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SWINE DYSENTERY: A SCANNING ELECTRON MICROSCOPIC INVESTIGATION

By

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TEIGE jr., JON, THOR LANDSVERK, ARVE LUND and HANS JØRGEN LARSEN: *Swine dysentery: a scanning electron microscopic investigation.* Acta vet. scand. 1981, 22, 218—225. — An investigation by scanning electron microscope was performed on colonic specimens from 4 conventionally reared pigs, 2 of which were suffering from swine dysentery (SD) after inoculation with Treponema hyodysenteriae in pure culture. In pigs with SD dilated cryptal openings surrounded by uneven mucosa with furrows and ridgeformations were found in areas with scattered pseudomembranes. The treponemes were numerous on the mucosal surface but seemed only to penetrate damaged epithelial cells. Freeze-fractured sections from the same pigs revealed dilated crypts where the microvilli were short, irregular and markedly reduced in number possibly due to change in cell kinetics. The present study seems to indicate that epithelial attachment and penetration of treponemes are of minor importance in SD pathogenesis.

swine dysentery; scanning electron microscopy; pseudomembraneous colitis; Treponema hyodysenteriae.

Swine dysentery (SD) is an acute-to-chronic disease which primarily affects weaned pigs and which is characterized clinically by diarrhea and pathomorphologically by a pseudomembraneous caecocolitis. For many years the etiology of SD was obscure. Studies during the last 10 years, however, have revealed that Treponema hyodysenteriae, is the primary etiological agent in this enteritic disorder (*Whipp et al.* 1979). This discovery has promoted interest in other aspects of the disease, recent studies include descriptions of pathomorphological lesions based on light and transmission electron microscopy, aspects of patho-

genetic and immunological mechanisms and influence of dietary factors (*Hughes et al.* 1975, 1977, *Schleicher* 1977, *Teige et al.* 1977, 1978, *Joens et al.* 1979, *Wilcock & Olander* 1979 a, b).

Two scanning electron microscopic (SEM) studies dealing with the lesions on the mucosal surface have so far been published (*Kennedy et al.* 1973, *Kennedy & Strafuss* 1976). The present report includes a description of SEM findings relating to surface epithelium and to freeze-fractured crypts in the colonic mucosa and, on the basis of the results, discusses some pathogenetic mechanisms in SD.

MATERIAL AND METHODS

Four conventionally reared pigs (Nos. 1—4), each weighing approximately 24 kg were used in this study. They originated from a herd without previous outbreaks of SD, fecal samples from which had shown no occurrence of Salmonella spp. Prior to inoculation rectal swabs were collected from the 4 animals and examined for the strongly hemolytic T. hyodysenteriae with negative result. The pigs were starved for 24 h and then each was fed contents of 10 petri dishes containing a pure culture of either T. hyodysenteriae (Pigs Nos. 1—3) or weakly beta-hemolytic treponemes (Pig. No. 4) on the TSA - S 400 medium (*Songer et al.* 1976), together with a small amount of phosphate-buffered saline. Pigs Nos. 1 and 2 developed watery bloodstained stools 17 days after inoculation and they were killed when this clinical condition had lasted for 2 days. The other 2 pigs which had no signs of SD were killed 62 days after inoculation. They served as controls. The specimens for the SEM investigation were collected under general anesthesia with pentobarbital just before killing. They were taken from the upper end of colon and from the apex of the spiral colon. The specimens were immediately fixed either in 2.8 % glutaraldehyde and 0.9 % paraformaldehyde containing 0.19 mol/l cacodylate buffer, or in a fixative containing 0.45 % glutaraldehyde and 0.35 % paraformaldehyde in 0.12 mol/l cacodylate buffer.

