Diarrhea Due to “Attaching and Effacing Escherichia coli (O 26)” Infection in a Calf
Tomokazu IJIMA, Masuo SUEYOSHI1), Toshibo YAMAMOTO, Keisuke YOSHIOKA, and Munetaka NAKAZAWA3)
Kenpoku Livestock Hygiene Service Center, Nakagachi-cho, 966-1, Mito, Ibaraki 310 and 1)Kyushu Branch Laboratory, National Institute of Animal Health, Chuzan-cho, 2702, Kagoshima 891-01, Japan
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Recently, enteropathogenic Escherichia coli was isolated from calves with dysentery [2, 4]. This bacteria attach closely to the enterocytes and efface the microvilli of the cells, and were named as “attaching and effacing E. coli” (AEEC) [3]. AEEC does not produce heat-labile or heat-stable enterotoxin. In Japan, atypical E. coli (serotype O5: K*: NM) was isolated from diarrheal feces of a calf and the attaching and effacing lesions were also demonstrated in the intestine of goats experimentally infected with the isolated E. coli [6]. This note describes the intestinal lesions of a calf spontaneously infected with AEEC O serogroup 26.

An 8-day-old male Japanese black calf showed diarrhea on April 18, 1989, and died on the next day in spite of treatment with oral electrolytes (Electro plus A, Pitman Moore Co., Ltd., Illinois, U.S.A.) and oxytetracycline (terramycin injectable solution, Pfizer Co., Ltd., Tokyo). The calf had been reared on a farm with 30 mature cows and 30 calves in Ibaraki prefecture.

The scraping of the intestinal mucosa from the dead calf was serially diluted, spread on DHL agar (Nissui Co., Ltd., Tokyo) and incubated at 37°C for 18 hrs. The rate of isolation of E. coli was 4.0 × 10^3, 2.0 × 10^2, 2.5 × 10^2 and 1.3 × 10^9 colony forming units per g of the samples from the ileum, jejunum, ileocecal junction and rectum, respectively. The isolated E. coli was identified as O serogroup 26, and negative for K 99, K 88, and 987P antigens. This bacteria produced the verotoxin but did not produce the heat-labile and heat-stable enterotoxin [1]. In addition, bovine diarrhea mucosal disease virus and calf rotavirus were not isolated from the feces or intestinal contents.

Gross lesions were confined to the intestine.

Patchy congestion was scattered in the serosa of the duodenum and in the mucosa of the jejunum. No lesions were observed in other organs. Histologically, the characteristic lesions were seen in the mucous membrane of the colon and rectum. The colonic lesion was the most severe in the intestine. The superficial epithelial cells were irregualr in shape, shrunken and degenerated, frequently showing desquamation (Fig. 1). A few neutrophils were present in the lamina propria and epithelial cell layer. Some cellular debris was also observed in the lamina propria. The intestinal crypts were considerably dilated with mucus. Numerous bacilli attached to the epithelium of the superficial mucosa and of the upper one-third of the crypt.

In addition, the villi were generally atrophic with degeneration of the superficial mucosal epithelial cells in the duodenum and jejunum. There were erosions, congestion of the lamina propria and dilation of the crypt in the ileum. Lymphocytes were decreased in number, showing necrotic changes, in the Peyer’s patches. The cecal enterocytes were markedly desquamative.

Immunohistologically, the avidin-biotin-affinity technique (ABA: Omnitag kit, Lipshaw, Co., Ltd., U.S.A.) was applied to paraffin sections. The rabbit antisera to E. coli (O 26) (Difco Laboratories, Detroit, Michigan, U.S.A.), were diluted at 1:5,120 for primary antibody. Numerous E. coli bacteria were observed diffusely on the epithelial surface of the superficial mucosa and of the upper one-third of the crypt in the colon and rectum (Fig. 2). These bacteria were present only focally on the mucosal surface in the small intestine and cecum.

