domestic animals to indirectly determine the presence of *Helicobacter*[22]. Similarly, an *in situ* urease assay can be performed on an open stomach recovered at slaughter using colour change as an indication of the presence of urease-producing bacteria[23]. Briefly, 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) is spread over the entire mucosal surface of the stomach. When the urease produced by *Helicobacter* splits urea, ammonia is produced causing a pH rise and this liberates the phenol red indicator. Colour changes from yellow-orange to a deep pink in areas where urease is being produced. Generally, the reaction occurs within about 2 hours. There is substantial agreement between this *in situ* urease test and histological observations[12].

Because culturing has been unsuccessful, alternative means of growing the bacteria have been used. Inoculation of mice with porcine gastric material has been shown to be quite effective in demonstrating the presence of "*Candidatus H. suis*"[24]. In one study[25], mouse inoculation was compared to histologic examination of carbol-fuchsin-stained slides. Of 70 pig stomachs examined, 54 were positive using the mouse inoculation technique versus only 17 of the 70 stomachs examined by histology, and only 14 of 70 positive using a rapid urease test.

There is a need for serological tests that would enable rapid screening of pig herds for *Helicobacter* organisms and readily available PCR tests.

Clinical disease

There have been several epidemiological studies showing an association between the presence of *Helicobacter*-like organisms and ulceration of the *pars oesophagea*. Barbosa et al (1995)[26] examined 32 pigs with grossly normal mucosa and 32 pigs with chronic ulceration of the *pars oesophagea*. Forty pigs (62.5%) were positive for *Helicobacter* and of these positive animals, 67.5% had ulcers. Of the 24 negative pigs, only 20.8% had ulcers. Similarly, Queiroz et al. (1996)[25] examined 20 pig stomachs with ulcers, 30 stomachs with parakeratosis, and 20 normal stomachs. *Helicobacter* were present in 100% of

stomachs with ulcers and 90% of stomachs with parakeratosis but only 35% of macroscopically normal stomachs.

On the other hand, Melnichouck et al. (1999)[12] found no relationship between the presence of *Helicobacter* and stomach lesions. These researchers examined four herds with *Helicobacter* prevalence ranging from zero in an SPF herd to 87.5% in a herd using continuous pig flow and practicing floor feeding. Stomach lesions were highest for the herd where *Helicobacter* could not be demonstrated and lowest in the herd with 87.5% prevalence of *Helicobacter*.

French researchers[10] examined 10 pigs from six different farms and found positive animals from all farms, and an overall prevalence of 65% infection based on histology. Again these researchers did not find an association between the presence of *Helicobacter* and the occurrence of stomach lesions.

In all likelihood, the mechanism responsible for ulceration of the *pars oesophagea* in pigs is very different from the pathogenesis of the human peptic ulcer, considering the major differences in gastric anatomy and physiology between the two species. It is highly unlikely that the swine ulcer problem will be solved by eradicating *Helicobacter* from herds. However, there may be other reasons to create *Helicobacter*-free herds, other than to control ulcer problems. The potential of pig-to-human spread of *Helicobacter heilmannii* has been suggested[11,27]. A survey of 177 patients with *H. heilmannii* infection found that *H. heilmannii* infection was strongly associated with contact with dogs, cats, cattle, or pigs[28]. Contact with pigs was found to be a greater risk factor than contact with other animal species. People with pig contact are almost five times more likely to be infected with *H. heilmannii* than those without pig contact. More studies are needed to determine if *Candidatus H. suis* is a zoonotic disease and more studies are needed to examine the role these bacteria play in swine gastric disease.

Summary

- *Helicobacter*-like organisms are present in swine and are likely very widespread in the general pig population.
- The prevalence can likely be controlled by techniques such as all-in—all-out management and good sanitation procedures.
- Organisms can be detected by histology, but distribution of colonies is patchy and false negatives can occur.
- The association between the presence of *Helicobacter*-like bacteria and the severity of stomach lesions has not been satisfactorily established. The small number of studies performed to date contradict each other.
- Infecting gnotobiotic pigs with *Helicobacter* organisms has not produced lesions in the *pars oesophagea*.

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