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Coronavirus-, Calicivirus-, and Astrovirus-Like Particles Associated with Acute Porcine Gastroenteritis

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ABSTRACT. Diarrheal stools of an acute porcine gastroenteritis were examined by electron microscopy and inoculation into cell cultures. Two types of virus, coronavirus-like and calicivirus-like, were detected by direct electron microscopy, and cytopathogenic astrovirus-like virus was isolated in ESK cell cultures. This is the first report on the detection of calicivirus- and astrovirus-like viruses in porcine diarrhea in Japan, and on the isolation of cytopathogenic astrovirus-like virus in cell cultures.—**KEY WORDS:** acute porcine gastroenteritis, astrovirus-like virus, calicivirus, coronavirus.

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In recent years, a direct application of electron microscopy to diarrheal stools of several animal species has led to discover a number of viruses and virus-like particles that include rotavirus, rotavirus-like particles, coronavirus, calicivirus, astrovirus and other small virus-like particles [1–4, 6, 8]. Because many of these viruses, except some cases, have not been isolated and cultivated in cell cultures so far, the direct electron microscopy of diarrheal stools is the only method available for their detection. In this report, coronavirus-like and calicivirus-like particles and astrovirus-like virus found in association with porcine diarrhea are described.

In February 1983, an outbreak of acute gastroenteritis occurred among pigs on a small farm in Tokushima Prefecture, where a total of 222 pigs (2 boars, 17 sows, 140 fattening pigs, and 63 suckling pigs) were reared. All pigs, regardless of age, developed clinical signs characterized by loss of appetite followed by vomiting and watery diarrhea, but death did not occur in pigs of any age. Transmissible gastroenteritis (TGE) virus or rotavirus was suspected as the causative agent of the disease. However, virological exami-

nations with immunofluorescence yielded negative results for both viruses. Furthermore, no seroconversion to TGE virus occurred in convalescent sera, indicating that the disease had been caused by neither TGE virus nor rotavirus. A 10% suspension of the mixed fecal samples from three 2-month-old pigs suffering from diarrhea was made with phosphate buffered saline and clarified by centrifugation at 7,000 rpm for 20 min at 4°C. The supernatant was filtered through a membrane filter with 450 nm pore size, laid on the cushion of 1.20 g/ml density sucrose solution and centrifuged at 111,700 xg for 2 hr at 4°C. The pellet was suspended in a drop of distilled water, placed on a 300 mesh collodion-coated grid, and stained with 0.2% phosphotungstic acid solution (pH 7.2) for 30 sec. The grid was examined with JEM-100 CX electron microscope (Nippon Denshi, Co.) at an acceleration voltage of 80 kV.

Two types of viral particles morphologically resembling coronavirus and calicivirus were observed. Fig. 1 shows coronavirus-like particles, which are round shape with petal-shaped projections. The diameter of these particles was between 120–140 nm, and the

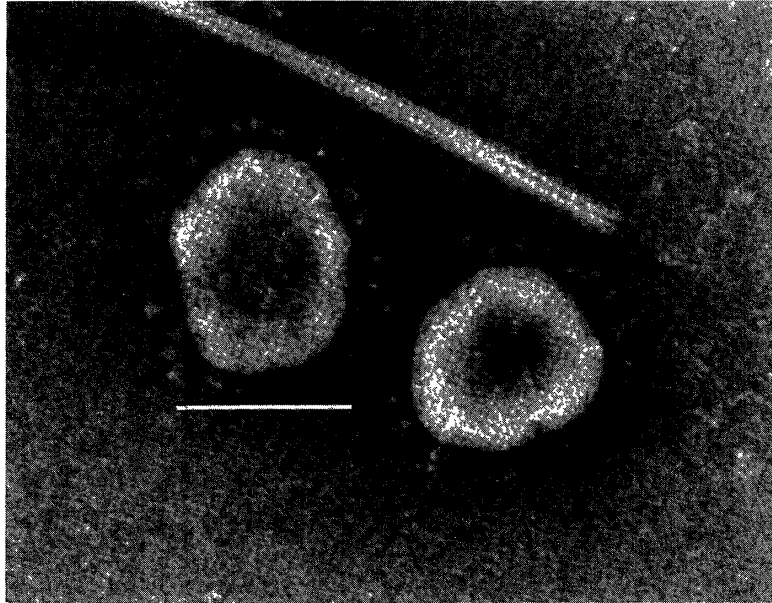


Fig. 1. Negatively stained coronavirus-like particles. Bar represents 100 nm.

