Colonic Infection by Bilophila wadsworthia in Pigs

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Bilophila wadsworthia is a common inhabitant of the human colon and has been associated with appendicitis and other local sites of inflammation in humans. Challenge-exposure or prevalence studies in laboratory and other animals have not been reported. B. wadsworthia is closely related phylogenetically to Desulfovibrio sp. and Lawsonia intracellularis, which are considered colon pathogens. We developed a PCR specific for B. wadsworthia DNA. Samples of bacterial DNA extracted from the feces of pigs on six farms in Australia and four farms in Venezuela were examined. Specific DNA of B. wadsworthia was detected in the feces of 58 of 161 Australian and 2 of 45 Venezuelan pigs, results comprising 100% of the neonatal pigs, 15% of the weaned grower pigs, and 27% of the adult sows tested. Single-stranded conformational polymorphism analysis of PCR product DNA derived from pigs or from known human strains showed an identical pattern. Histologic examination of the intestines of weaned B. wadsworthia-positive pigs found no or minor specific lesions in the small and large intestines, respectively. B. wadsworthia is apparently a common infection in neonatal pigs, but its prevalence decreases after weaning. The possible role of B. wadsworthia as an infection in animals and in human colonos requires further study.

Bilophila wadsworthia is a slow-growing, asaccharolytic, and obligately anaerobic bacillus, making it somewhat difficult for routine culture and identification (1, 2). It has been cultured from the colon or feces of 50 to 60% of healthy adult humans, but generally in low numbers (ca. 10^3 to 10^6 CFU/g [wet weight]) (1, 2). It has been strongly associated with pathogenic infections of intra-abdominal sites, such as appendicitis and cholecystitis (4), as well as extra-intestinal sites, such as otitis (10, 23). However, challenge-exposure studies in laboratory animals have not been reported. Endotoxin and procoagulant activities have been identified in B. wadsworthia (15), and an in vitro study suggested that it may be able to attach to epithelial cells of the colon (8). Separate subgroups or strains of B. wadsworthia have been indicated by DNA fingerprinting studies (9). The possible isolation of B. wadsworthia from healthy or diseased nonhuman hosts has not previously been reported. Molecular studies, such as the identification of possible invasion or attachment genes or receptors, have not been reported.

Pigs are widely used as an animal model in comparative dietary and other biomedical studies. Healthy weaned pigs have a typical complex bacterial flora in their colon, including a remarkable variety of anaerobes (18, 19, 20). Pigs with enteric diseases can develop alterations in this flora, with elevations of primary enteropathogens, such as Brachyspira hyodysenteriae or Lawsonia intracellularis, followed by elevations in the levels of other bacteria of non or doubtful pathogenicity, such as Acetivibrio ethanoligenes or Campylobacter mucosalis, respectively (12, 19). The mere association of an organism with enteric disease is therefore not proof of its pathogenicity. B. wadsworthia is a member of the Desulfovibrionaceae and is closely related (>90% 16S ribosomal DNA [rDNA] sequence homology) to both Desulfovibrio sp. and L. intracellularis. The latter organism has been isolated from the intestines of a wide variety of host species, particularly pigs, rabbits, and hamsters (6, 13) and has recently been identified in rhesus macaque monkeys (10). It causes marked proliferation of immature epithelial cells in the intestinal epithelium in the colon or small intestine of infected animals (6, 14).

The aims of this study were to establish the prevalence of B. wadsworthia infection in healthy pigs in various age groups and to compare these organisms to human isolates.

MATERIALS AND METHODS

Pig feces samples. Feces were collected from pigs in six convenience-selected farms in Australia and four such farms in Venezuela (Table 1). The diet and housing of the pigs in both countries were similar; pigs were fed commercial meal diets of cereal base with added soybeans and vitamins. The farms were commercial enterprises using standard husbandry practices. The numbers of samples per age group are given in Table 1. The clinical features of all -positive pigs found no or minor specific lesions in the small and large intestines, respectively. B. wadsworthia is apparently a common infection in neonatal pigs, but its prevalence decreases after weaning. The possible role of B. wadsworthia as an infection in animals and in human colonos requires further study.

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**RESULTS**

*B. wadsworthia* DNA identification. Eubacterial DNA was identified in DNA extractions from each fecal specimen in this study. The identification of *B. wadsworthia* DNA in the feces of pigs is given in Table 1. No specific clinical signs were noted in infected pigs.

