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# Pathogenicity of Intestinal Spirochetes Associated with Porcine Colonic Spirochetosis

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### Summary and Implications

*We reported on a new species of intestinal spirochete bacterium, Serpulina pilosicoli, associated with a diarrheal disease of grower-finisher swine, called porcine colonic spirochetosis (PCS). In this report we show that Serpulina pilosicoli, associated with outbreaks of PCS in the United States, attached to the cecal surface of chicks while Serpulina innocens, a non-pathogenic intestinal spirochete, did not. These findings support a role for Serpulina pilosicoli as a cause of diarrhea and reduced feed efficiency in swine.*

### Introduction

Because 80 percent of the total feed cost is associated with the grower-finisher phase of pork production, improved feed efficiency during that period can generate significant cost savings and increase profits. It is known that approximately 76 percent of the basal energy requirement of the pig is derived from absorption of volatile fatty acids (VFA) by the large intestine. In fact, the concentration of VFA produced from microbial fermentation in the lower intestine of swine ranges between 180 and 200 mmoles/L, a range similar to that found in the rumen contents of cattle. In contrast to cattle where most of the VFA absorption takes place in the abomasum, VFA are absorbed from the large intestine of swine. The quantity of VFA absorbed from the pig's lower intestine depends primarily upon the surface area available.

Presently, all weakly β-hemolytic intestinal spirochetes (WBHIS) inhabiting the lower intestine of swine are assigned to the non-pathogenic spirochete species, *Serpulina innocens*. However, certain WBHIS are clearly associated with a non-fatal wasting diarrheal disease of growing swine, called porcine colonic spirochetosis (PCS). We found that the WBHIS associated with PCS are distinct but related to *Serpulina innocens* and we proposed the name *Serpulina pilosicoli* to describe these spirochete bacteria (Table 1). Comparative analyses of *Serpulina pilosicoli* isolated from swine in the United States, Canada, the United Kingdom, and Australia indicated the worldwide distribution of PCS. Because the newly identified *Serpulina pilosicoli* can attach and invade the wall of the lower intestine, we hypothesized that it has the potential to reduce feed efficiency by disrupting VFA absorption. In the present study, we examined several isolates of *Serpulina pilosicoli* for attachment to the surface of chicks' ceca. Attachment of *Serpulina pilosicoli* to the ceca of chicks is indicative of pathogenicity associated with reduced surface area available for absorption of nutrients.

### Materials and Methods

*Serpulina pilosicoli* were obtained from swine with clinical signs or lesions of PCS in Nebraska (n=2), Iowa (n=2), and California (n=3). After confirming the identity of *Serpulina pilosicoli*, using structural, biochemical, and genotypic analyses (Table 1), the spirochetes were compared with *Serpulina innocens* for attachment to the ceca of chicks. One-day-old chicks were inoculated by crop gavage with either sterile medium or medium containing either *Serpulina innocens* or *Serpulina pilosicoli*. On day 7, 14 and 21 post-inoculation, the ceca of control chicks and *Serpulina*-inoculated chicks were collected for bacteriologic and histopathologic examinations.

**Table 1. Differentiating features of *Serpulina* spp. isolated from the intestine of swine.**

| Characteristic | *S. hyodysenteriae* | *S. innocens* | *S. pilosicoli* |
|---|---|---|---|
| Hemolysis | Strong | Weak | Weak |
| Number of periplasmic flagella | 8 to 12 | 10 to 13 | 4 to 7 |
| Indole production | Positive | Negative | Negative |
| Hippurate hydrolysis | Negative | Negative | Positive |
| 16S rDNA PCR† | Negative | Negative | Positive |
| Associated hosts | Swine<br>Dogs<br>Mice<br>Birds<br>Guinea pigs | Swine<br>Dogs | Swine<br>Dogs<br>Mice<br>Guinea pigs<br>Non-human primates<br>Humans<br>Birds |
| Associated condition | Swine dysentery | Not pathogenic | Colonic spirochetosis |

†PCR = polymerase chain reaction.

**Table 2. Attachment of spirochetes to the ceca of chicks challenge-exposed with porcine weakly β-hemolytic intestinal spirochetes.**

| Isolate | Number of isolates tested | Days post-inoculation† | | | |
|---|---|---|---|---|---|
| | | 7 | 14 | 21 | Total |
| *Serpulina pilosicoli* | 7 | 13/20 | 10/13 | 8/9 | 31/42 |
| Infection rate (%) | | 65 | 77 | 89 | 74 |
| *Serpulina innocens* | 1 | 0/3 | 0/5 | 0/6 | 0/14 |
| Infection rate (%) | | 0 | 0 | 0 | 0 |
| Sterile medium | Not applicable | 0/7 | 0/3 | 0/6 | 0/16 |

†Number positive for attachment/number challenge-exposed, as determined by histologic examination.