Specimens for freeze fracture were dehydrated in ethanol and placed in gelatin capsules containing ethanol. These capsules were submerged and fractured in liquid nitrogen (*Humphreys et al.* 1978). The tissue fragments were thawed in ethanol, equilibrated in acetone and critical point dried with carbon dioxide

as the transitional fluid. The dried specimens were attached to metal stubs with colloidal silver and coated with gold in a vacuum evaporator. The coated specimens were examined with a Jeol 50 A scanning electron microscope with an accelerating voltage of 5—10 kV. Photographs were recorded on Polaroid Type 52 film. Colonic sections for light microscopy were fixed in 10 % neutral buffered formalin, embedded in paraffin, sectioned at about 5 microns and stained with haematoxylin and eosin and by the Martius scarlet blue (MSB) mehtods (*Lendrum et al.* 1962). The selective medium of *Songer et al.* was used for bacteriological examination of colonic samples.

RESULTS

Macro- and light microscopical examinations revealed normal colonic mucosa in the control pigs. The animals with SD were somewhat emaciated and exhibited oedematous hyperemic mucosa and mucohemorrhagic colon contents. In pig No. 2 scattered pseudomembranes were found, these were especially numerous towards the apex of the spiral colon. The main findings by light microscopy were increased length and dilation of the colonic crypts, cellinfiltrations and hyperemia in the lamina propria, a decreased number of goblet cells and small pseudomembranes on the mucosal surface. The MSB-method revealed few red-stained hyaline thrombi in capillaries beneath these membranes.

The SEM-examination in the control pigs showed a relatively smooth, slightly undulating colonic surface with narrow orifices of the crypts of Lieberkühn (Fig. 1). The freeze-fractured sections revealed narrow crypts containing a material of mucous origin (Fig. 2). Tightly spaced microvilli covered the surface of the epithelial cells both on the colonic surface and within the apical and middle portions of the crypts (Fig. 3).

In pigs with SD dilated orifices of the crypts were seen in areas with minor pseudomembraneous lesions (Fig. 4). These orifices contained cellular debris, mucus and often spirochetes (Fig. 5). The orifices were often surrounded by uneven mucosa containing circular or irregular furrows which displayed small ruptures in some areas (Fig. 6). Ridge formations could be seen in the probable extrusion zone for the epithelial cells midway between the cryptal openings. The spirochetes, measuring approximately 9 microns in length and 0.35 microns in diameter,