Specimens from the colon fixed in 10% buffered formalin were examined by a transmission electron microscope. The superficial mucosal epithelial cells were irregular in shape and arrangement. Numerous E. coli bacteria were attached to their cell surface membranes (Fig. 3). Some cell membrane formed cup invagination and pedastal-like protrusion associ-
ated with the bacteria (Fig. 4). The bacteria were also demonstrated within the goblet cells (Fig. 5) but not in the lamina propria. Most microvilli were lost on the superficial epithelial cells and remaining ones were disoriented. The *E. coli* bacteria were short rod in shape and had no pili. Specimens from the colon fixed in 10% buffered formalin were observed by a scanning electron microscope. The mucosal surface was roughened with numerous short rod-shaped bacilli, and had decreased number of microvilli or abnormal ones (Fig. 6).

The isolated *E. coli* was O serogroup 26 and did not produce any enterotoxins. This result suggested that the *E. coli* did not belong to the enterotoxigenic *E. coli* usually associated with calf diarrhea. However, the bacteria produced verotoxin.

On the basis of the results obtained here, the isolated *E. coli* was regarded as the AECC that was named by Moon et al. [3]. The lesions in the large intestine in the present case were similar to those due to the AECC previously reported [2, 4, 6]. However, the isolate *E. coli* was O serogroup 26 and differed from atypical *E. coli* previously demonstrated [2, 4, 6].

*E. coli* was seen within enterocytes of rabbits [5] and pigs [3]. *E. coli* was demonstrated in the goblet cell of the colon in this case. This findings may suggest hypersecretion of mucus from the goblet cells stimulated by the bacteria.

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REFERENCES


Fig. 1. Colon. Superficial mucosal epithelial cells are irregular in arrangement, shrunken and desquamative. Crypts are dilated. H & E stain. ×160.

Fig. 2. Colon. Numerous *E. coli* bacteria adhere to superficial epithelial cells. ×160. ABA stained with the antiserum to *E. coli* (O 26).

Fig. 3. Colon. Numerous bacilli attach closely to irregular-shaped and desquamated epithelial cells in the mucosal surface. The epithelial cells have cytoplasmic cup (arrows) and pedestal-like protrusion. N=Neutrophil. Bar=5 μm.

Fig. 4. Apical portion of a colon surface epithelial cell. Short rod-shaped bacilli attach to pedestal-like cytoplasmic protrusions (arrows). The bacteria (B) have no pili. Bar=1 μm.

Fig. 5. Goblet cell in the colon. Some bacilli (arrows) are present in a partially evacuated goblet cell (G). Bar=5 μm.

Fig. 6. Colon. Numerous bacilli adhere to the mucosal surface. Remaining microvilli are oriented abnormally. E=eroded cells. Arrow=pedestal-like cytoplasmic protrusion. Bar=10 μm.
要約

“Attaching and Effacing Escherichia coli (O 26)”の自然感染による子牛の下痢症（短報）：飯島知一・末吉益雄11・山本敏弘・吉岡貴美・中澤宗生11（茨城県北家畜保健衛生所，11農林水産省家畜衛生試験場九州支場）——下痢を呈し、急死した9日齢の子牛の大腸から多数のO血清群26の大腸菌が分離された。この大腸菌はエンテロトキシン非産生で、ペロトキシンを産生した。組織学的には、大腸の粘膜表面は凹凸が著明で表層粘膜上皮細胞は変性し、一部剝離・脱落していた。免疫組織化学的に観察したところ、抗O群26血清に陽性の大腸菌が粘膜表面に多数付着していた。また、電子顕微鏡で観察したところ、菌の付着した表層上皮細胞表面は微細毛が消失し、小窩または台座形成が認められた。分離した大腸菌は“Attaching and Effacing E. coli (AEEC)”であると思われた。この報告はO血清群26のAEECに自然感染し、下痢を呈した子牛の日本における最初の報告である。