Serological Comparison of Cytopathogenic and Non-Cytopathogenic Bovine Viral Diarrhea-Mucosal Disease Viruses Isolated from Cattle with Mucosal Disease

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ABSTRACT. Ten cattle that died with mucosal disease were examined for bovine viral diarrhea-mucosal disease (BVD-MD) viruses. Both cytopathogenic (CP) and non-cytopathogenic (NCP) BVD-MD viruses were isolated concomitantly from 9 of them, and only a CP virus was recovered from the other. Then each pair of CP and NCP viruses was compared serologically by a serum neutralization test. Each pair of CP and NCP viruses from the same cattle was found to be serologically indistinguishable, although a minor antigenic difference was observed among the groups of the paired viruses. These results seem to support the hypotheses that mucosal disease occurs in persistently infected cattle which were induced by in utero infection with NCP virus when they are superinfected with CP virus, and the antigenic homology in CP and NCP BVD-MD viruses may be an important factor in the pathogenesis of the disease.—KEY WORDS: antigenicity, BVD-MD virus, mucosal disease, persistent infection.

The common form of bovine viral diarrhea-mucosal disease (BVD-MD) virus infection in cattle is mild or subclinical disease, that is characterized by transient fever, inappetence, and leukopenia with or without diarrhea, with the exception of the fatal form of mucosal disease. When pregnant cattle are exposed to non-cytopathogenic (NCP) BVD-MD virus, however, transplacental infection occurs frequently and virus causes various congenital anomalies of fetuses [20]. Most interestingly, when pregnant dams are infected with NCP BVD-MD virus at the early period of pregnancy, fetuses may become immunotolerant to the infected virus, and subsequently calves that are apparently normal but persistently infected with NCP virus may be born [10, 13, 16].

Mucosal disease that is characterized clinically by severe diarrhea mixed with blood and mucus is the sporadic but fatal form of BVD-MD virus infection. Usually normal cattle exposed to the virus isolated from the cases of mucosal disease are not affected with the disease. The disease can be reproduced experimentally in cattle persistently infected with NCP BVD-MD virus by exposure to cytopathogenic (CP) virus [2–5, 18]. Furthermore, both NCP and CP viruses are recovered from cases of naturally occurring mucosal disease [1, 6, 8, 12]. These facts appear to indicate that persistently infected cattle induced by in utero infection with NCP virus may be population at high risk of developing mucosal disease, as suggested by Malmquist [11] and others [15, 19]. The pathogenesis of mucosal disease, however, remains obscure.

In the present study, the antigenicity and biotype of BVD-MD viruses isolated from naturally occurring mucosal disease were investigated in order to gain information on the pathogenesis of the disease.
MATERIALS AND METHODS

Subjects: Ten cattle, approximately 6 to 12 months old, affected with severe diarrhea mixed with blood and mucus were investigated. Pathological examination on the digestive organs of all subjects revealed the presence of typical lesions of mucosal disease. The ileum, mesenteric lymph nodes, spleen, lung, brain, and thyroid obtained from affected cattle were used for the isolation of BVD-MD viruses.

Cell culture: Bovine fetal muscular (BFM) cell cultures were used for virus isolation and a neutralization test. BFM cell cultures were prepared by the ordinary tissue culture method, as described previously [17].

Viruses: The virus used as the challenge virus in an interference test was the CP Nose strain [9]. The NCP No. 12 [14], NCP KS86–1 [17] and the CP Nose strains were used as the reference viruses in the neutralization test.

Virus isolation: NCP BVD-MD virus was assessed by its ability to interfere with the multiplication of CP virus in cell culture [7]. Four to 10 tubes of BFM cell cultures were inoculated with 0.1 ml of serial 10-fold dilutions of tissue suspensions. After adsorption for 1 hour at 37°C, the cultures received 0.5 ml of the maintenance medium, and were incubated at 37°C. The cultures were observed daily for cytopathic effect (CPE), and culture media were harvested for cloning of CP virus when CPE appeared. Five days after inoculation, the remaining cultures were washed twice with Eagle’s minimum essential medium, refed with 0.5 ml of the maintenance medium containing 10^4.0TCID_{50}/ml of the CP Nose strain, incubated for 3 days at 37°C, and observed for CPE. The cultures without CPE were considered as being infected with interfering NCP BVD-MD virus. BVD-MD viruses isolated were purified by either terminal dilution or plaque method on 4 occasions, and designated as follows: 1-CP and 2-NCP. These mean CP virus isolated from calf No. 1, and NCP virus from calf No. 2, respectively.