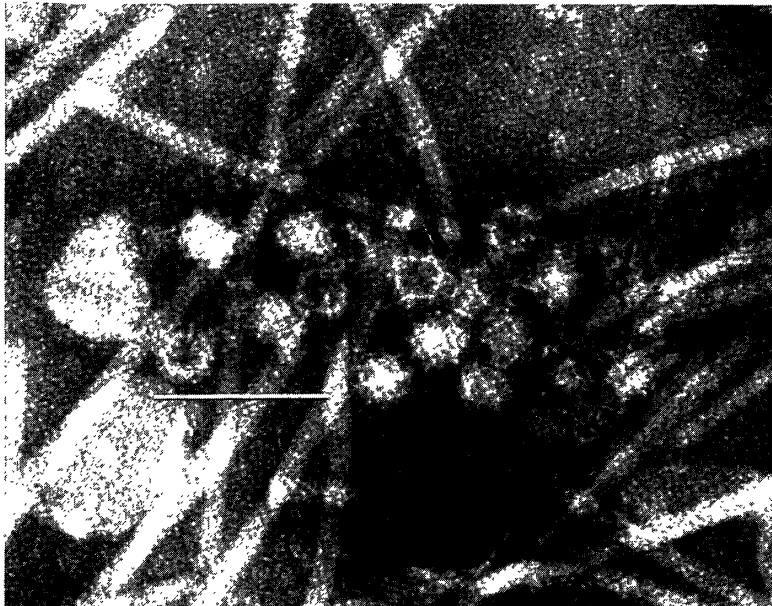


Fig. 2. Calicivirus-like particles in a sample stained with 0.2% phosphotungstic acid (pH 7.2). Bar shows 100 nm.

projections were 14 to 17 nm in length. Fig. 2 shows calicivirus-like particles. The shape of these particles resembles that of sea urchin. The diameter of the particles was approximately 35–40 nm. They had spiky projection on the surface. These morphological characteristics of coronavirus-like and calicivirus-like particles identify with the previous re-

ports [2, 5, 7, 8].

A portion of fecal suspension was inoculated with 0.5 $\mu\text{g/ml}$ of trypsin into cell cultures of ESK, which is an established cell line derived from porcine embryonic kidney [9]. After the 2nd passage in ESK cell cultures, a cytopathogenic agent was isolated. As shown in Fig. 3, electron microscopic examination

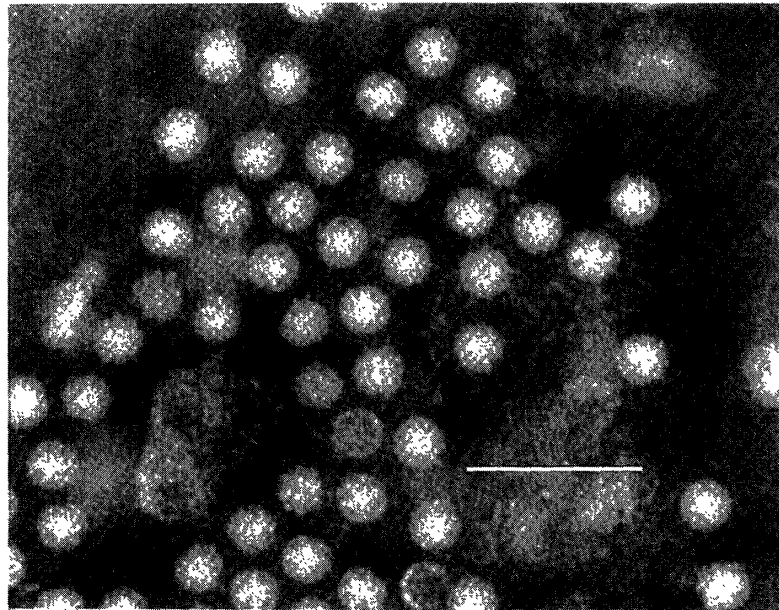


Fig. 3. Astrovirus-like particles in a sample prepared from culture fluid of infected ESK cells and stained with 0.2% phosphotungstic acid (pH 7.2). Bar shows 100 nm.

