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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The pestivirus genus within the family Flaviviridae consists of single-stranded RNA viruses that infect a wide range of domestic and wild animals. The genus includes viruses, such as BVDV and classical swine fever virus, that have a major impact on commercial animal production. Currently, there are 4 recognized species within the pestivirus genus. However, 4 additional species have been proposed on the basis of phylogenetic analysis. These 4 putative species (listed in chronological order of discovery) are giraffe (isolated from one of several giraffes in the Nanyuki District of Kenya that were affected with mucosal disease–like clinical signs), HoBi-like viruses (first isolated from fetal bovine serum in samples originating in Brazil and subsequently from samples originating in Southeast Asia and Europe), pronghorn (isolated from an emaciated blind pronghorn antelope in the United States), and Bungowannah (isolated after an outbreak of disease in pigs in Australia that resulted in stillbirth and neonatal death). Three of the 4 putative species (giraffe, pronghorn, and Bungowannah) have been isolated from only 1 animal or during an outbreak of disease in 1 geographic region. In contrast, viruses from the HoBi-like putative species (also known as atypical bovine pestiviruses, atypical HoBi-like viruses, and BVDV-3) have been isolated from animals originating in South America, Southeast Asia, and Europe.
The first HoBi-like virus, D32/00 HoBi, was isolated from a batch of fetal calf serum that originated in Brazil. 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, Thailand for 1, and Italy for 1. 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.

The isolation of HoBi-like viruses from aborted fetuses, a calf apparently persistently infected with BVDV, and cattle after an outbreak of respiratory tract disease indicates that infection with these viruses may result in clinical disease. The isolation of HoBi-like viruses from fetal bovine serum 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 disease; however, researchers were not able to replicate this clinical syndrome in controlled experimental conditions. Use of HoBi-like strains to experimentally induce infections in cattle and sheep resulted in mild clinical disease characterized by moderate hyperthermia and reduced leukocyte counts. In contrast, experimentally induced infection of pigs resulted in severe respiratory tract disease indicates that infection with these viruses may result in clinical disease.

The range of virulence among BVDV isolates, as determined on the basis of clinical signs in animals with acute uncomplicated infections, is most pronounced among viruses from the BVDV2 species. Clinical signs after infection with HV BVDV strains include pyrexia (rectal temperatures > 40°C, with temperatures as high as 41.7°C) for 3 or more days and decreases of > 40% in the number of circulating lymphocytes and 60% in the number of platelets. In contrast, clinical signs after infection with LV BVDV strains include a mild and short-term pyrexia (rectal temperatures between 39.2°C and 40.0°C for 1 to 3 days), a small decrease (ranging between 20% and 40%) in the number of circulating lymphocytes, and no significant decrease in the number of platelets.

The objective of the study reported here was to determine whether the clinical signs after exposure to a HoBi-like virus would be similar to the clinical signs after exposure to field strains of noncytopathic BVDV. Therefore, we intended to evaluate the clinical signs after exposure to a HoBi-like virus or HV or LV BVDV2 viruses in controlled conditions.

Materials and Methods

Animals—Colostrum-deprived Holstein bull calves were procured from a local dairy at birth. Calves were raised for the first 2 weeks after birth in individual crates. Calves were fed a commercial infant formula for the first 48 hours after birth. After calves were 2 weeks old, they were housed in groups on the basis of experimental group. Feeding and housing in bio-level-2 containment facilities were performed as described elsewhere. Handling and treatment of calves was conducted in accordance with the Animal Welfare Act as amended (7 USC, 2131-2136).

Before the beginning of the study, buffy-coat samples obtained from the calves were tested for BVDV or HoBi-like viruses via virus isolation. Calves were also tested for antibodies against BVDV or HoBi-like viruses in serum and for BVDV antigen in ear notch samples via a commercial antigen-capture ELISA used in accordance with the manufacturer’s directions.

Viruses—Three noncytopathic pestiviruses were used. Viral strain BVDV2-1373 was used as the HV BVDV strain. It was isolated from BVDV2-1373 obtained from a widespread outbreak of severe acute BVDV infection in Ontario, Canada, in 1993. BVDV2-RS886 was used as the LV BVDV strain. It was isolated from a healthy calf persistently infected with BVDV. In another study, infection of cattle with this strain resulted in a 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. 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.

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. Cells were grown in complete cell culture medium composed of minimal essential media supplemented with 10% fetal bovine serum, l-glutamine (final concentration, 1.4mM), 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. Viral titers were determined via dilution on bovine turbinate cells. Endpoints were based on monoclonal antibodies that bound the E2 protein of BVDV2-1373, BVDV2-RS886, and D32/00 HoBi.

Exposure to virus—Calves were assigned to treatment groups (6 calves/group). Of the 24 color-strain 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, 1:128 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.
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 10⁴ 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 14. 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.

