Comparison of acute infection of calves exposed to a high-virulence or low-virulence bovine viral diarrhea virus or a HoBi-like virus

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Objective—To compare acute infection of cattle exposed to a high-virulence (HV) bovine viral diarrhea virus (BVDV), low-virulence (LV) BVDV, or HoBi-like virus.

Animals—24 Holstein bull calves.

Procedures—Colostrum-deprived 2- to 4-week-old calves, free of BVDV antigen and antibodies, were allocated into 4 groups (6 calves/group). Calves in 3 groups were exposed to an LV BVDV strain (BVDV2-RS886), an HV BVDV strain (BVDV2-1373), or a HoBi-like virus (D32/00 HoBi), whereas calves in the fourth group were not exposed to a virus but were cohoused with calves exposed to the HoBi-like virus. Circulating WBCs, platelets, rectal temperature, and presence of virus in the blood were monitored.

Results—Infection of calves with any of the 3 viruses resulted in reduced numbers of circulating WBCs. Pyrexia was detected in all calves exposed to HV BVDV or LV BVDV but in only 3 of 6 calves exposed to the HoBi-like virus. Diarrhea was observed in 6 of 6 calves exposed to the HoBi-like virus, 2 of 6 calves exposed to the LV BVDV, and 6 of 6 calves exposed to the HV BVDV. The HoBi-like virus was transmitted from acutely infected calves to naïve cohorts.

Conclusions and Clinical Relevance—The HoBi-like viruses are an emerging species of pestivirus isolated from water buffalo and cattle in South America, Southeast Asia, and Europe but not from cattle in the United States. Understanding the clinical course of disease caused by HoBi-like pestiviruses will be important for the design of surveillance programs for the United States. (Am J Vet Res 2013;74:438–442)
The first HoBi-like virus, D32/00 HoBi, was isolated from a batch of fetal calf serum that originated in Brazil.1,2 Subsequently, 9 other HoBi-like viruses have been isolated. Of these 10 reported HoBi-like viruses, the known or deduced origin is Brazil for 8,3 Thailand for 1,4 and Italy for 1.5 Although viral genomic sequences similar to those of HoBi-like viruses have been amplified from fetal bovine serum labeled as originating in the United States and Australia, replicating virus has not been isolated from samples originating in these 2 countries.6

The isolation of HoBi-like viruses from aborted fetuses7,12,13 a calf apparently persistently infected with BVDV,14 and cattle after an outbreak of respiratory tract disease9 indicates that infection with these viruses may result in clinical disease. The isolation of HoBi-like viruses from fetal bovine serum15 raises the concern of spread of these viruses via biological products that contain or are produced using fetal bovine serum.

A HoBi-like virus has been isolated from cattle with severe respiratory tract disease6; however, researchers were not able to replicate this clinical syndrome in controlled experimental conditions. Use of HoBi-like strains to experimentally induce infections in cattle14,15 and sheep16 resulted in mild clinical disease characterized by moderate hyperthermia and reduced leukocyte counts. In contrast, experimentally induced infection of pigs resulted in moderate hyperthermia and reduced leukocyte counts. In another study,17 infection of cattle with this strain resulted in low-grade pyrexia that lasted for 1 or 2 days and a transient decrease in circulating lymphocytes. Behavior and food intake were unaffected by the infection, and all calves recovered. The HoBi-like virus used was D32/00 HoBi.1 It was the first HoBi-like virus described in the literature and was isolated from a batch of fetal bovine serum imported into Switzerland from Brazil.4

All 3 viral strains were propagated in bovine turbinate cells that had been tested and were found to be free of BVDV and HoBi-like viruses.19 Cells were grown in complete cell culture medium composed of minimal essential media supplemented with 10% fetal bovine serum, 1-glutamine (final concentration, 1.4 mM), and gentamicin (final concentration, 50 mg/L). Fetal bovine serum was tested and found to be free of BVDV and HoBi-like viruses and antibodies against BVDV or HoBi-like viruses.23 Viral titers were determined via dilution on bovine turbinate cells.25 Endpoints were based on monoclonal antibodies that bound the E2 protein of BVDV2-1373, BVDV2-RS886, and D32/00 HoBi.25