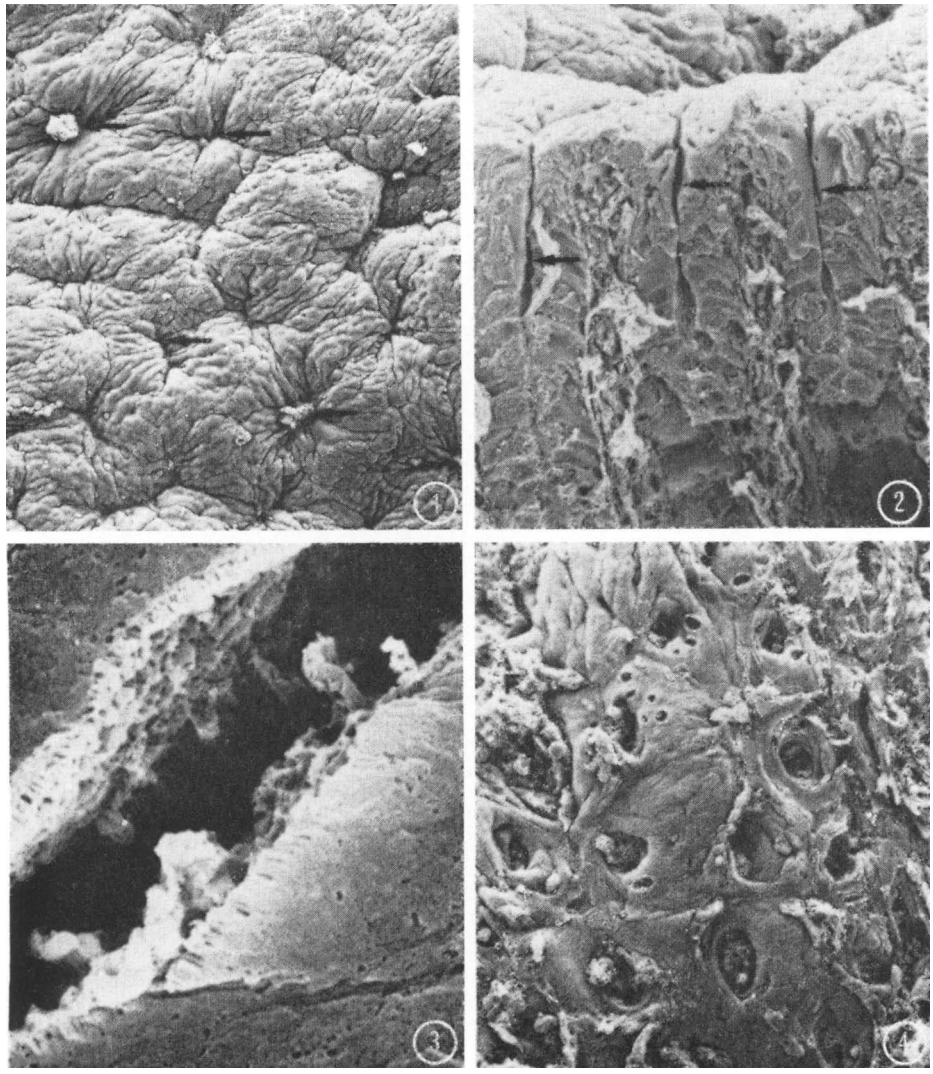

Figure 1. Control pig. Scanning electron micrograph (SEM) of the colon showing a relatively smooth surface. Arrows point to narrow orifices of the crypts. × 300.

Figure 2. Control pig. SEM, freeze-fractured specimen of the colonic mucosa. Arrows point to narrow crypts of Lieberkühn which contain some mucus. × 450.

Figure 3. Control pig. High power SEM, freeze-fractured sections of the colonic mucosa. Tightly spaced microvilli can be seen on the epithelial surface. × 10.000.

Figure 4. SD-pig. SEM of the colonic surface showing dilated orifices of the crypts. F indicates small fibrinous membranes overlying the mucosa. × 300.

