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A Case of Neonatal Calf Diarrhea Associated with Natural Infection with Rotavirus

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(Received 29 November 1984/Accepted 7 March 1985)

ABSTRACT. A case of neonatal calf diarrhea associated with natural infection with rotavirus was investigated morphologically. No obvious gross lesions could be seen. Histologic examination revealed degeneration and desquamation of epithelial cells near the tips of villi in the small intestine, and degeneration and necrosis of follicle-associated epithelial cells in the ileum. Electron microscopically, atrophy and loss of microvilli, and budding of virus particles into cisternae of rough endoplasmic reticulum and tubular structures were observed. Among the changes observed, the presence of the tubular structures appeared to be characteristic of viral infection.—**KEY WORDS:** neonatal calf diarrhea, rotavirus.

Jpn. J. Vet. Sci. 47(3): 517–521, 1985

Rotavirus is one of the causative agents of neonatal calf diarrhea. Since Mebus *et al.* [3] have first isolated the virus from faecal materials of calves in 1969, many virological and pathological investigations have been done of experimentally induced calf diarrhea [2]. Morin *et al.* [5] and Pearson *et al.* [7] reported pathologic findings of naturally infected cases. However, these authors did not mention electron microscopic findings in their cases. This paper deals with results obtained by pathologic and ultrastructural investigations on a case of neonatal calf diarrhea associated with natural infection of rotavirus.

The present case was a Holstein calf, female, 4 days of age, and born on a farm of Chiba prefecture in September, 1983. On the 3rd day after birth, the animal showed diarrhea, depression and loss of appetite, and was slaughtered on next day. After autopsy tissue specimens for light microscopy were fixed in 10% formalin solution, embedded in paraffin, sectioned at about 6 μ m, and stained with hematoxylin and eosin (H-E). In addition, specimens from the intestine were fixed in 5% glutaraldehyde, postfixed in 1% os-

mium tetroxide, and embedded in Epon 812. Ultrathin sections were cut, and stained with 6% uranyl acetate followed by Sato's lead solution. Virus particles obtained from faecal materials were examined by negative staining technique, and by reversed passive hemagglutination test using ROTA CELL manufactured by Nissui Seiyaku CO., LTD, Tokyo, Japan.

In negatively stained preparations, virus particles had wheel-like morphology with a wide hub and short spokes, and measured about 65 nm in diameter (Fig. 1). The antigenicity of the virus was identified with that of rotavirus.

There were no conspicuous gross lesions, except thinning of the intestinal wall, petechiae of the rectal mucosa and lungs, and atrophy of the thymus.

Microscopic lesions were mainly confined to the small intestine, especially the ileum. That is, degeneration and desquamation or shedding of the villous epithelial cells, edema and proliferation of histiocytes in the lamina propria, and decrease of lymphocytes in lymphatic follicles were recognized (Fig. 2).

Fig. 1. Viral particles seen in faecal materials. Negative staining with 4% phosphotungstic acid. $\times 180,000$.

Fig. 2. Desquamation and shedding of villous epithelial cells and decrease of lymphocytes in follicle of the ileum. H-E, $\times 128$.

Degeneration and desquamation of the epithelial cells were particularly conspicuous near the tips of villi. The follicle-associated epithelial cells in the ileum exhibited necrotic changes with atrophy and pyknosis (Fig. 3). The rectum merely showed hemorrhage in the lamina propria, and no inflammatory changes were present there. In the other organs, there were decrease in lymphocytes and atrophy of

lymphatic follicles in such lymphatic tissues as the spleen, lymph node and thymus, and suppurative bronchopneumonia.

Electron microscopically, virus particles could be recognized in the villous epithelial cells of the jejunum, ileum, colon and rectum. Especially, a large number of them were detected in the absorptive cells and follicle-associated epithelial cells (Fig. 4).

Fig. 3. Atrophy and pyknosis of follicle-associated epithelial cells (arrow) in lymphatic follicle (LY) of the ileum. Arrow heads show absorptive cells. H-E, $\times 560$.

Fig. 4. Atrophy and fusion of microvilli, and virus particles in cisternae of rough endoplasmic reticulum (rER) in villous epithelial cells of the ileum. $\times 12,000$.

Fig. 5. Budding of virus particles into cisternae of rER (arrow) and tubular structures in cytoplasm. M: mitochondria. N: nucleus. $\times 36,000$.

They filled up in dilated cisternae of rough endoplasmic reticulum (rER) and the perinuclear cisternae, and occasionally the particle budded from the rER membrane into the cisternae (Fig. 5). Virus particles in the process of budding measured about 80 nm in diameter, whereas particles lying free in cisternae of rER measured usually about 65 nm. Most of them had an electron dense core. Infected epithelial cells showed atrophy and fusion of their microvilli (Fig. 4), and occasionally appeared to be electron dense masses as a whole. Moreover, a large number of tubular structures, about 50–55 nm in width, were observed in the nucleus and cytoplasm (Fig. 5). These tubules were present only in infected cells, suggesting a characteristic manifestation of viral infection. An electron dense granular or fibrous material, so-called viroplasm, was rarely observed in the cyto-

plasm of infected epithelial cells. The viroplasm released virus particles from its margin (Fig. 6). There were many bacteria in the epithelial cells of the ileum, especially follicle-associated epithelial cells, but attachment or invasion of bacteria to these cells was hardly seen.

According to the previous reports on neonatal calf diarrhea induced experimentally with rotavirus [4, 9–11], it seemed that infection of rotavirus alone caused diarrhea but death seldom occurred [10]. On the other hand, the disease showed high mortality in natural infection, and this was usually attributed to mixed infection of such agents as coronavirus, adenovirus and toxigenic *Escherichia coli* [5, 7]. In the present case, no virus particles except those of rotavirus were detected by the negative staining of faecal materials, but mixed infection of bacteria was

Fig. 6. Viroplasm (V) releasing virus particles (arrow) from its margin in the cytoplasm of infected epithelial cells. $\times 37,500$.

demonstrated in thin sections by electron microscopy. Therefore, it was difficult to know whether intestinal lesions of the present case were caused by rotavirus alone or by mixed infection with bacteria. However, histologic and ultrastructural changes observed in the present case were similar to those described in single infection of rotavirus [1, 4, 6, 8, 9], and it was considered that such changes might be enough to cause diarrhea. Torres-Medina [11] emphasized the changes of follicle-associated epithelium in cases of mixed infection with rotavirus and bacteria. In the present case, degeneration and necrosis of cells as well as virus particles were observed not only in epithelial cells near the tips of villi but also in follicle-associated epithelial cells, suggesting that the changes of follicle-associated epithelium were also caused by rotavirus.

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要 約

新生子牛のロタウイルスによる下痢症例（短報）：蛭間正己・井出誠弥・久米常夫¹⁾（北里研究所附属家畜衛生研究所，²⁾北里大学獣医畜産学部）——新生子牛のロタウイルスによる下痢症例を組織学および超微形態学的に観察した。従来記載されている腸管病変に加え，follicle associated epithelial cell の障害も観察され，電顕的には管状物がみられ，ウイルス感染にともなう特異的な変化とみなされた。