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# BRIEF COMMUNICATIONS

## Calfhood Coronavirus Enterocolitis: A Clue to the Etiology of Winter Dysentery

H. J. Van Kruiningen, L. H. Khairallah, V. G. Sasseville, M. S. Wyand, and J. E. Post

Recently we published a review of winter dysentery and documented findings in spontaneous and experimental disease in New England dairy cattle.[10] The histologic features of winter dysentery were characterized for the first time. A lesion of the colon, particularly the spiral colon, was recognized. It consisted of pyknosis, karyorrhexis, granular degeneration, hydropic degeneration, and hyaline droplet degeneration of crypt epithelial cells. This scattered focal destruction of epithelial cells produced two characteristic elements: a moth-eaten appearance of the mucosa at low magnification and striking nuclear pyknosis and cytoplasmic hyaline droplets at high magnification (Fig. 1). A cow that died had widespread changes of this type as well as reparative dilated crypts. These features were recognized as unique and suggestive of "virus-induced enterocolitis."

While our work was in progress, investigators from Japan[9]

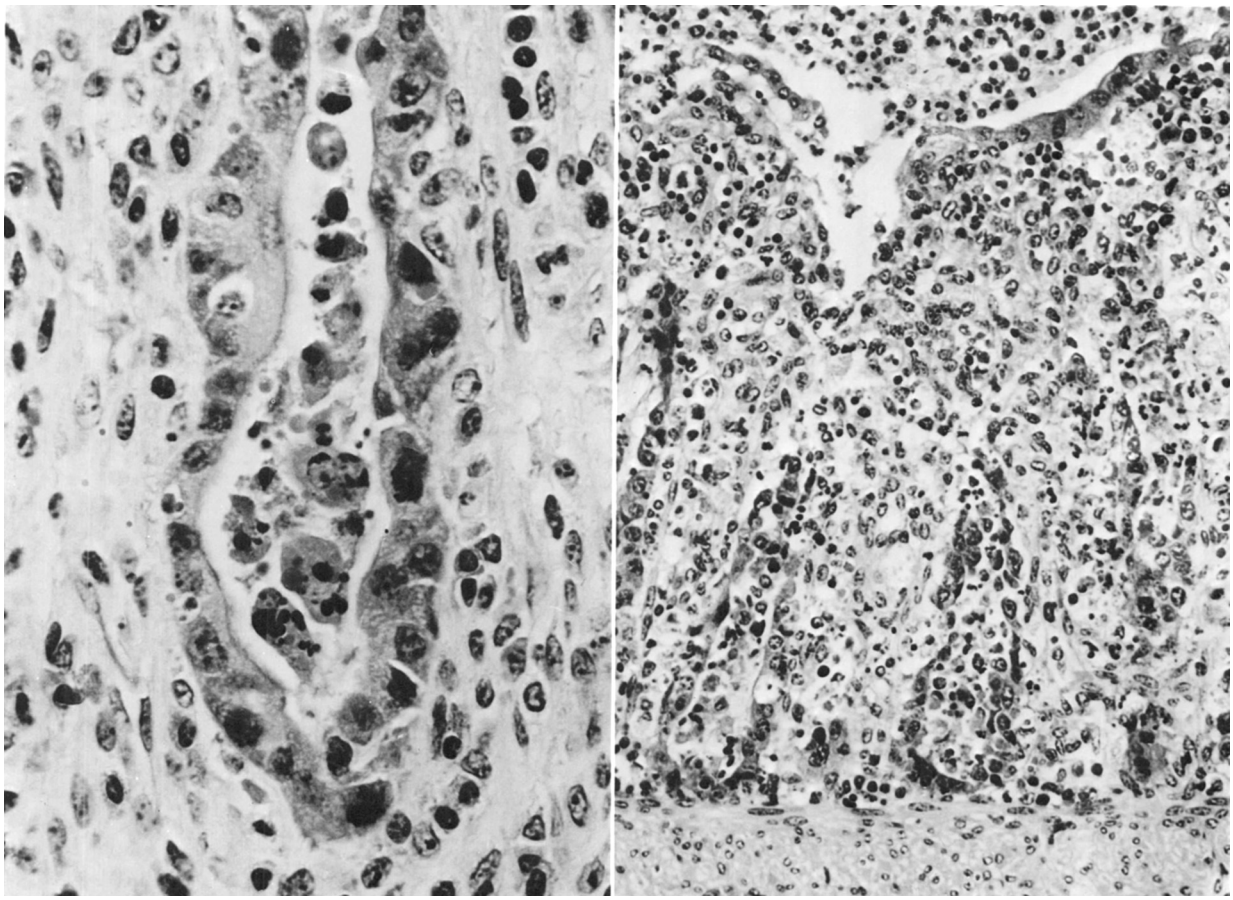

**Fig. 1.** Colonic crypt from spontaneous case of winter dysentery. Epithelial cells with various degenerative and necrotic changes. Within crypt lumen, dying cells contain karyorrhectic nuclei and hyaline droplets. HE.

