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Short Communication

EPIZOOTIC DIARRHOEA OF ADULT CATTLE ASSOCIATED WITH A CORONAVIRUS-LIKE AGENT

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ABSTRACT

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An epizootic of acute diarrhoea of adult cattle occurred in Japan during the winter of 1976 to 1977. A majority of adult cattle clinically diagnosed as having the disease, showed significant rises in antibody titres to bovine coronavirus, whereas only a small minority showed serological evidence of recent infection with calf rotavirus, bovine adenovirus type 7, parainfluenza virus type 3, or bovine viral diarrhoea-mucosal disease virus. A coronavirus-like agent was detected by electron-microscopy in faecal material from a cow with diarrhoea, and was subsequently isolated in primary bovine kidney cell cultures.

INTRODUCTION

The aetiology of diarrhoea in the bovine animal is complex, and a variety of viruses have been associated with the syndrome. The purpose of the present report is to give serological evidence for an aetiological role of bovine coronavirus in an epizootic diarrhoea of adult cattle in Japan, with supportive electron-microscopical and cell culture studies.

MATERIALS AND METHODS

Herds concerned. In ten herds from central and western areas of Japan, 150 of 200 adult cows developed diarrhoea during the winter of 1976—1977. Milk production declined by an average 20% during the outbreak and for 3—4 weeks during recovery periods.

Serology. Paired serum samples were taken during the acute and convalescent stages of the syndrome, and were tested for haemagglutination-inhibiting (HI) or neutralizing (NT) antibodies against reference strains of bovine coronavirus (BVC) using techniques described by Sharpee et al. (1976) and Stair

et al. (1972), of calf rotavirus (NCDV, Fernelius et al., 1972), of bovine adenovirus type 7 (BAV-7 — Inaba et al., 1968), of parainfluenzavirus type 3 (PIV-3 — Inaba et al., 1963) and of bovine viral diarrhoea-mucosal disease virus (BVD-MD — Omori et al. 1967).

Electron microscopy (EM). A faeces sample taken during the acute phase was homogenised at 5% *v/v* in phosphate buffered saline (PBS) at pH 7.2. The suspension was clarified by centrifugation at 5000 *g* for 20 min, and the supernatant fluid was again centrifuged at 20,000 *g* for 30 min. A final centrifugation of the resulting supernatant fluid was made at 100,000 *g* for 2 h, and the pellet was resuspended in 1/50 original volume of PBS, and clarified at 20,000 *g* for 10 min. A drop of the supernatant fluid was examined by the phosphotungstic acid negative staining technique.

Virus isolation and immunofluorescence. Primary bovine kidney (BK) cell cultures were prepared in 100 × 11 mm tubes (Sato et al., 1978), and monolayers were infected with 0.1 ml of faeces extract prepared as above. After an adsorption period of 2 h at 37°C, infected cultures were washed 3 × with Earle's solution, fed with 0.5 ml of maintenance medium, and incubated at 37°C in a roller drum. Cultures were routinely checked for immunofluorescence at each passage, using a rabbit antiserum to bovine coronavirus (kindly supplied by Dr. C.A. Mebus, Lincoln, Nebraska, U.S.A.) conjugated to fluorescein isothiocyanate as described by Sato et al. (1978).

Serial ten-fold dilutions of the supernatant fluids were passaged weekly to fresh BK monolayers which were checked for cytopathic effect and immuno-fluorescence.

RESULTS

As shown in Table I, 59% of 100 paired sera showed significant seroconversion between acute and convalescent sera, as measured by the HI technique for antibody to BCV. Only a low percentage showed sero conversions to other viruses tested. Of 210 cattle tested for coronavirus antibody from 8 of the farms concerned, 139 (66.2%) showed levels of HI antibody to coronavirus greater than titre of 160 (Table I).

From diarrhoea faeces of an affected cow, EM preparations revealed the presence of numerous particles 60 to 120 nm in diameter, with the morphology of coronaviruses (Fig.1). Similar particles were observed in sucrose density gradient preparations (Sato et al., 1978) of fluids from infected BK cultures.

No cytopathic effect (CPE) or convincing immuno-fluorescence was seen during the first passage in vitro of faeces extract. From the second to the eighth passage in vitro fluorescent cells increased in number, and from the eighth passage CPE became obvious, characterised by syncytia formation and granularity of cells. This CPE, which became more distinct on further passage, was neutralised by rabbit antiserum to bovine coronavirus.

TABLE I

Seroconversion in paired serum of cattle with epizootic diarrhoea

Prefecture	BCV[1]	BRV[2]	BAV-7[3]	PIV-3[4]	BVD-MD[5]
Shizuoka	38/65*	3/65	5/65	7/65	3/65
Chiba	11/21	8/21	4/21	0/21	2/21
Tochigi	4/5	0/5	0/5	0/5	0/5
Shimane	6/9	0/9	0/9	0/9	0/9
Total	59/100	11/100	9/100	7/100	5/100

* No. of seroconverted cows/no. of cows tested.
[1] Bovine coronavirus
[2] Bovine rotavirus
[3] Bovine adenovirus type 7
[4] Parainfluenza virus type 3.
[5] Bovine viral diarrhoea-mucosal disease virus

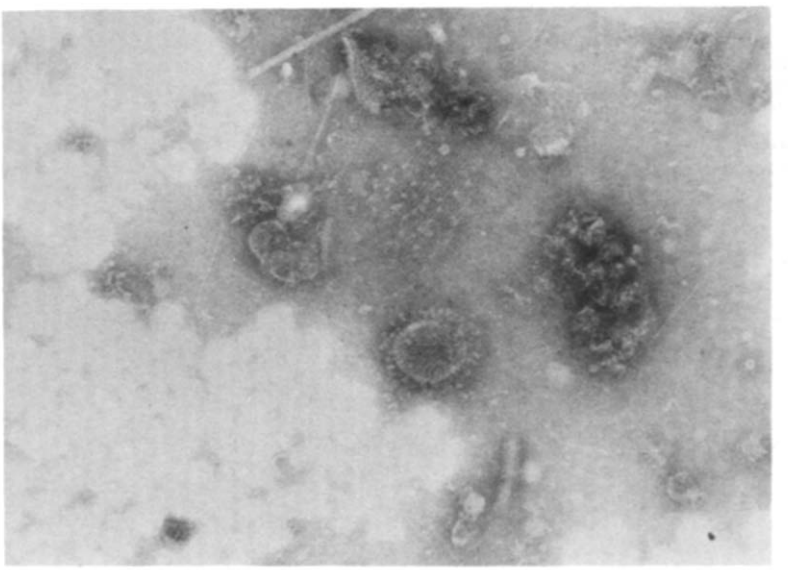

Fig.1. Electron micrograph of bovine coronavirus-like particle obtained from faecal extract. Negative stain. × 100,000.

DISCUSSION

Whilst there are numerous reports of coronavirus-like agents associated with calf diarrhoea (Stair et al., 1972, Bridger et al., 1978), only Horner et al. (1976) and more recently Durham et al. (1979) have reported such viruses in association with diarrhoea in adult cattle. Whilst the evidence is suggestive that the coronavirus-like particles are active during the diarrhoea, it still remains to be proven that they are indeed the aetiological agent of such diarrhoea.

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