The Infectivity and Pathogenicity of a Group 2 Bovine Coronavirus in Pups

Takashi KANESHIMA1), Tsutomu HOHDATSU1)*, Ryoko HAGINO1), Sakiko HOSOYA1), Yui NOJIRI1), Michiko MURATA1), Tomomi TAKANO1), Maki TANABE1), Hiroshi TSUNEMITSU2) and Hiroyuki KOYAMA1)

1)Department of Veterinary Infectious Disease, School of Veterinary Medicine and Animal Science, Kitasato University, Towada, Aomori 034–8628 and 2)Viral Diseases Research Team, National Institute of Animal Health, Tsukuba, Ibaraki 305–0856, Japan

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ABSTRACT. Canine respiratory coronavirus (CRCoV), which is more closely related to the bovine coronavirus (BCoV), has recently been detected in dogs. In this study, we examined whether BCoV was capable of infecting and exhibiting pathogenicity in dogs. Three 1-month-old pups were oronasally given field isolates of BCoV, and were kept together with 2 control animals. As a result, increases in BCoV-neutralizing antibody titers were confirmed in all pups in the challenged and control groups. Moreover, the virus gene was also detected in oral and rectal swabs by RT-PCR. These results indicate that BCoV infects dogs, and easily infects other dogs that are kept together. However, no clinical symptoms such as respiratory symptoms and diarrhea were observed.

NOTE. Virology

KEY WORDS: BCoV, Coronavirus, CRCoV.

In this study, we inoculated three 1-month-old pups with BCoV oronasally, and examined whether BCoV 1) infected the pups, and 2) caused respiratory or gastrointestinal symptoms in them. In addition, we kept these 3 pups together with 2 control pups to examine whether the BCoV infection became established in the control pups. Five 1-month-old, coronavirus antibody-negative beagle pups that had been kept in a temperature-controlled, isolated animal room for use in animal experiments were used.

A 75% emulsion of diarrheal stools obtained from two colostrum-deprived calves that had been inoculated with a BCoV-positive diarrheal stool was used as the Japanese BCoV field isolate. This diarrheal stool emulsion had been confirmed to be positive for BCoV by detecting the hemagglutination/esterase (HE) gene via PCR and the viral antigen by electron microscopy and ELISA (Bio-X Diagnostics, Belgique), and causes diarrhea in calves.

Dogs Nos. 1, 2, and 3 were oronasally administered 1 ml of the diarrheal stool emulsion, and kept together with 2 control dogs, Nos. 4 and 5. They were observed for clinical symptoms every day, and rectal and oral swabs and blood samples were taken every day in the first week, every 2 to 3 days in the second and third weeks, every 3 to 4 days in the fourth and fifth weeks, and every 7 days thereafter. In these samples, leukocyte and lymphocyte counts were determined, the viral gene was detected by RT-PCR, and anti-BCoV antibodies were detected by a neutralization test. In addition, from day 58 after virus administration, 10 mg/kg, 5 mg/kg, and 3 mg/kg of the immunosuppressive drug methylprednisolone acetate (Pfizer, Tokyo) were injected intramuscularly on days 1, 2, and 3, respectively, into all pups. After the administration of the immunosuppressive drug, rectal and oral swabs and blood samples were taken every 3 to 4 days.

The HE gene of BCoV was detected by RT-PCR according to the method of Kaneshima et al. [8]. Anti-BCoV neutralizing (NT) antibodies were detected...
by the method of Kaneshima et al. [8] using the Mebus strain of BCoV and HRT-18 G human rectal tumor cells.

Figure 1 shows chronological changes in NT antibody titers after the administration of the BCoV-positive diarrheal stool emulsion. At the time of BCoV administration (on day 0), all pups in the challenged and control groups had NT antibody titers of less than 1:5. However, in the challenged group, dog Nos. 3, 1, and 2 had an NT antibody titer of 1:5 on days 9, 11, and 14, respectively. Thereafter, the NT antibody titers rose gradually until day 45, when dog Nos. 1, 2, and 3 had titers of 1:40, 1:20, and 1:20, respectively. In the control group, dog No. 4 had an antibody titer of 1:5 on day 9, and dog No. 5, 1:20 on day 20. Thereafter, the antibody titers rose rapidly to 1:160 on day 45 in the 2 pups. The HE gene of BCoV was detected in rectal and oral swab samples by RT-PCR. In the challenged group, the rectal swab from dog No. 2 on day 1 and that from dog No. 3 on day 5 were positive for the HE gene. In the control group, the oral swab on day 5 and the rectal swab on day 11 from dog No. 5 were positive for the HE gene. All other samples were negative.

After the administration of the BCoV-positive diarrheal stool emulsion, all 3 pups in the challenged group had a transient loss of appetite, but had no clinical symptoms such as fever, respiratory symptoms, and diarrhea, and the leukocyte and lymphocyte counts varied slightly within normal limits. Similar to the dogs in the challenged group, dog Nos. 4 and 5 in the control group had no clinical symptoms.

From day 4 after the administration of methylprednisolone acetate, marked decreases in lymphocyte counts and NT antibody titers (Fig. 1) were observed. However, no clinical symptoms such as fever, respiratory symptoms, and diarrhea were noted, and no viruses were detected in rectal or oral swab samples.

CRCoV has been shown to be 98.8% and 98.4% homologous to BCoV and HCoV-OC43, respectively, in terms of the nucleotide sequence of the polymerase gene. It has also been reported that the amino acid sequence of the spike protein of CRCoV is 96.0% and 95.2% homologous to those of BCoV and HCoV-OC43, respectively [5]. The research group of Erles et al. (the Royal Veterinary College) has recently isolated CRCoV using HRT-18G cells, established an ELISA system for the detection of antibodies to CRCoV antigen, and reported that the results of CRCoV antigen detection were well correlated with those of BCoV antigen detection [10]. The cross-reactivity of BCoV antigen with canine antibodies in ELISA suggests its genetic and immunological similarity to CRCoV. In this study, we showed that the oro-nasal administration to pups of a field isolate of BCoV pathogenic in calves (causing diarrhea) resulted in the production of NT antibodies, and that the BCoV gene was detected in oral and rectal swab samples; in other words, BCoV was infective for pups. We also showed that the 2 control pups became positive for BCoV-neutralizing antibodies, indicating that contact infection with BCoV occurs readily among dogs.

During the period of the experiment, all 5 dogs remained free from respiratory symptoms and gastrointestinal symptoms such as diarrhea, suggesting that the pathogenicity of BCoV in dogs is very weak. Monoinfection with CRCoV causes mild or no respiratory symptoms, but superinfection with other pathogens is considered to aggravate the symptoms [3–5]. Erles et al. [5] detected CRCoV most frequently in dogs with mild respiratory symptoms, but less frequently in dogs with more severe symptoms. This observation suggests that the role of CRCoV in canine infectious respiratory disease (CIRD) is to facilitate the invasion into the body of other pathogens causing more severe respiratory symptoms, thereby aggravating the disease. We speculate that since this study involved experimental monoinfection with BCoV, no symptoms developed. In future studies, an experimental mixed infection with CIRD-related pathogens

![Fig. 1. Chronological changes in BCoV-neutralizing antibodies. Black circle, Dog No. 1; Black triangle, Dog No. 2; Black square, Dog No. 3; White triangle, Dog No. 4; and White square, Dog No. 5.](image-url)
and BCoV needs to be performed to examine the involvement of group 2 coronaviruses in CIRD.

REFERENCES