**Fig. 2.** Histologic section of spiral colon from calf with coronavirus enterocolitis. Extensive crypt epithelial cell pyknosis, karyorrhexis, lysis, and regenerative hyperplasia identical to that occurring in winter dysentery. Loss of crypts leukocytosis, tattered surface epithelium, and granulocytic exudate at the surface. HE.

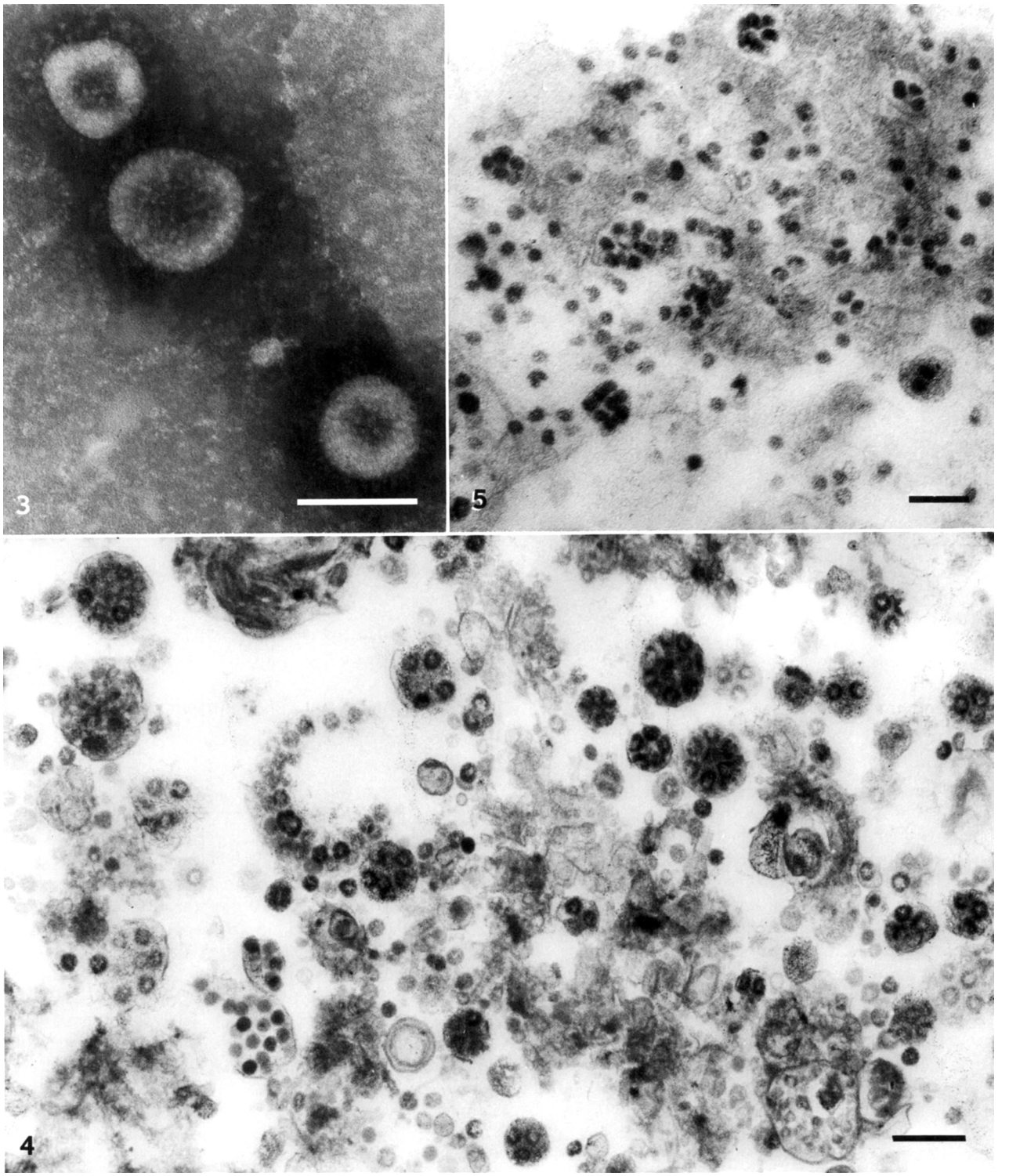


**Fig. 3.** Negatively stained coronavirus particles in intestinal contents from affected calf. Size from 95–115 nm, 130–150 nm with peplomeres. Bar = 116 nm.

**Fig. 4.** Numerous coronavirus particles and "virus factories" within degenerate epithelial cell from damaged colonic mucosa of calf. Formalin-fixed tissue, virus particles 60 nm in diameter. Bar = 200 nm.