Exposure to virus—Calves were assigned to treatment groups (6 calves/group). Of the 24 colostrum-deprived calves, 22 were seronegative for D32/00 HoBi strain or BVDV prior to the start of the experiment. Two calves had neutralizing serum antibody titers (determined via a virus neutralization test) against BVDV and D32/00 HoBi in samples obtained at birth. For those 2 calves, one had titers of 1:64 against BVDV2, 1:128 against BVDV1, and 1:8 against D32/00 HoBi, and the other calf had titers of 1:128 against BVDV2, 1:256 against BVDV1, and 1:4 against D32/00 HoBi. These 2 calves plus 4 calves seronegative for BVDV2, BVDV1, and D32/00 HoBi were assigned as the control group. The remaining 18 calves were assigned to 3 virus-exposure treatment groups.

Calves in the 3 virus-exposure groups were inoculated with LV BVDV, HV BVDV, or HoBi-like virus,
respectively, via the nasal route (fluids were instilled directly into the nasal passages) with 5 mL of cell culture lysate that contained a virus load of 10^6 TCID/mL. Calves in the control group were mock inoculated with 5 mL of cell culture lysate prepared from noninfected cells. Day of inoculation was designated as day 0. The experiments were conducted in replicates (3 calves/group in each replicate) because of space limitations in biosecurity level 2 and 3 containment facilities and to determine reproducibility of results.

Calves inoculated with BVDV were housed in separate rooms equipped with high-efficiency particulate air filters. Calves inoculated with the HoBi-like virus and the control calves were housed in the same room, which also had high-efficiency particulate air filters, beginning 24 hours after inoculation. Cohousing of these 2 groups allowed us to evaluate virus transmission. Biosecurity measures maintained throughout the study included no sharing of equipment and the requirement that all animal caretakers shower before moving between rooms.

Monitoring of clinical signs—Calves were observed at least twice daily for signs of clinical disease. A health-scoring system for calves was used. Rectal temperatures were obtained daily from day –2 to day 0. Baseline rectal temperature for each calf was determined by calculating the mean daily temperature recorded for the 2 days preceding and > 0.6°C higher than the baseline rectal temperature; baseline rectal temperature for each calf was recorded as a rectal temperature > 0.6°C higher than the baseline rectal temperature; baseline rectal temperature for each calf was determined as day 0. Pyrexia was defined as pyrexia, as determined on the basis of the highest temperature recorded, differed on the basis of virus and on days –2, 2, 4, 6, 8, 10, 12, 14, and 18 for calves exposed to the HV and LV BVDV isolates. These time points represented the typical first observation of clinical signs (day 3 or 4), midpoint of clinical disease (days 6 through 9), recovery (days 10 through 14), and seroconversion (days 14 to 18), as determined on the basis of previous studies. Nasal swab specimens were collected from only the control calves and calves exposed to the HoBi-like virus; these specimens were collected on days –2, 3, 6, 9, 11, and 13.

Results—A decrease in the number of circulating WBCs was detected in all calves from each of the 3 viral-inoculated groups (Figure 1). This decrease was substantially larger in calves inoculated with HV BVDV than in calves inoculated with LV BVDV or the HoBi-like virus. Only calves infected with HV BVDV developed thrombocytopenia.

Pyrexia was detected in all calves inoculated with LV BVDV or HV BVDV and in 3 of 6 calves inoculated with the HoBi-like virus (Table 1). The severity of pyrexia, as determined on the basis of the highest rectal temperature recorded, differed on the basis of the virus used for inoculation. Although there was variation among calves inoculated with the same virus, some patterns were evident. Rectal temperatures > 41.1°C were detected only in calves inoculated with HV BVDV. No calf inoculated with the HoBi-like virus had a rectal temperature > 40.0°C. Similarly, the duration of pyrexia differed on the basis of virus and calf (Figure 2). For calves inoculated with LV or HV BVDV, the duration of pyrexia ranged from 1 to 13 days and 7 to 13 days, respectively. In calves inoculated with the HoBi-like virus that became pyreptic, pyrexia was detected for only 1 day.

All of the calves inoculated with HV BVDV developed diarrhea. None of the calves inoculated with the HoBi-like virus developed diarrhea.