Blood samples for PCR assay, determination of the number of circulating lymphocytes and platelet counts, and measurement of serum neutralizing antibodies were collected on days –2, 3, 6, 9, 11, 13, and 18 for the housed control calves and calves exposed to the HoBi-like virus and on days –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-url) —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 1—Number of calves exposed to a HoBi-like pestivirus, LV BVDV, or HV BVDV that became pyreptic or developed diarrhea.

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>No pyrexia</th>
<th>39.4°–40.0°C</th>
<th>40.0°–41.1°C</th>
<th>&gt; 41.1°C</th>
<th>Diarrhea</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exposed to HoBi-like virus</td>
<td>3/6</td>
<td>3/6</td>
<td>0/6</td>
<td>0/6</td>
<td>0/6</td>
</tr>
<tr>
<td>Exposed to LV BVDV</td>
<td>0/6</td>
<td>0/6</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>4/6</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).
The virus D32/00 HoBi was detected in at least 1 blood sample collected from day 3 through 14 for calves inoculated with the HV BVDV. Rate of detection of D32/00 HoBi in buffy-coat samples was higher than the rate of detection in nasal swab specimens. The virus D32/00 HoBi was detected via PCR assay in at least 1 blood sample collected from all experimentally inoculated calves between days 3 and 9. In contrast, D32/00 HoBi RNA was detected in nasal swab specimens from 3 of 6 inoculated calves during the same sampling period.

The virus D32/00 HoBi was detected in at least 1 blood sample or nasal swab specimen collected from each of the control calves between days 6 and 13. One of the control calves initially seronegative for BVDV and the HoBi-like virus had a rectal temperature of 39.4 ºC higher than the baseline rectal temperature; baseline rectal temperature was calculated as the mean of the rectal temperatures recorded for the 2 days preceding and the day of viral exposure (days –2 through 0). Pyrexia is defined as a rectal temperature > 0.6 ºC higher than the baseline rectal temperature.

None of the inoculated calves seroconverted. Virus was detected in blood samples collected on day 3 or 4 and day 9 or 10 from calves inoculated with HoBi-like virus and LV BVDV. Virus was detected from day 3 through 14 for calves inoculated with the HV BVDV. None of the inoculated calves seroconverted. Virus was detected in blood samples collected on day 3 or 4 and day 9 or 10 from calves inoculated with HoBi-like virus and LV BVDV. Virus was detected from day 3 through 14 for calves inoculated with the HV BVDV.

Rate of detection of D32/00 HoBi in buffy-coat samples was higher than the rate of detection in nasal swab specimens. The virus D32/00 HoBi was detected via PCR assay in at least 1 blood sample collected from all experimentally inoculated calves between days 3 and 9. In contrast, D32/00 HoBi RNA was detected in nasal swab specimens from 3 of 6 inoculated calves during the same sampling period.

The virus D32/00 HoBi was detected in at least 1 blood sample or nasal swab specimen collected from each of the control calves between days 6 and 13. One of the control calves initially seronegative for BVDV and the HoBi-like virus had a rectal temperature of 39.4 ºC on day 5 and was found dead on day 6. Postmortem examination of that calf did not reveal gross lesions of the respiratory or gastrointestinal tracts, and tissues appeared normal during histologic examination. However, D32/00 HoBi was isolated from buffy-coat samples collected from heart blood harvested during necropsy. Isolation of D32/00 HoBi was confirmed by sequencing the 5′ untranslated region of the isolated virus. Of the 3 remaining control calves that were initially seronegative for BVDV and the HoBi-like virus, 1 seroconverted (as defined by a 4-fold increase in virus neutralization titer against the HoBi-like virus) by day 18. By day 18, there was no change in the titers of one of the calves initially seropositive for BVDV and the HoBi-like virus. In contrast, the other calf that was initially seropositive for BVDV and the HoBi-like virus had a 4-fold increase in the virus neutralization titer against D32/00 HoBi by day 18.

**Discussion**

Similar to infection with LV BVDV strains, infection with HoBi-like strains results in low-grade, short-term pyrexia and a decrease in the number of circulating lymphocytes. A study conducted to compare infection with a HoBi-like virus and an HV BVDV1 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 infection with 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 BVDV has infected the herd is the birth of a calf persistently infected with BVDV. Although the initial acute infection of pregnant cattle with BVDV may not have a noticeable impact on herd health, the presence of the resulting persistently infected animal can have a major impact on the health and performance of cohorts. Similarly, the impact of the introduction of HoBi-like viruses into a naïve population may not be realized until calves persistently infected with HoBi-like virus are born in the herd or introduced into feedlots or milking herds.

Analysis of the results of the present study suggests 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