**Fig. 5.** Numerous coronavirus particles and "virus factories" within a degenerate epithelial cell from damaged colonic mucosa of cow with winter dysentery. Bar = 200 nm.

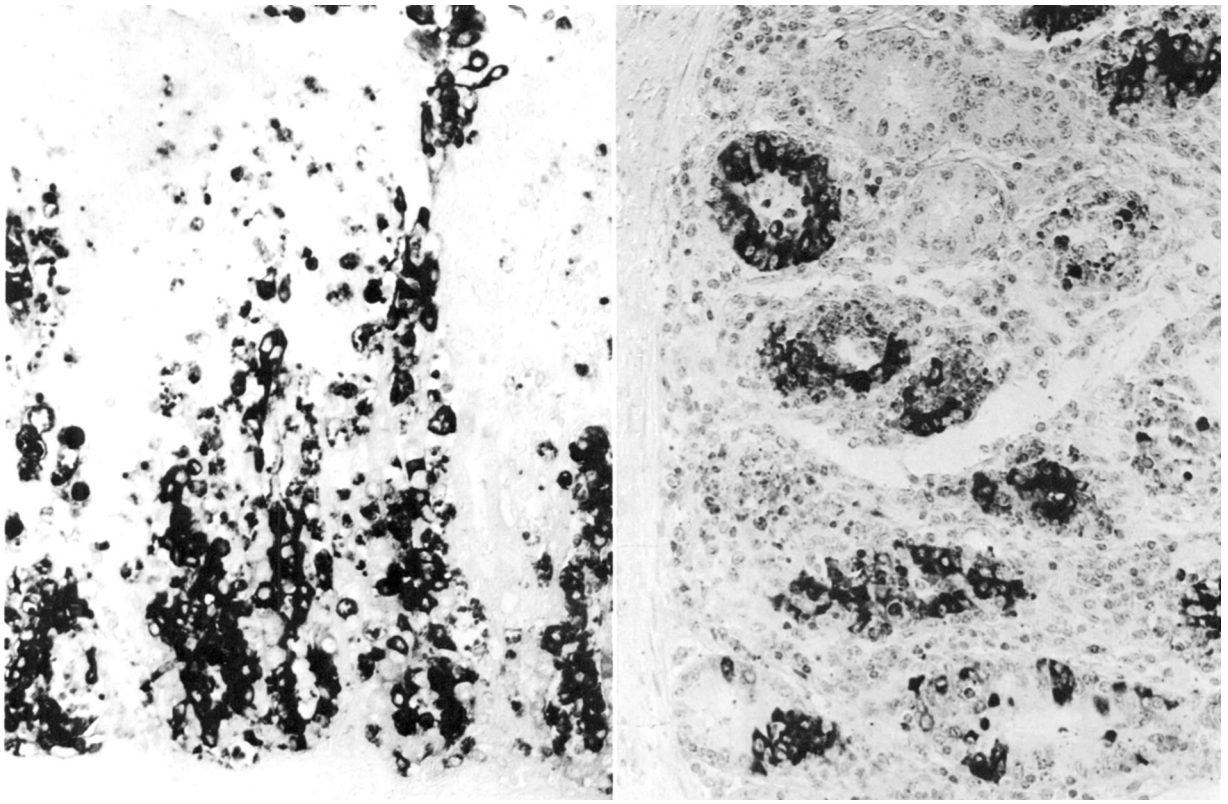

**Fig. 6.** Extensive immunoperoxidase reactivity with coronaviral antigen in crypt epithelial cells from spiral colon of calf. PAP.

**Fig. 7.** Immunoperoxidase reactivity with coronaviral antigen in crypt epithelial cells from spiral colon of cow with winter dysentery, muscularis mucosae at left. PAP.

and France[3] reported demonstration of coronavirus-like agents in the feces of adult cattle with "epizootic diarrhea" and "winter dysentery," respectively. Recently, a report from Colorado documented a high incidence of shedding of coronavirus particles in the feces of normal dairy cattle during the winter stabling season.[1] Although the histologic changes of calfhood coronavirus enterocolitis had been well documented in the literature,[2,4–7] an association between the latter disease and winter dysentery escaped recognition until the present authors had an opportunity to view the calfhood lesions firsthand and to make the necessary comparisons.

In February 1986, a 9-day-old, 39 kg, white and black Holstein calf was presented for autopsy following 4 days of diarrhea, the last day of which had been bloody. The calf became dehydrated and weak, and died enroute to the laboratory, thus providing a post-mortem specimen essentially free of autolysis. At autopsy, the calf was thin and dehydrated, with sunken eyes; the spiral and distal colon contained bloody pseudomembranous casts. Other organs were free of disease.