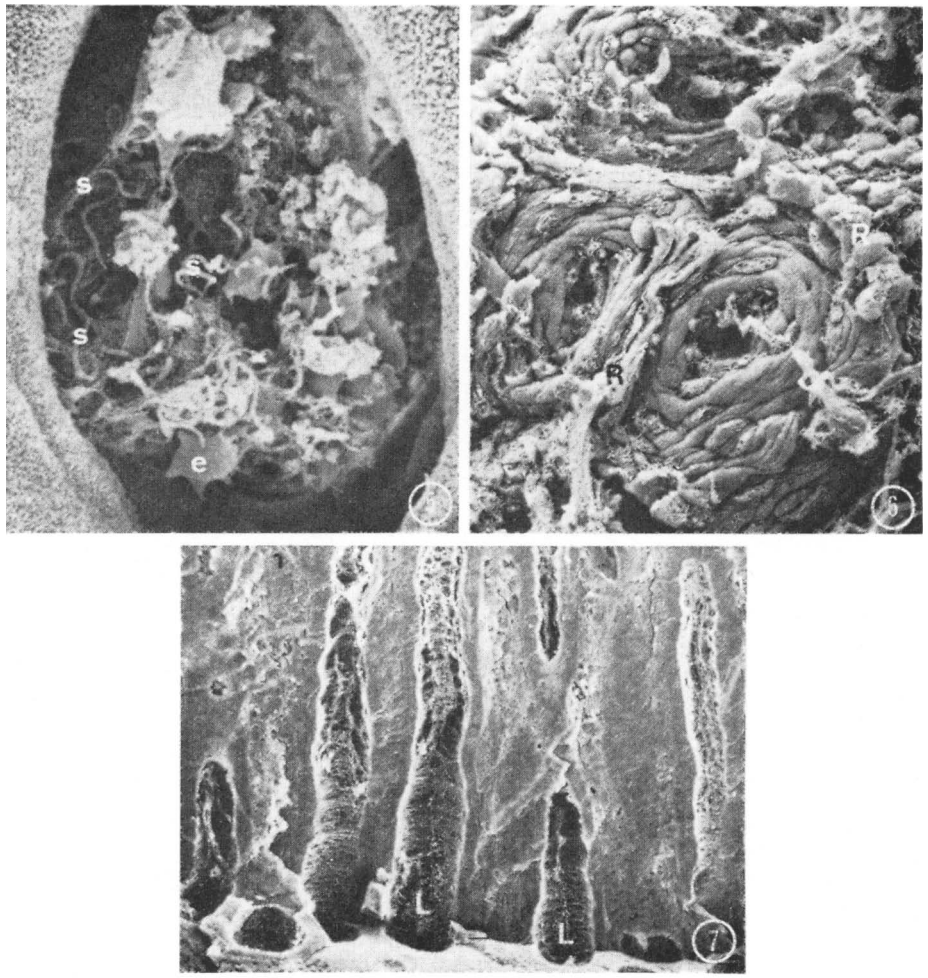

Figure 5. Close-up view of one of the orifices seen on the preceding micrograph. The dilated orifice contains mucus, erythocytes (e) and spirochetes (s). × 3,000.

Figure 6. SD-pig. SEM of the colonic surface showing dilated orifices surrounded by swollen mucosa containing circular or irregular furrows. Ridge formations (R) can be seen midway between the orifice. × 600.

Figure 7. SD-pig. SEM, freeze-fractured section of the colonic mucosa. The crypts are elongated and dilated and contain some filamentous material of mucous origin. L indicates the lower portion of the crypts. × 300.