Antisera: Antisera were prepared in cattle by inoculation with 2 ml of respective viruses titering about 10^0.0TCID_{50}/ml. Sera were obtained 4 to 5 weeks after inoculation, and used in the neutralization test.

Neutralization Test: The neutralization test was carried out with the microtitration method as described previously [17]. The neutralizing antibody titer was expressed as the reciprocal of the highest serum dilution that had neutralized virus.

RESULTS

Virus isolation: CP virus was recovered most often from the ileum, mesenteric lymph nodes, and spleen. On the other hand, the lung, thyroid, and brain were more suitable for the isolation and segregation of NCP virus than the ileum, mesenteric lymph nodes, and spleen, although the later tissues also contain NCP virus. It was difficult to segregate NCP virus from the ileum, mesenteric lymph nodes, and spleen, because those tissues contained a large amount of CP virus.

As indicated in Table 1, both CP and NCP BVD-MD viruses were isolated concomitantly from 9 of 10 cattle tested. Only CP virus was obtained, but NCP virus was not segregated, from the other calf, in which the tissues other than the ileum and spleen were not available for examination.

Antigenical characterization of isolates: Antisera to the paired viruses isolated from 2 cattle with mucosal disease were prepared, and their antigenic properties were compared by the cross-neutralization test. Although a minor difference in antigenicity was existent between the groups of the paired viruses, each pair of CP and NCP
viruses isolated from the same calf was antigenically indistinguishable (Table 2).

Then 9 pairs of CP and NCP viruses recovered from affected cattle were examined antigenically by the neutralization test using antisera to the isolates 1-CP and 2-CP, and the CP Nose, the NCP No. 12 and KS86-1 strains of the reference viruses. As indicated in Table 3, CP and NCP BVD-MD viruses isolated from the same animals showed similar antigenicity to each other. However, a slight difference in antigenicity was observed among the groups of paired viruses isolated from the different cattle.

**DISCUSSION**

Both CP and NCP BVD-MD viruses were isolated from naturally occurring mucosal disease with high frequency. This result agrees with the previous reports [1, 6, 8, 12] and supports the hypothesis that persistently infected cattle induced by in utero infection with NCP BVD-MD virus may later succumb to mucosal disease [2, 3, 4, 5, 11, 15, 19].

CP BVD-MD virus was recovered most frequently from the small intestine and lymphoid tissues, while NCP virus was segregated more easily from other tissues. This is probably due to the presence of a higher proportion of CP virus in the small intestine and lymphoid tissues, and may suggest that a tissue tropism of CP and NCP viruses is different.

### Table 1. Isolation of BVD-MD viruses from cattle affected with mucosal disease

<table>
<thead>
<tr>
<th>Biotype of virus isolated</th>
<th>No. of cattle</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytopathogenic and non-cytopathogenic</td>
<td>9</td>
</tr>
<tr>
<td>Cytopathogenic</td>
<td>1</td>
</tr>
<tr>
<td>Non-cytopathogenic</td>
<td>0</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>10</strong></td>
</tr>
</tbody>
</table>

### Table 2. Cross-neutralization test of the paired viruses isolated from 2 cattle affected with mucosal disease

<table>
<thead>
<tr>
<th>Virus&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Antiserum 1-CP</th>
<th>Antiserum 1-NCP</th>
<th>Antiserum 2-CP</th>
<th>Antiserum 2-NCP</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-CP</td>
<td>4,096&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1,024</td>
<td>1,024</td>
<td>1,024</td>
</tr>
<tr>
<td>1-NCP</td>
<td>4,096</td>
<td>2,048</td>
<td>512</td>
<td>2,048</td>
</tr>
<tr>
<td>2-CP</td>
<td>1,024</td>
<td>128</td>
<td>1,024</td>
<td>4,096</td>
</tr>
<tr>
<td>2-NCP</td>
<td>2,048</td>
<td>512</td>
<td>1,024</td>
<td>8,192</td>
</tr>
</tbody>
</table>

<sup>a</sup> Numbers, CP and NCP indicate No. of cattle tested, and cytopathogenic and non-cytopathogenic viruses, respectively.