The SSCP reactions of the PCR products of *B. wadsworthia* DNA from pigs and control bacteria of human origin are shown in Fig. 1. PCR products from DNA extracted from pig feces showed an SSCP pattern similar to that of the human control bacterium, thus indicating species identity.

**TABLE 1. Identification of *B. wadsworthia* DNA in pig feces**

<table>
<thead>
<tr>
<th>Farm Location</th>
<th>No. of PCR-positive pigs/ no. of pigs tested</th>
</tr>
</thead>
<tbody>
<tr>
<td>SA, Vic, NSW</td>
<td></td>
</tr>
<tr>
<td>1 SA</td>
<td>NT</td>
</tr>
<tr>
<td>2 SA</td>
<td>NT</td>
</tr>
<tr>
<td>3 SA</td>
<td>10/10</td>
</tr>
<tr>
<td>4 Vic</td>
<td>NT</td>
</tr>
<tr>
<td>5 NSW</td>
<td>10/10</td>
</tr>
<tr>
<td>6 NSW</td>
<td>10/10</td>
</tr>
<tr>
<td>7 to 10 Venezuela</td>
<td>NT</td>
</tr>
<tr>
<td>Total (%)</td>
<td>30/30 (100)</td>
</tr>
</tbody>
</table>

DISCUSSION

We have established, by DNA detection techniques, that *B. wadsworthia* is harbored in the pig. This is the first record of this organism in nonhuman sources. It is apparently a common infection in neonatal pigs, being detected in 30 piglets tested from three farms. The source of this infection is likely to be the vagina, teats, or skin of the mothers of the piglets, which were contaminated with feces, but clear transmission data are lacking. The prevalence of the infection was reduced after the pigs were weaned onto solid food, with fewer adult pigs being found positive. However, we did demonstrate the organism in several Australian and in two Venezuelan pig farms, indicating that infection was not restricted to a single continent. We had no data to explain the variation in carriage between different pig farms. It is possible that variations in diet components may have been responsible. It is also possible that the cereal-based diet of weaned pigs or the milk-based diet of neonatal pigs acts to reduce or promote the carriage of *B. wadsworthia*, respectively, but there is no direct evidence of this. The stable microflora in adults may also act to reduce the carriage of this organism. Although the diet, gut length, and colonic flora of pigs are similar to those of humans, we have found no similar studies of the age-based prevalence of *B. wadsworthia* in humans and so cannot extrapolate our results further. It is also possible that humans, other animals, or environmental sources play a larger role in transmission. There is a lack of data on other sources of *B. wadsworthia*; it may be a common infection in many young animals.

The histologic and bacteriologic examination of positive colonies did not indicate that *B. wadsworthia* was a primary cause

![FIG. 1. SSCP reactions of nested-PCR products from *B. wadsworthia* DNA from human and porcine sources. SSCP patterns from pigs 3, 15, and 25 from farm 1 (lanes 1 to 3), pigs 1 and 13 from farm 2 (lanes 4 to 5), suckling pigs 1 and 3 from farm 6 (lanes 6 to 7), grower pig 7 and breeder pig 5 from farm 6 (lanes 8 to 9), and a human cultured strain of *B. wadsworthia* (ATCC 51580) (lane 10).](image-url)
of significant lesions in the pigs examined. Pigs are widely used models of gastroenteric pathology in humans, including models of bacterial infections (11); however, the limited nature of the investigation does not allow the exclusion of \textit{B. wadsworthia} as an enteric pathogen in either species. It is possible that \textit{B. wadsworthia} is part of the normal bacterial flora of both species but that it may play a pathogenic role when in high numbers in an enteric site or in an abnormal site. The use of animal models involving \textit{B. wadsworthia} as a secondary agent may help to resolve this pathogenesis, particularly in relation to dietary changes.

The present study further indicates that extraction of DNA from feces and its use in specific PCRs can give valuable noninvasive information on enteric organisms. The use of SSCP analysis was considered a robust and straightforward method to show identity of PCR products to control DNA material. The SSCP results indicate the validity of the PCR DNA product results in this study of fecal bacteria. Studies of other complex microbial communities, such as soil and compost, have also indicated that SSCP techniques offer clear validation of PCR product DNA for bacterial identity (17, 22).

**REFERENCES**