Representative tissues were fixed in 10% buffered formalin, processed for paraffin embedding, sectioned at 6 $\mu$m, and stained with hematoxylin and eosin (HE). Selected sections were stained by Giemsa, Shorr, Feulgen, periodic acid-Schiff (PAS), and Brown and Hopps methods. Lesions were confined to the small intestine, colon, and regional lymph nodes. Colonic mucosa was severely damaged and coated with a thick granulocyte-rich bloody fibrinopurulent pseudomembrane. Colonic crypts had extensive epithelial cell pyknosis and karyorrhexis, with hyaline degeneration of the cytoplasm. Focally, there was thinning of crypt epithelium with crypt dilation, aggregates of karyorrhectic, degenerate cells filled many crypt lumina, and there was widespread regenerative epithelial hyperplasia (Fig. 2). These changes occurred diffusely in some sections and focally in others. The mucosa of affected areas had hyperemia of capillary beds and hemorrhage near the surface, focal moth-eaten appearance where cells had undergone necrosis and/or vacuolar degeneration, loss of many colonic glands, with collapse of remaining connective tissue elements, and some plasmacytosis. Lymphoid nodules of the submucosa were unaffected.

Sections of the small intestine contained only focal crypt damage. Small numbers of cryptosporidia were present among villi. Peyer's patches were normal. Regional mesenteric lymph nodes had marked sinus histiocytosis, and karyorrhexis of macrophages within cortical sinuses.

Electron microscopy of intestinal contents from this calf revealed numerous coronavirus particles (Fig. 3), and examination of formalin-fixed intestine showed numerous aggregates of coronavirus within vacuoles in damaged colonic epithelial cells (Fig. 4). A direct fluorescent antibody method employing bovine convalescent anti-coronavirus antibody

on frozen sections of colon, counterstained with Evan's blue, demonstrated striking fluorescence of damaged colonic epithelial cells throughout the length of the crypts. Sections treated with an anti-virus diarrhea antibody failed to fluoresce. A search of the pseudomembrane in this case with the Brown and Hopps stain revealed bacterial colonies of two types: some consisted of large, gram-positive rods (consistent with *Clostridium* spp.); others were smaller, gram-negative rods (*Enterobacteriaceae*). Bacteriologic culture for *Salmonella,* a frequent cause of pseudomembranes, was negative.

With the similarity of this calfhood coronavirus enteritis to the lesions of winter dysentery in mind, case material from the four cows with winter dysentery which were autopsied in 1980–1981 was reviewed. Formalin-fixed sections of spiral colon from one case were rinsed in phosphate buffered saline, and then 3 $mm^3$ cubes were cut from the mucosal ridges and prepared for electron microscopy. Examination revealed numerous coronavirus particles and virus factories within degenerate epithelial cells in damaged crypt lumina (Fig. 5). The viruses were similar in size and structure to those identified in the calf.

Then, antibody to calfhood coronavirus which had been raised in rabbits (courtesy of R. Sharpee, Norden Labs, Omaha, NE) was used in an immunohistochemical search for virus in trypsinized paraffin-embedded sections of affected spiral colon and mesenteric lymph node of each of the four cases of winter dysentery, and in similar tissues from the calf with enteritis. Using a 1 : 160 dilution of the primary antibody, the peroxidase-antiperoxidase (PAP) technique was applied (Dako Corp., Santa Barbara, CA), with 3-amino-9-ethyl-carbazole as substrate. Antibody from non-immunized rabbits was applied to corresponding serially cut control sections. Crypt epithelial cells from the calf with coronavirus enteritis gave extensive PAP reaction with a patchy distribution (Fig. 6). Crypt epithelial cells from each of the four cattle with winter dysentery gave a similar positive PAP reaction, although it was focal, occurring in degenerate cells in damaged crypts, in macrophages, and in clusters of intact crypt cells (Fig. 7). Sections treated with antibody from non-immunized rabbits gave no PAP reactions, and spiral colon sections from eight control cattle reacted with primary antibody and control serum were equally negative. Mesenteric lymph nodes from the calf and the four cattle with winter dysentery were negative.

We believe the striking similarity of the histologic features of the calfhood coronavirus enterocolitis reported here and the changes occurring in winter dysentery, documented earlier, as well as the positive immunohistochemistry and electron microscopy, provide convincing evidence that a bovine coronavirus is the cause of winter dysentery—at least as seen by us in New England. Recovery of the virus from tissues we have saved, as well as from cases of winter dysentery which will be studied in the future, will be important in determining if the agent of this severe calfhood enterocolitis and winter dysentery is identical to or different from the coronavirus[2,4,8] responsible for conventional, non-bloody calfhood diarrhea.

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Request reprints from Dr. H. J. Van Kruiningen, Department of Pathobiology, University of Connecticut, Storrs, CT 06268 (USA).