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## Short Communication

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### Coronavirus as an Agent of Neonatal Calf Diarrhea in a Chinese Dairy Cattle Farm

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*With one figure and one table*

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#### Summary

Neonatal calf diarrhea is a significant health problem in dairy industry in the whole world. Although the aetiological agents of neonatal diarrhea are diverse, it is obvious that viruses play an important role. One of them is bovine coronavirus (BCV). Since BCV was discovered (MEBUS et al., 1969), there have been many published reports describing the virus and its importance in calves. In China, we first investigated the seroepidemiological status of BCV infections in cattle (YAO et al., 1990). The results showed that the incidence of infections with BCV in cattle in different Chinese regions are high. Further work on direct diagnosis of BCV as a causative agent of diarrhea was therefore necessary. In the last years we have developed diagnostic methods including a monoclonal antibody-based ELISA to detect BCV and other diarrheal viruses. In this study, the diagnosis of an outbreak of BCV in Chinese dairy cattle is described.

#### *Clinical Aspects*

In early 1989, an outbreak of calf diarrhea occurred in a dairy herd in Nanjing, southeastern China, affecting calves up to two months of age. The morbidity was 98.9% (78 out of 79 calves) and the mortality was 10.1% (8 out of 79 calves). Of 78 affected calves, 73 were 1–20 days old and all of the 8 calves that died belonged to this group. The duration of diarrhea was approximately three–eight days. In this dairy farm, 73 newborn calves were derived from cows imported from Canada, the others from native cows. The diarrhea originated from the imported calves and then rapidly spread to the whole herd. The main manifestation of the disease was serious watery diarrhea with yellowish feces accompanied by lack of appetite and extreme weakness, but normal temperature. In some of the diseased calves, foamy mucus and flocks of undigested milk could be observed in the

faeces. Treatment with antibiotics had no effect. Infusion of electrolyte-glucose solutions was to a certain extent useful in the treatment of affected calves.

#### *Aetiological Diagnosis*

Autopsy of dead calves showed that the small intestine was distended with yellowish mucus and watery contents and the mucosal surface was defect.

Nine fecal samples from sick calves and one piece of small intestine containing the contents from a dead calf were collected for bacteriological and virological examination. For virological diagnosis, the samples were diluted 1:10 in phosphate-buffered saline (PBS), thoroughly suspended and centrifuged at 2.200 g for 30 min. The supernatants were tested by the following methods: I. ELISA for detection of rotavirus antigen as described (BACHMANN, 1979). II. HA-HI for detection of bovine coronavirus antigen as described by BALJER et al. (1987) and LU et al. (1988), i. e. erythrocytes of rat or mice were used. BCV-specific hyperimmune serum was produced in rabbits. III. ELISA for detection of BCV-antigen as described by CZERNY and EICHHORN (1989). Additionally, monoclonal antibodies to BCV were produced in Nanjing (XU, 1988) and used in an ELISA. Here, a mixture of two monoclonal antibodies was coated onto microplates as catching antibodies. Rabbit hyperimmune serum and a commercial HRP-conjugated anti-rabbit IgG-antiserum were used as detecting antibodies. IV. For electron microscopy, grids were prepared according to standard techniques. V. Bacteriological examination for detection of *Salmonella* and *E. coli* was carried out following routine methods with SS-Agar. VI. Detection of cryptosporidia was performed as described by BALJER et al. (1987).

#### **Results**

All tested samples were negative for rotavirus, cryptosporidia, *Salmonella* and *E. coli*. When tested for the presence of BCV-antigen, four out of ten samples were positive by HA-HI and six were positive by ELISA. The gut content was positive both by HA-HI and ELISA. Furthermore, typical viral particles resembling coronaviruses were seen by EM both in Nanjing and in Munich (Fig. 1 and Table 1).

#### **Discussion**

Based on the epidemiological observations and the results of aetiological diagnosis it should be concluded that bovine coronavirus was the causative agent for this outbreak of calf diarrhoea in a dairy farm in Nanjing. It is suggested that introduction of the imported cows which might carry a new causative agent or a new, highly pathogenic viral strain resulted in the occurrence of serious diarrhoea, as the sickness appeared first in imported newborn calves.

Table 1. Results of BCV diagnosis with different methods

Sample No.	HA-Titer	Confirmed as BCV by HI	ELISA-Titer
2022	1:160	+	1:640
2046	<1:10	-	<1:10
2134	<1:10	-	<1:10
2144	1:40	-	1:160
2166	1:40	-	<1:10
2170	<1:10	-	<1:10
BH	<1:10	-	1:80
WH	1:2560	+	>1:2560
177	1:80	+	1:320
177-gut	1:2560	+	>1:2560

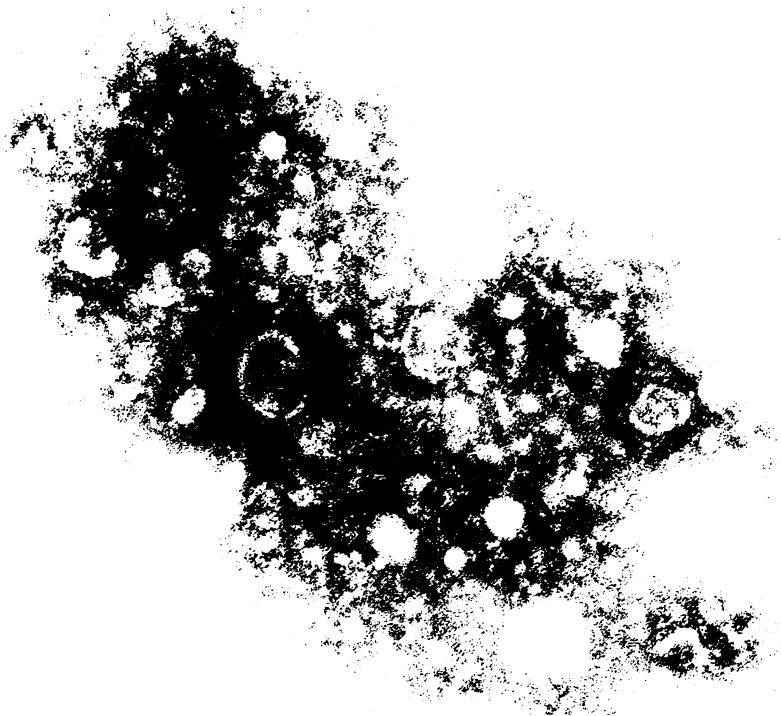


Fig. 1. Coronaviral particles in a fecal sample from a calf with diarrhea. Magnification: 31,500 $\times$

Newborn calf diarrhea induced by bovine coronavirus is observed worldwide. The present communication reports, for the first time in China, by complete diagnosis, coronavirus as an aetiological agent of neonatal calf diarrhea in a chinese dairy farm. The complete diagnostic methods by means of EM, HA-HI and ELISA to detect BCV, which we employed here, increased the reliability of the examination. By electron microscopy, coronavirus-like particles can be found in nearly every fecal sample. Therefore, to diagnose BCV EM-technique alone is not sufficient as false positive results will probably frequently occur. Additionally, the ELISA turned out to be more sensitive and specific than HA-HI for the detection of BCV antigen in feces.

In China, the participation of rotaviruses, cryptosporidia, *Eimeria* spp. and bacteria in diarrheal problems in calves has been reported (LU et al., 1988; SONG et al., 1985). Now, the participation of BCV is also established. The rapid diagnosis, prevention and attempts to the control of neonatal calf diarrhea with special reference to the possible occurrence of antigenetically different BCV-strains awaits further investigation.

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