<sup>b</sup> Neutralizing antibody titers

Antigenic similarity of CP and NCP viruses isolated from the same calf affected with mucosal disease was first reported by Howard et al. [8]. They have tested 5 pairs of CP and NCP viruses isolated from different outbreaks, and have found that both viruses were antigenically indistinguishable within each group. The results of this study using 9 paired viruses were coincident with those reported by Howard et al. [8].

In the previous study [18], we have found that 3 of 4 persistently infected cattle succumbed to mucosal disease or severe chronic diarrhea after exposed to the CP BVD-MD viruses that are antigenically different from the NCP persistent viruses, and the CP viruses isolated from carcasses at necropsy were antigenically different from the challenge viruses but similar to the NCP persistent viruses. Furthermore, Brownlie et al. [5] have reported that all persistently infected cattle challenged with homologous CP virus succumbed to mucosal disease within 2 to 3 weeks, but only 2 of 6 cattle developed the disease with a prolonged incubation period of 98 and 146 days when challenged with heterologous CP virus. These facts suggest that antigenic homology of CP and NCP viruses may be an important factor in the pathogenesis of mucosal disease.
Little is known, however, about the origin of the CP viruses that are recovered from cattle with mucosal disease and antigenically identical to the NCP persistent viruses. On the basis of evidence that BVD-MD viruses, other than those from mucosal disease, are almost always NCP, Howard et al. [8] have suggested that a mutation occurs from the NCP form to the CP one in the virus causing persistent infection, and this causes mucosal disease and account for the antigenic similarity of CP and NCP isolates from naturally occurring mucosal disease. Another speculation for this is that a mutation of antigenicity or biotype in CP or NCP virus may occur in persistently infected cattle during superinfection with CP virus, and the CP mutant with the same antigenicity as the NCP persistent virus may cause mucosal disease.

Further studies on the genetics and mutation of BVD-MD virus, and on the interaction between NCP and CP viruses in the course of superinfection are expected to elucidate the pathogenesis of mucosal disease.

ACKNOWLEDGEMENTS. We thank Mr. N. Nishioka for his technical assistance, the veterinary officials of Rumoi, Kamikawa, Ishikawa, Tokachi, Nemuro and Abashiri Livestock Hygiene Centers of Hokkaido Prefecture for their cooperation in collection of materials of mucosal disease, and Dr. O. Itoh, National Veterinary Assay Laboratory, for his kind supply of antiserum to the No. 12 strain of BVD-MD virus.
REFERENCES


要約

粘膜病牛から分離した細胞病原性ウイルス性下痢—粘膜病ウイルスの血清学的性状：清水実綾・村上喜一

佐藤国雄（家畜衛生試験場北海道支場，1日目家畜保健衛生所）—粘膜病発病牛1頭についてウイルス学的
検査を実施した。9頭からは細胞病原性と非細胞原性の牛ウイルス性下痢—粘膜病ウイルス二種が分離された。1
頭からは細胞病原性ウイルスのみが分離されたが，分離材料を選択することにより非細胞病原性ウイルスの分離
も可能と思われた。分離ウイルスの血清学的性状を中和試験で調べたところ，発病牛によって多少の差異が認め
られたが，同一個体から分離した細胞病原性と非細胞病原性ウイルスは類似した抗原性状を示した。以上の成績
は，粘膜病は非細胞病原性ウイルスの子宮内感染により誘導された持続感染牛に発生し，非細胞病原性の持続感
染ウイルスと細胞病原性ウイルスの抗原性状の一致が粘膜病の病理に関係するという仮説を支持するものと思わ
れる。