NOTES

Isolation and Propagation of Spirochetes from the Colon of Swine Dysentery Affected Pigs

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RÉSUMÉ

Les auteurs ont isolé du côlon des porcs atteints de dysentérie aiguë des spirochètes anaérobies de petite et de grande dimensions, en culture pure. D'après l'examen au microscope électronique, les plus gros spirochètes ressemblaient à ceux que certains rapports ont déjà mentionnés comme envahisseurs des cellules epitheliales du colon, au tout début des lésions de dysenterie porcine.

The anaerobic spirochetes, which are commonly present in the large intestines of man and animals, remain poorly understood because of difficulties of *in vitro* isolation and cultivation. Spirochetes have been isolated from the ceca of mice (3), but no consistent attempt has been made to isolate and identify these organisms from the large intestines of other animals. In the past three years, spirochetes have been associated with the lesions of swine dysentery (1, 5, 6, 8) but reportedly have been grown only in symbiotic culture systems (4, 7). Four strains of a small spirochete and four strains of a large spirochete which were from the colons of pigs affected with swine dysentery have been isolated and propagated in the following manner.

The spirochetes were isolated from four pigs submitted to the Iowa Veterinary Diagnostic Laboratory, Ames, Iowa. Each pig originated from a different herd and ranged in age from eight to 14 weeks. All pigs had mucohemorrhagic enteritis confined to the large intestine. Microscopic lesions in the colon included congestion and edema of the submucosa and mucosa. Musocal crypts were distended with mucus. A mucofibrinous pseudomembrane covered a shallow layer of necrotic tissue at the luminal surface of the mucosa in most sections examined.

A 30 cm section of the colon from each pig was opened longitudinally. The epithelium was removed with the edge of a microscope slide, placed in a mortar and emulsified with a pestle in phosphate buffered saline, 0.01M, pH 7.4 (PBS) at a dilution of 1:4. The emulsant was centrifuged for ten minutes at 60 x g at 4°C. The supernatant fluid was passed through an epoxy glass membrane filter with an average pore diameter (APD) of 5.0u at 10 pounds per square inch (psi) pressure. The filtrate was then passed

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through a series of cellulose acetate membrane filters with an APD of 8.0u, 5.0u, 3.0u, 1.2u, 0.8u, 0.65u and 0.45u at 10 psi pressure. Passage of the supernatant through this series of filters was necessary to facilitate passage through the 0.45u filter and to insure the presence of the spirochetes in the filtrate.

Phase microscopic observation of this material revealed the presence of organisms resembling *Vibrio* spp. and two types of spirochetes which differed morphologically. The spirochetes were motile and helically coiled. The large spirochete was loosely coiled while the small spirochete was tightly coiled.

Four 10-fold dilutions of the filtrate were made in PBS. For isolation of the small spirochete, two-tenths ml of each dilution was inoculated into pre-reduced, anaerobically sterilized (PRAS) E-agar in roll tubes (2) under deoxygenated carbon dioxide. The roll tubes were incubated at 37°C for five to eight days. For isolation of the large spirochete, two-tenths ml of each dilution was placed into sterile petri dishes. Tryptose agar base containing 5% citrated bovine blood (TBA) at 45°C was poured into the petri dishes. The TBA was allowed to solidify and the petri dishes were placed in an anaerobic container[1] and incubated at 37°C for five to ten days. The *Vibrio* spp. grew in both PRAS E-agar and TBA but the dilution in PBS of the filtrate material or spirochete colonies resulted in the separation of the Vibrio from the spirochetes.

The small spirochete grew initially as a small pinpoint colony which gradually spread concentrically. Colonies of the small spirochete were picked and placed in PRAS E-broth medium (2) under deoxygenated CO_2. The organism grew readily in PRAS E-broth and could also be adapted to grow in PRAS peptone-yeast extract broth (2) containing inactivated swine serum under deoxygenated nitrogen. Preliminary electron microscopic observations (Fig. 1) revealed the presence of either one or two axial fibrils originating from each end of the protoplasmic cylinder. The axial fibrils overlap in the center of the protoplasmic cylinder in a "1-2-1" or "2-4-2" arrangement and the cell diameter was 0.24 to 0.30u. The small spirochetes resemble the "immobile spirochete" (type b) as described by Taylor (4).

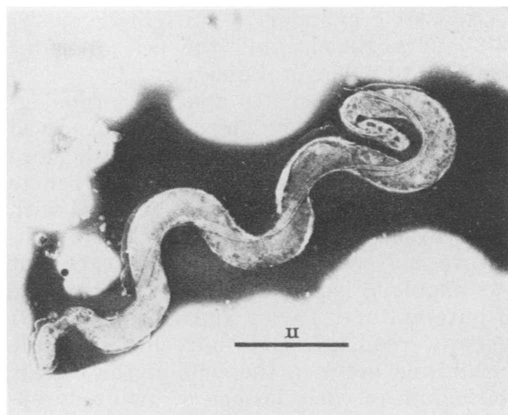

Fig. 1. Electron micrograph of small spirochete with axial fibrils in 2-4-2 arrangement. Phosphotungstic acid stain. X14,000.

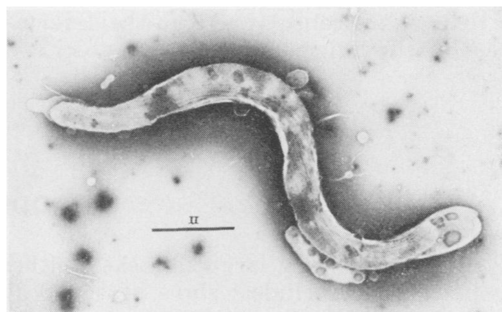

Fig. 2. Electron micrograph of large spirochete with axial fibrils in 7-14-7 arrangement. Phosphotungstic acid stain. X10,000.

The large spirochete caused hemolytic zones in the TBA and occasionally small white colonies were present in these zones. Phase microscopic observations of these hemolytic zones revealed the presence of large numbers of the organism. Attempts to grow the large spirochete in tryptose broth medium have failed. Electron microscopic observations of the large spirochete organism revealed the presence of seven to nine axial fibrils originating from each end of the protoplasmic cylinder (Fig. 2). The axial fibrils overlap in the center of the

[1]Gas Pak Anaerobic Jar, BBL, Division of Bioquest, P.O. Box 175, Cockeysville, Maryland, 21030, U.S.A.

protoplasmic cylinder in a "7-14-7" or "9-18-9" arrangement and the cell diameter was 0.29 to 0.38u in diameter.

The large spirochetes appear morphologically to resemble the spirochetes observed by Taylor (4) and Taylor and Blakemore (5). Taylor (4) reported that a spirochete (type a) was seen only in pigs affected with swine dysentery. He also was able to subculture spirochetes in mixed culture but was unable to isolate any type of spirochete in pure culture. Taylor and Blakemore (5) recently reported that spirochetes invade within and between the cells of the colonic epithelium in early lesions of swine dysentery. These spirochetes were 0.35 to 0.37u in diameter and most contained 13 axial fibrils.

The isolation and propagation in pure culture of both the small and large spirochetes should facilitate the determination of their significance, if any, in the development of the lesions of swine dysentery.

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ADDENDUM

Inoculation of a large spirochete either alone or in combination with other infectious agents produced clinical signs of swine dysentery in experimental pigs. *See* Vet. Med. small Anim. Clin. January 1972.