Electron Microscopy of Clostridium perfringens in the Intestine of Neonatal Pigs with Necrotic Enteritis

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ABSTRACT. Electron microscopy on the necrotic enteritis of newborn pigs caused by Clostridium perfringens revealed many bacilli in and around the necrotic villi and endospore formation in the intestine of the diseased piglets. The spores were characterized by the thick cortex and radiating outer spore coat.—KEY WORDS: Clostridium perfringens, necrotic enteritis.

Necrotizing or necrotic enteritis of newborn pigs caused by Clostridium (C1.) perfringens has been reported in England [6], Hungary [12], the United States [4], Denmark [9] and Japan [3, 14]. However, no electron microscopic studies have been made on C1. perfringens in the intestine of the piglets affected with this disease. Recently, necrotic enteritis occurred in newborn pigs in Kagoshima Prefecture. This paper describes the light and electron microscopic findings of the animals, with special reference to the ultrastructure of C1. perfringens.

During January and February, 1983, 70% of piglets farrowed by 20 sows died within 2 days after birth and the rest of them died within 5 days on a farm. Two moribund (autopsy Nos. 3412 and 3413) and 4 dead (autopsy Nos. 3414-3417) piglets were submitted and autopsied 1.5 hours after death. The bacteria isolated from the intestine of the 6 piglets were Gram-positive and anaerobic, and identified as C1. perfringens from biochemical properties [11], but the serotype was uncertain. Grossly, 1 moribund and all dead piglets showed almost the same lesions which consisted of red or black coloration in the mucous membrane with yellow thin pseudomembrane and edematous thickening of the wall, some with scattered bubbles on the serous membrane in the lower jejunum and upper ileum. One dead piglet (autopsy No. 3417) showed peritonitis with ascites. No gross lesions were seen in the organs other than the digestive tract.

In February, 1984, the same disease broke out on the same farm, and 80% of piglets farrowed by 5 sows died. One dead piglet (autopsy No. 4023) was pathologically examined, in which no bacteriological study was done. Grossly, the same lesions were observed as those seen in the piglets of previous outbreak.

Specimens of major organs were fixed phosphate buffered formalin and routinely processed to paraffin wax and sections were stained with hematoxylin and eosin. Selected sections from the intestine were stained with MacCullum-Goodpasture staining.

Histologically, lesions in the dead animals were principally found in the jejunum and ileum, and consisted of necrosis of the mucosa, hemorrhage, hyperemia and edema in the submucosa, and frequent vacuoles with gas in the wall (Fig. 1). The necrotic area were surrounded with macrophages and a few neutrophils. A number of large, rod, Gram-positive and sometimes Gram-negative bacilli
were attached to the necrotic villi. Some areas of the mucous membrane in the colon also were necrotic.

Numerous Gram-positive bacilli were observed in the intestinal content of all 7 piglets. Neutrophil infiltration was seen in the mesenteric lymph nodes of all cases. There was no lesion in the organs other than digestive tract.

Necrotic and intact villi were mixed in one moribund case (autopsy No. 3413) (Fig. 2), in which large rod bacilli were observed more numerous in the lamina propria rather than around the villi as seen in the dead ones and the same changes in the submucosa as seen in the dead ones were associated, though they were mild. In the other moribund case (autopsy No. 3412), there was no lesion such as necrosis of villi and hemorrhage and edema in the submucosa.

For electron microscopy, the formalin-fixed tissues from the jejunum of 2 dead piglets (autopsy Nos. 3414, 4023) were postfixed with 1% osmium tetroxide and sections were stained with uranyl acetate and lead citrate.

A number of bacilli were seen in and around the necrotic villi. The bacilli had two different cell walls, smooth electron-dense and undulating tri-laminar (Fig. 3). The bacilli with smooth electron-dense cell wall were 0.7~0.9 μm in diameter. Some of them had spores in their cytoplasm and the sporangium was 0.8~1.5 μm. Various stages of spore formation were observed (Figs. 3, 5). Firstly the core wall was formed and the nucleus increased in density, but the spore
Fig. 3–6. Electron microscopy of the bacteria seen in the jejunum of piglets.

Fig. 3. A bacillus with a spore. The nucleus (N) of the spore is surrounded by a core wall (CW) and a thin cortex (CX). An electron-dense inner spore coat (IC) is being formed. Two bacilli with undulating trilaminar cell wall are seen in left side of photograph. Autopsy No. 4023. ×36,000.

cortex was not discernible (Fig. 3). After that, the spore cortex, being electron-opaque, increased in thickness, the electron-dense inner spore coat, sometimes with electron-dense granular material, was formed, and the outer spore coat with characteristic radiating structures were observed (Figs. 4, 5). The cell wall were frequently surrounded by fine flocculent materials (Fig. 5).

Bacteria with undulating tri-laminar cell wall had rhabdosesomes in the center and ribosomes at the periphery. Bacteriophages were found in some bacteria (Fig. 6). They were hexagonal in shape, 0.11 μm in diameter, and had an electron-dense core in the center and a sheath at one side. Mature bacteriophages were also found near the punctured bacteria.

Light microscopical studies having been done so far did not suggest the existence of spores in the intestine of piglets [2–4, 6, 9, 12, 14], however, present electron microscopic study revealed that spores of *C. perfringens* were already formed in the intestine. Mature spores of *C. perfringens* had structures in the following order from the inner side: (1) nucleus or core, (2) core wall, (3) cortex, (4) inner spore coat, and (5) outer spore coat. These structures were basically the same as those having been reported [1, 5, 7, 8, 13]. Mature spores were formed in 7 to 12 hours in *C. perfringens* cultured *in vitro*, and remained in the sporangium [10]. Present electron microscopic study confirmed
that spores remained in the sporangium, and revealed that the membrane described as the cell wall [10] was composed of inner and outer spore coats.

Electron microscopic studies have been done on sporulation of *Clostridium pectinovorum* [7], *Clostridium butyricum* [13], *Clostridium botulinum* [13], Bacillus (B.) *megaterium* [5] and *B. cereus* [5]. The outer spore coat of *Clostridium pectinovorum* had lamellar structure [7]. *Clostridium butyricum* and *Clostridium botulinum* had the outer spore coat of three layered structures [13]. The spore of *Clostridium sporogenes* had thin cortex and the outer spore coat without radiating structure [8]. The outer spore coat of the spore of *B. megaterium* and *B. cereus* was lack of radiating structure [5]. The spore of *Clostridium perfringens* had thick cortex and outer spore coat with radiating structure. These features made it possible to distinguish the spore of *Clostridium perfringens* from those of other bacteria.

In one moribund case (autopsy No. 3413), the bacilli were observed in the lamina propria rather than around the villi. The significance of this finding is uncertain. But it is possible that the bacilli invade and proliferate at first in the lamina propria and then provoked villous necrosis.

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REFERENCES


要約

新生豚の壊死性腸炎でみられた *Clostridium perfringens* の電顕像（短報）：久保正法・渡瀬 弘（家畜衛生試験場九州支場）——1983年2-3月と1984年2月に鹿児島県で発生した新生豚のクロストリジウム感染症について光顕および電顕的に観察した。組織学的には小腸経毛は壊死に際し、その内外へ多数のグラム陽性菌がみられた。電顕的には、グラム陽性菌は特徴的な細胞壁をもち、細胞質内の芽胞では厚い皮質と放射状構造をした外芽胞膜が特徴的であった。