![Figure 1](image)

**Figure 1**—Mean ± SEM decrease in the percentage of WBCs in calves (6/group) after exposure to a HoBi-like pestivirus, LV BVDV, or HV BVDV. Results represent the greatest percentage decrease from baseline values (number of circulating WBCs in samples obtained 2 days before viral exposure). The experiment was performed in 2 replicates (R1 and R2), with 3 calves/group in each replicate.

<table>
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<th>Experimental group</th>
<th>No pyrexia</th>
<th>39.4°C–40.0°C</th>
<th>40.0°C–41.1°C</th>
<th>&gt; 41.1°C</th>
<th>Diarrhea</th>
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<td>3/6</td>
<td>0/6</td>
<td>0/6</td>
<td>0/6</td>
</tr>
<tr>
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<td>2/6</td>
<td>4/6</td>
<td>0/6</td>
<td>2/6</td>
</tr>
<tr>
<td>Exposed to HV BVDV</td>
<td>0/6</td>
<td>0/6</td>
<td>4/6</td>
<td>2/6</td>
<td>6/6</td>
</tr>
</tbody>
</table>

Values reported are No. of affected calves/No. of calves in the experimental group. Pyrexia was defined as a rectal temperature > 0.6°C higher than the baseline rectal temperature; baseline rectal temperature for each calf was determined by calculating the mean daily temperature recorded for the 2 days preceding and the day of viral exposure (days –2 through 0).
pyrexia and a decrease in the number of circulating lymphocytes. A study conducted to compare infection with a HoBi-like virus and an HV BVDV strain revealed that although infection with both viruses resulted in lymphocytopenia, infection with the HoBi-like virus resulted in milder clinical disease. It was suggested by the authors of that study that infection with HoBi-like viruses was similar to infection with LV BVDV. Although similar clinical signs were observed in the present study, it appeared that effects of infections with the HoBi-like virus are milder than those after infection with a field strain of LV BVDV2. Furthermore, acute infection with a HoBi-like strain resulted in transmission to naïve control calves of the present study. Severe clinical disease was not observed in inoculated calves, but 1 calf in the control group died. Although D32/00 HoBi was isolated from the control calf that was found dead on day 6, it was not possible to establish that viral infection was the cause of death.

It is not known whether there is a range in virulence among HoBi-like viruses. Furthermore, if there is a range in virulence, it is not known where D32/00 HoBi would be within that range. The clinical response to infection with BVDV depends on host factors, such as immune status, pregnancy status, secondary infections and environmental stress, and virulence of the infecting BVDV. It is likely that the clinical signs after infection with HoBi-like strains will be affected by the same factors. Although the origin of HoBi-like viruses is unknown, initial isolation of these viruses from water buffalo and from cattle within regions with water buffalo populations has led to speculation that HoBi-like viruses were introduced to naïve cattle populations from water buffalo. If this were the case, the virulence of HoBi-like viruses may change as these viruses adapt to a new host species.

Development of mild clinical signs after infection does not mean that introduction of HoBi-like viruses into cattle populations would be of little consequence. Low-virulence strains predominate among BVDV, and many acute BVDV infections are subclinical. Frequently, the first indication a producer may have that infection with HoBi-like viruses is that infections attributable to HoBi-like viruses cannot be readily discerned in field conditions solely on the basis of clinical signs after acute infection. Authors of a recent study suggested that the current means of sur-
veillance for exposure and vaccination programs may be inadequate for effective containment after introduction of HoBi-like viruses into new regions. In that study, the threshold of detection for HoBi-like viruses for commercial antigen-capture ELISA kits designed to detect BVDV was similar to that for BVDV. However, commercial ELISA kits designed to detect antibodies against BVDV failed to detect between 22.2% and 66.7% of bovine sera with HoBi-like-specific neutralizing antibodies. In addition, sera from cattle vaccinated with a commercial killed-virus vaccine containing BVDV1 and BVDV2 antigens had low neutralizing activity against HoBi-like viruses. These results suggest that surveillance for exposure to HoBi-like viruses and the control of infections attributable to HoBi-like viruses require the development of new diagnostic reagents and reformulation of current vaccines.

References