on purified viral material prepared from the culture fluids of infected ESK cells revealed the presence of viral particles morphologically resembling astrovirus [2, 3]. The astrovirus-like particles have a spherical shape with 28–30 nm in diameter, and a visible subunit structure with 5- or 6-pointed star which looks white is evident.

TGE virus and rotavirus have been well defined as causative agents of nonbacterial gastroenteritis in pigs. However, virological and serological examinations indicated that both viruses had not been involved in the case investigated. Electron microscopy on the diarrheal specimens revealed the presence of coronavirus-like and calicivirus-like particles, and isolated astrovirus-like virus in ESK cell cultures. Recently, a new coronavirus, so-called porcine epidemic diarrhea virus, which is immunologically distinguishable from TGE virus, has been recognized as one of the etiological agents of porcine viral gastroenteritis in several countries [5, 7]. In Japan, Takahashi *et al.* [10] have reported on enteric coronavirus associated with porcine diarrhea. Although relationship between por-

cine epidemic diarrhea virus and coronavirus found in our case remains obscure, it is possible to suspect that coronavirus-like particles detected might play a major role in induction of gastroenteritis studied in present report. Calicivirus and astrovirus have been detected in diarrhea of pigs [2, 8], human and other animals [1, 3, 4, 6]. In addition to these viruses, rotavirus-like particles, that are morphologically similar to rotavirus but share no common antigen with rotavirus, have been detected in porcine diarrhea [2, 8]. These seem to indicate that viral agents causing porcine gastroenteritis are very complex. However, their etiological significance as cause of nonbacterial gastroenteritis as well as their virological characteristics are still undefined since many attempts to propagate these viruses in cell culture have failed. Further investigations on these viruses should be carried out to recognize their importance in induction of gastroenteritis.

This is the first report on the detection of calicivirus-like and astrovirus-like particles in porcine diarrhea in Japan, and on the cytopathogenic astrovirus-like virus in cell

cultures. The details of isolation and characterization of cytopathogenic astrovirus-like virus will be described elsewhere.

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REFERENCES

1. Assaad, F.A., and Schild, G.C. 1983. *Arch. Virol.* 76: 275-288.
2. Bridger, J.C. 1980. *Vet. Rec.* 107: 532-533.
3. Bridger, J.C., Hall, G.A., and Brown, J.F. 1984. *Infect. Immun.* 43: 133-138.
4. Cukor, G., and Blacklow, N.R. 1984. *Microbiol. Rev.* 48: 157-179.
5. De Bouck, P., and Pensaert, M.B. 1980. *Am. J. Vet. Res.* 41: 219-223.
6. Holmes, I.H. 1979. *Prog. Med. Virol.* 25: 1-36.
7. Pensaert, M.B., and De Bouck, P. 1978. *Arch. Virol.* 58: 243-247.
8. Saif, L.J., Bohl, E.H., Theil, K.W., Cross, R.F., and House, J.A. 1980. *J. Clin. Microbiol.* 12: 105-111.
9. Sugimori, T., Morimoto, T., Miura, Y. and Sazawa, H. 1969. *Natl. Inst. Anim. Health Q. (Jpn.)* 9: 55-64.
10. Takahashi, K., Okada, K., and Oshima, K. 1983. *Jpn. J. Vet. Sci.* 45: 829-832.

要 約

コロナウイルス, カリチウイルスおよびアストロウイルスの関係した豚の急性胃腸炎: 白井淳資・清水実嗣・福所秋雄 (農林水産省家畜衛生試験場)——急性胃腸炎を示す豚の下痢便を検査したところ コロナウイルス様およびカリチウイルス様粒子が電顕により, またアストロウイルス様粒子が接種後細胞変性を示した ESK 培養細胞上清中に見出された。