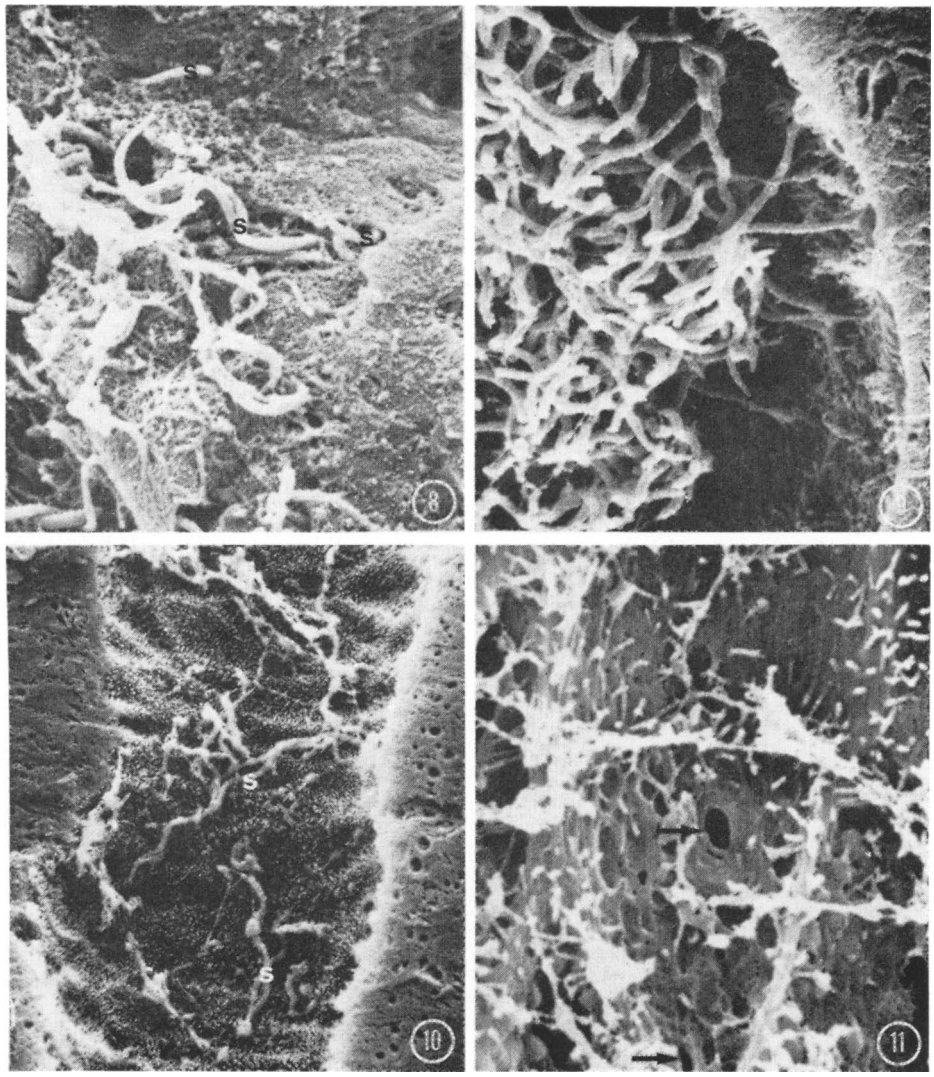

Figure 8. SD-pig. SEM of damaged epithelial cells on the colonic surface with only minor remnants of microvilli. Spirochetes (s) penetrating the cellular membrane can be seen. × 6,000.

Figure 9. SD-pig. High-power SEM of a freeze-fractured section of a colonic crypt. Tangled masses of spirochetes are seen within the crypts. Only few of them seem to be in contact with the epithelial surface which otherwise shows few, short and irregular microvilli. × 6,000.

Figure 10. SD-pig. SEM of freeze-fractured section showing dilated crypt with few spirochetes (s) in the lumen. Reduced number of microvilli on the epithelial surface. × 3,000.

Figure 11. SD-pig. High-power SEM showing epithelial surface in one of the dilated crypts. Arrows point to ruptures in the cellular membrane. Microvilli are sparse. × 10,000.

were sometimes numerous on the mucosal surface and within the pseudomembranes. They were also found in epithelial ruptures, moreover they seemed able to penetrate damaged epithelial cells (Fig. 8). The number of microvilli was reduced on these cells and on cells in the extrusion zone but seemed normal in the remaining mucosal surface showing apparently intact epithelial cells.

Freeze-fractured sections of the colonic mucosa in SD pigs revealed dilated crypts (Fig. 7). This feature was somewhat more conspicuous in the lower portions of the crypts. The crypts often contained some irregular filamentous matter of mucous origin. Tangled masses of spirochetes were also often noted within the dilated crypts (Fig. 9 and 10). A few of these organisms seemed to be stuck to the epithelial surface (Fig. 9). Spirochetes penetrating the luminal plasma membrane of the cryptal epithelium were not found. However, high magnification showed many pores of different size in the same membrane (Fig. 11). Most of the cryptal microvilli were short, irregular and their number markedly reduced (Figs. 9, 10 and 11).

The bacteriological examination revealed growth of T. hyodysenteriae in colonic samples from pigs Nos. 3 and 4, but not from the other animals.

DISCUSSION

The main findings upon SEM-examination of the freeze-fractured colonic mucosa were dilated crypts of Lieberkühn with relatively few, short and irregular microvilli on the epithelial surface. There were numerous spirochetes within the crypts, some of these organisms adhered to the epithelial surface but none were found to penetrate the cell membrane. The SEM findings on the mucosal surface were otherwise in accordance with the description given by *Kennedy et al.* (1973) and *Kennedy & Strafuss* (1976). It should, however, be pointed out that dilated cryptal orifices and swollen, rough surface with circular furrows were typical alterations in areas of the mucosa with slight pseudomembraneous lesions.

The present study revealed that the spirochetes were invading apparently necrotic cells on the mucosal surface. Areas both on the surface and in the crypts with intact epithelial cell membranes did not seem to be invaded. These findings appear to be

in accordance with the observations of *Taylor & Blakemore* (1971) who only found spirochetes within and between damaged epithelial cells; they suggested that the presence of the microbes in these locations was due to an opportune invasion.