## Results

Grossly, the ceca of chicks inoculated with either sterile medium or medium containing either *Serpulina innocens* or *Serpulina pilosicoli* had no notable changes; the cecal contents were yellowish brown, foamy and semisolid. Histologically the ceca of chicks inoculated with sterile medium or medium containing *Serpulina innocens* had tall columnar epithelium without spirochetes. In contrast, the ceca of chicks inoculated *Serpulina pilosicoli* had spirochetes attached along the surface (Table 2). The infection rate of the *Serpulina pilosicoli*-inoculated chicks increased from 65 percent after 7 days, to 77 percent after 14 days and 89 percent after 21 days. No spirochetes were found by culture of ceca from chicks given either sterile medium or medium containing *Serpulina innocens* at any time post-inoculation. In contrast, large numbers of WBHIS were isolated from the ceca of chicks challenge-exposed with *Serpulina pilosicoli*.

## Discussion

Detailed phenotypic and genotypic characterization of intestinal spirochetes has lead to major advances in our understanding of the molecular epidemiology of spirochetal diarrhea in humans and animals. Complete agreement was found between structural, biochemical, and genotypic analyses and the results of attachment to the ceca of chicks. On the bases of these results, we concluded that *Serpulina pilosicoli* has the potential to cause reduced surface area for absorption of nutrients from the gut lumen. We found that the infection rates of chicks increased over time, such that it took 21 days to reach 89 percent of the chicks with spirochetes attached to the surface of the ceca.

Porcine colonic spirochetosis is characterized clinically by watery to mucoid diarrhea without blood, or so called "cow-pie scours". Although diarrhea can affect up to 20 percent of swine in the grower-finisher phase of production, depression of weight gain is the most significant finding with PCS and this can result in a significant delay in reaching market weight. Additional animal care is a major problem associated with PCS in all in/all out management systems because of the uneven sizes of the pigs causing disruption of pig flow.

The pathologic changes in chicks inoculated with *Serpulina pilosicoli* were similar to those present in the early stages of colonic spirochetosis in humans, swine and other animals. In swine, the large intestine contains abundant watery-green or yellow mucoid materials and variable degree of exudation and surface erosions are sometimes visible. Early in the infection spirochetes attach by one of their ends along the surface of the lower intestine producing what appears as a dark fringe when examined histologically. The loss of surface area in the large intestine of pigs accounts for the reduced feed efficiency and increased numbers of days to market. Over longer periods of time, infection with *Serpulina pilosicoli* causes inflammation of the wall of the lower intestine, a lesion referred to as "non-specific colitis". Based on the changes observed in the ceca of the chicks, this change must take longer than 21 days to develop.

Because of the diversity of WBHIS normally present in the intestinal tract of swine and the widespread occurrence of non-pathogenic WBHIS, isolation of WBHIS from swine is only suggestive of PCS. Demonstration of spirochetal attachment to the surface of the lower intestine confirms the diagnosis of PCS; however, necropsy of pigs is not routinely performed in uncomplicated cases of PCS. Assessment of several laboratory procedures for rapid and accurate identification of *Serpulina pilosicoli* indicates that a preliminary identification of the spirochete can be made on the basis of positive hydrolysis of hippurate. Definitive identification of *Serpulina pilosicoli* requires amplification of a specific 16S rDNA gene sequence by polymerase chain reaction. Why is it then that *Serpulina pilosicoli* is only reported sporadically as a cause of diarrhea and colitis in swine? This may be attributable to either *(1)* failure to use appropriate techniques for primary isolation of the spirochete from intestinal specimens, *(2)* failure to identify the spirochete from diagnostic specimens, or *(3)* failure to identify lesions of PCS in tissues submitted for diagnostic evaluation. *Serpulina pilosicoli* also has been isolated from stool samples obtained from children with diarrhea in developing countries and from immunocompromized adult individuals infected with the human immunodeficiency virus in more developed countries. Because *Serpulina pilosicoli* are isolated from humans and swine affected with PCS, it raises the possibility that these spirochetes may be zoonotic and have a public health significance.

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