# NOTE

# An Outbreak of Swine Diarrhea of a New-Type Associated with Coronavirus-Like Particles in Japan

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Recently, outbreaks of epizootic diarrhea, clinically similar to transmissible gastroenteritis (TGE), were reported in England [5, 15], Belgium [6, 11], Canada [14] and West Germany [8]. In these countries, laboratory investigations including serological and immunofluorescent examinations revealed no evidence of TGE virus (TGEV) infection; however, coronavirus-like particles were detected by electron microscopy [5, 8, 11, 14]. These particles were shown to be antigenically distinct from the known porcine coronaviruses, TGEV and hemapplutinating encephalomyelitis virus (HEV). According to the facts, it is suggested that some other virus morphologically similar to the coronavirus TGEV is associated with the diarrheal syndrome of swine of all ages. This would be the first report on such an outbreak in Japan.

In Feburuary, 1982, a sudden outbreak of epizootic diarrhea was observed on a farm keeping approximately 2,500 swine in Iwate Prefecture. Almost all swine of various ages suffered from diarrhea within a short term of about a week. They showed watery diarrhea and vomiting. Deaths occurred in piglets of less than 20 days old. The mortality rate was over 20% among 400 piglets. Aging seemed to decrease the mortality rate. The pigs weanling to adult recovered

from diarrhea in 2 to 5 days. TGE was suspected of because of the resemblance in clinical and epizootiological features.

Three affected piglets of 14 days old were autopsied, and histologic and virologic examinations were performed. At autopsy, hyperemia of the gastric serosa and dilation of the small intestinal tract observed. Histologic examinations of the small intestines revealed markedly shortened villi and infiltration of neutrophils in the lamina propria (Fig. 1). The direct fluorescent antibody test for TGEV antigen [12] was performed on cryostat sections of the small intestine and on the tissue culture of porcine kidney cell lines (CPK) [10] inoculated with the intestinal homogenate of an affected piglet as well as passaged blindly three times without cytopathic effect. TGEV-specific fluorescence was demonstrated on any specimen. Furthermore, no neutralizing antibody against TGEV was demonstrated in the serum of recovered pigs. It was concluded that no TGEV was involved in this outbreak.

In an attempt to make etiologic diagnosis, formalin-fixed intestinal specimens from three affected piglets were subsequently processed for electron microscopy. Upon negative staining [11] of the intestinal contents, numerous coronavirus-like particles were detected in all

three specimens. The particles, shown in Fig. 2, had typical morphology of coronavirus. They were pleomorphic, 120 to 155 nm in diameter including the projections of approximately 15 to 22 nm in length. The projections formed a single fringe radiating from the core. None of the rotaviruses or any other virus except bacteriophages was recognized. Electron microscopic examination of the ultrathin section of the jejunum disclosed many virions in the cytoplasmic vesicles of degenerative epithelial cells (Fig. 2).

TGEV and rotavirus are known to be specifically associated with swine diarrhea. The former affects swine of all ages [7], whereas the latter is associated with only piglets [2, 3, 16]. At the present time, only two coronaviruses, TGEV and HEV, are known to be implicated in swine disease in Japan. TGE prevails throughout the country every year, and diagnosis is made by isolation or detection of the TGEV antigen. As already mentioned, TGEV was excluded from the cause of the diarrhea in this outbreak. HEV causes encephalomyelitis or so-called vomiting and wasting disease in suckling piglets [1, 4, 13]. Although serological survey showed an evidence of the presence of HEV [9], no isolation of HEV has been made in Japan. Clinical and epizootiologic features of this outbreak, diarrhea of all ages, were evidently distinct from that of HEV infection. The possibility that the coronavirus-like particles detected in the intestinal specimens were HEV was unlikely for this reason.

In conclusion, the present data strongly suggest that there is a new disease represented by diarrhea due to coronavirus-like particle, similar to those described in foreign countries [5, 6, 8, 14, 15].

When diarrhea clinically similar to TGE occurs, etiologic investigation is required for further precise diagnosis.

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### EXPLANATION OF FIGURES

Fig. 1. Marked shortening and depletion of the jejunal villi with moderate infiltration of neutrophols in the lamina propria. Hematoxylin and eosin stain. ×136.

Fig. 2. Coronavirus-like particles (arrow) within membrane-bound vesicles in the cytoplasm of a

jejunal epithelial cell.

N: nucleus. Uranyl acetate and lead citrate stain.  $\times 28,500$ 

Inset: Coronavirus-like particle in the intestinal content. Phosphotungstic acid staining.  $\times 193,200$ .

## 要 約

コロナウイルス様粒子の関与する新しい豚の下痢症の 日本における発症例 (短報): 高橋喜和夫・岡田幸助<sup>1)</sup>・大島寛一<sup>1)</sup> (水沢家畜保健衛生所, <sup>1)</sup>岩手大学農学部家畜病理学教室) ——1982 年 2 月岩手県内の1養豚場において, 臨床および疫学的に豚伝染性胃腸炎 (TGE) に似た下痢症が発生した。免疫螢光法, 組織培養および血清学的検索により TGE ウイルスの関与は否定されたが, 電顕的検索では腸内容に多教のコロナウイルス様粒子が検出され, また小腸上皮皮細胞の細胞質空胞内にもウイルス様粒子が観察された。以上から, コロナウイルスが関与する新しい豚の下痢症が, 日本においても存在することが示唆された。