Adhesion and multiplication of bacteria on the intestinal epithelium according to *Walker et al.* (1980), are important primary steps in the establishment of enteropathogenic infections like E. coli-diarrhea in piglets. SEM examination in these cases revealed a large number of bacteria attached to the intestinal villi, which otherwise remained fairly normal (*Walker et al.*). *Knop et al.* (1979) have demonstrated that T. hyodysenteriae adheres to epithelial cells in an in vitro system but without the appearance of any alterations in cellular morphology or change in the cell surface at the site of attachment. No correlation has, however, hitherto been demonstrated between this characteristic and enteropathogenicity. The present study revealed that only a small percentage of the treponemes appearing in the cryptal lumina seemed to adhere to the epithelial surface. This observation therefore indicates that epithelial attachment of treponemes is not a very prominent feature in SD and thus strongly diverges from SEM findings in some other intestinal infections (*Walker et al.*).

At least two explanations can be proposed concerning the reduction of microvilli in the present case. Firstly, this morphological feature may be a result of destructive effects of the treponemes and their possible toxins as shown with other bacteria (*Takeuchi* 1967, *Staky et al.* 1969, *Johnson & Barthold* 1979). Secondly the hyperplasia of the cryptal epithelium which apparently occur in SD may alter the cell kinetics, resulting in a decreased differentiation of the epithelial cells. Poor development of microvilli in apical cryptal portions are also found in the ulcerative colitis and celiac disease of man (*Eastwood & Trier* 1973, *Barthold* 1979).

Dilation of crypts was a conspicuous finding in the pigs with colonic lesions. Solid material seemed to occupy only a smaller part of many cryptal lumina which therefore may also contain a great deal of fluid. Fluid accumulation has been induced in ligated loops of colon inoculated with enteropathogenic treponemes (*Whipp et al.* 1978), and it is therefore possible that the dilation of the crypts in the present study may be due to an increased secretion from the epithelium. Decreased absorption

in the colon is another functional change in pigs with SD. These events may cause circulatory disturbances including stasis in the colonic mucosa. It has previously been proposed that this last phenomenon contributes to development of the erythrocytic "thrombi" demonstrated in the intestinal lesions of SD cases (*Teige & Nordstoga* 1979).

According to *Specht* (1977), desquamation of older epithelial cells is located to a zone approximately midway between orifices of neighbouring crypts. In the present material cellular ridge formations could be seen in the same area, thus probably indicating an increased extrusion of senescent epithelial cells in this part of the colonic surface in pigs with SD.

Attempts to detect cytotoxic effects produced by T. hyodysenteriae have been unsuccessful because the development of pathological lesions indicating SD both with in vitro and in vivo systems so far seem to require the presence of live pathogenic treponemes (*Whipp et al., Wilcock & Olander* 1979 b). On the other hand, the present study seems to indicate that epithelial penetration of these bacteria is not a primary event in the development of SD. In view of the cryptal findings it may also be suggested that the importance of epithelial attachment of treponemes seems questionable in the same disease. The role of T. hyodysenteriae in SD pathogenesis is therefore still obscure.

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SAMMENDRAG

Svinedysenteri: En skanning elektron-mikroskopisk undersøkelse.

Det er utført en skanning elektron-mikroskopisk undersøkelse av kolonprøver fra 4 konvensjonelt oppdrettede griser. To av dyrene hadde utviklet svinedysenteri etter poding med en renkultur av Treponema hyodysenteriae. Hos disse to grisene kunne det i områder med spredte pseudomembraner sees dilaterte kjertelåpninger omgitt av en ujamn slimhinne med furer og små kamdannelser. Treponema-bakterier var tallrike på slimhinneoverflaten og de syntes bare å penetrere skadete epitelceller. Fryse-frakturerte snitt fra de samme grisene viste dilaterte kjertler hvor det var korte uregelmessige mikrovilli som var sterkt reduserte i antall. Dette sistnevnte forholdet antas å skyldes forandringer i cellekinetikken. Denne undersøkelsen synes å indikere at epitelial tilheftning og penetrasjon av treponema-bakterier er av liten betydning for patogenesen ved svinedysenteri.

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