Extensive Colonization of the Porcine Colonic Epithelium by a Spirochete Similar to *Treponema innocens*

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Specimens of colonic mucosa from two pigs with diarrhea were examined by light and electron microscopy. The epithelial surfaces of both pigs were extensively colonized by large spirochetes morphologically compatible with *Treponema hyodysenteriae* or *Treponema innocens*. The microorganisms were intimately attached end-on to the luminal cells. A weakly beta-hemolytic spirochete similar to *T. innocens* was isolated from the colon of one of the pigs.

Swine dysentery is a mucohemorrhagic diarrheal disease caused by *Treponema hyodysenteriae*. The disease is characterized by inflammation and necrosis of the large intestinal mucosa with excess mucus production. To date, most investigations have examined lesions (1, 4, 5, 11, 16) and colonization (12) of the colonic mucosa following experimentally induced swine dysentery. *Treponema innocens* is considered part of the normal flora of swine (13). This spirochete is morphologically identical to *T. hyodysenteriae* and can be differentiated from the pathogenic species by hemolysin production. In this paper, we report the extensive colonization of the colonic mucosa of a pig naturally infected with a spirochete similar to *T. innocens*.

Two 5-week-old pigs (individuals A and B) from two local herds, with clinical signs of diarrhea, pneumonia, and decreased growth rate after weaning, were submitted to the Diagnostic Laboratory, Faculté de Médecine Vétérinaire, St-Hyacinthe, Canada, for euthanasia and necropsy. They had been treated with different antibiotics without resolution of the problem. At necropsy, the two pigs were emaciated. Lesions were limited to the digestive system. The colon of individual A contained clear liquid material. The colon contents of individual B were liquid, although no other gross lesions were observed.

Histological examination of Formalin-fixed large intestines from both pigs, sectioned at 6 μm and stained with hematoyxlin-phloxin-safran, revealed lesions compatible with porcine dysentery. The intestinal lumen contained mucus, necrotic cells, bacterial colonies, and a small number of *Balantidium coli*. Multifocal erosions of the mucosa were observed. The colonic glands were mildly dilated by mucus, with mild hyperplasia of goblet cells. The lamina propria contained a mild, diffuse, mononuclear infiltrate. A large number of gram-negative spirochetes were intimately attached to the superficial epithelium, giving a brush border, ciliated appearance to these cells (Fig. 1A). Similar bacteria were observed in the cytoplasm of the glandular epithelium after Warthin-Starry staining.

Glutaraldehyde-fixed intestinal segments were prepared for both scanning and transmission electron microscopy as previously described (3, 10). Scanning electron microscopic examination revealed large spirochetes on the epithelial surfaces of both individuals (Fig. 1B and 2A). The colon segments were so heavily colonized by spirochetes that the underlying mucosal surface was scarcely discernible. The form and size of these spirochetes were compatible with *T. hyodysenteriae* or *T. innocens* (6-8, 13). Each organism, with a serpentine form, was approximately 0.2 to 0.3 μm in diameter and 7 to 8 μm long. Transmission electron microscopic examination of colon segments from both individuals demonstrated many spirochetes intimately attached to the apical cell membrane of enterocytes which were devoid of microvilli (Fig. 1C and 2B). We observed polar attachment of the spirochetes (brush border aspect) to the porcine colonic epithelium. Periplasmatic flagella were clearly seen (Fig. 1D). *T. hyodysenteriae* and *T. innocens* cells are known to possess seven to nine periplasmatic flagella which are inserted into each of the cell poles and overlap near the middle of the cell (7, 13).

On examination of a Gram-stained direct smear of the colonic mucosa from individual A, 15 to 20 spirochetes were observed per microscopic field. An attempt to cultivate the organism on a selective medium (tryptic soy agar with 200 μg of spectinomycin per ml) was successful, and a weakly beta-hemolytic spirochete similar to *T. innocens* was isolated. Unfortunately, the colon from individual B was not submitted for bacteriological examination.

The pathogenesis of swine dysentery is associated with the proliferation of *T. hyodysenteriae* in the colon and its penetration of the colonic mucosa (6). Spirochetes are found in the lumen and crypts of the colon at all stages of the disease but are most numerous in the acute phase. They invade epithelial cells, goblet cells, and occasionally the lamina propria. Electron microscopic examination of segments of colonic mucosa from swine with experimentally induced dysentery has demonstrated spirochetes in close proximity, but not visibly attached, to the luminal surface or massed in the crypts (5-7). Albassam et al. (1) found large numbers of unattached spirochetes, either in place or partially extruded, between and surrounding necrotic epithelial cells. Knoop et al. (14) observed several types of treponemal attachment to isolated swine intestinal epithelial cells in vitro. *T. innocens* has been primarily considered a normal inhabitant of the swine colon, without any defined pathogenicity (13). However, some authors have reported lesions of a mild, mucoid colitis associated with a weakly beta-hemolytic treponeme (2). A case similar to ours, involving a

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FIG. 1. (A) Superficial colonic mucosa of pig B, showing numerous spirochetes intimately attached to the surface of the enterocytes (Warthin-Starry stain; magnification, ×180). (B) Scanning electron micrograph of a critical-point-dried preparation of the colonic mucosa of pig B, showing extensive colonization of the mucosal surface by large spirochetes. (C and D) Transmission electron micrographs of thin sections of the same preparation (bars = 1 μm). Numerous spirochetes are attached end-on to the luminal cells (C). At higher magnification (D), periplasmic flagella (arrow) and intimate contact between the bacterial cells and the mucosal surface are seen.
weakly beta-hemolytic spirochete, was recently reported by Spearman et al. (15).

This study demonstrated an extensive colonization by a treponeme similar to *T. innocens* and showed this bacterium to be intimately attached end-on to colonic luminal cells during the course of a natural infection in swine. In humans, cases of massive infestation of the colonic epithelium with similar organisms have been reported (9).

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**LITERATURE CITED**