Efficacy of a combination viral vaccine for protection of cattle against experimental infection with field isolates of bovine herpesvirus-1

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Objective—To determine whether a combination viral vaccine containing a modified-live bovine herpesvirus-1 (BHV-1) would protect calves from infection with virulent field strains of BHV-1 for weeks or months after vaccination.

Design—Randomized controlled trial, performed in 2 replicates.

Animals—63 weaned 4- to 6-month-old crossbred beef calves seronegative for antibody against BHV-1.

Procedures—Calves were randomly allocated to 1 of 2 treatment groups. Control calves (n = 10/replicate) received a combination modified-live mixed viral vaccine without BHV-1, and treatment calves (20 and 23/replicate) received a combination modified-live mixed viral vaccine containing BHV-1. Each group was challenged via aerosol with 1 of 2 field strains of BHV-1, 30 days after vaccination in replicate 1 and 97 days after vaccination in replicate 2. After challenge, calves were commingled in 1 drylot pen. Clinical signs, immune responses, and nasal shedding of virus were monitored for 10 days after challenge, after which the calves were euthanatized and tracheal lesions were assessed.

Results—Vaccination stimulated production of BHV-1–specific IgG antibody that cross-reacted with field isolates of BHV-1. Treatment calves had significantly fewer signs of clinical disease, shed less BHV-1, had less or no weight loss after challenge, and had fewer tracheal lesions than control calves for at least 97 days after vaccination.


Respiratory disease in beef and dairy cattle has been causally associated with BHV-1 since the 1950s. Soon after isolation of BHV-1 from clinically infected cattle in 1956, modified-live and inactivated vaccines were developed and applied in bovine practice. They are still used widely to control respiratory disease in cattle, particularly in feedlot cattle. Despite this common preventive practice of vaccination, considerable economic losses associated with BHV-1 infection continue. In recent years, there have been increases in the numbers of reports of outbreaks of BHV-1 in feedlot cattle vaccinated against the agent (so-called vaccine breaks) that take place late in the feedlot stage of production and are apparently not vaccine brand–specific. This has led to questions concerning the efficacy of available BHV-1 vaccines for protecting cattle from circulating BHV-1 strains. Despite evidence to the contrary, individuals in some segments of the livestock industry still believe that BHV-1 vaccine failures are largely attributable to the possibility that modern vaccines that contain historical strains of BHV-1 do not stimulate protective responses against circulating strains of BHV-1 because the latter are believed to be genetically and antigenically quite different from the strain of BHV-1 contained in the vaccine. The purpose of the study reported here was to determine whether vaccination with a combination viral vaccine containing a modified-live strain of BHV-1 derived from the Cooper strain of BHV-1 (one of the original isolates used in vaccines) would stimulate protective immune responses in calves experimentally infected with 2 virulent field strains that had been isolated during outbreaks of BHV-1 attributed to vaccine failure and that had variable genetic similarity with the vaccine strain.

Materials and Methods

Animals—In October 2006, sixty-three 4- to 6-month-old crossbred beef calves were obtained from...
a local pastured commercial herd that was not vaccinated against BHV-1 and contained calves that were historically seronegative for antibody against BHV-1. All calves were weaned on the same day and moved to a large, dry corral (approx 18 X 18 m). Tilmicosin (10 mg/kg [4.5 mg/lb], SC) was administered to each, and a blood sample was obtained via jugular venipuncture for detection of antibody against BHV-1 by use of an ELISA. All calves were confirmed as seronegative and were kept commingled in the drylot pen, isolated from other cattle until vaccination. The cattle were maintained and treated in accordance with the guidelines of the Canadian Council of Animal Care.

Experimental design—The experiment was conducted in 2 replicates. For each replicate, calves were randomly assigned to 1 of 2 treatment groups by means of a generalized randomized block design, with blocking based on body weight, in blocks of 3 (1 control and 2 treatment calves/block). Control calves (n = 10/replicate) were assigned to vaccination with a combination viral vaccine containing modified-live parainfluenza virus 3, bovine respiratory syncytial virus, and bovine viral diarrhea virus types 1 and 2 but not BHV-1. Treatment calves (20 and 23/replicate) were assigned to vaccination with the same combination modified-live viral vaccine that included BHV-1. During the vaccination phase of the study, to reduce the potential immunizing effect that shedding of vaccine virus by treatment calves might have on control calves, 7 to 10 calves from each block were randomly allocated to 1 of 3 pens (approx 10 X 10 m) separated by 2 m, with the constraint that there be 2 pens that contained 10 treatment calves each and 1 pen that contained 10 control calves. During the challenge phase of the study, all calves were commingled and housed in 1 pen; accordingly, calves became the experimental units during that phase.

In the first experimental replicate, calves were vaccinated 8 days after weaning. In the second replicate, calves were vaccinated 59 days after weaning. After vaccination, each group of calves was moved to identical adjacent pens that were separated by approximately 2 m to prevent nose-to-nose contact and thereby reduce the potential transmission of vaccine virus via nasal shedding.

BHV-1 strains—The challenge inoculum for the first replicate was a virulent field strain of BHV-1 (P3) that had been recently (2004) isolated from a feedlot steer with respiratory tract disease in Texas. The challenge inoculum for the second replicate was also a virulent field strain (P1) that had been recently (2003) isolated from a feedlot steer with respiratory disease in Texas. Both strains had been propagated in bovine viral diarrhea virus–free Madin-Darby bovine kidney cells, and the product was aliquoted and stored at –80°C. The inocula were confirmed to be devoid of bacterial contaminants, Mycoplasma spp., parainfluenza virus 3, and bovine viral diarrhea virus by use of standard diagnostic methods. The P3 inoculum contained approximately 10⁸ TCID₅₀/mL, the P1 inoculum contained approximately 10⁷ TCID₅₀/mL. The Cooper (wild-type) strain of BHV-1, the genetically and antigenically similar vaccine strain C-13, the temperature-sensitive mutant Cooper strain (temperature-sensitive vaccine strain), another strain from a recent (2004) outbreak of BHV-1 infection attributed to vaccine failure in Texas (P2), a recent (2004) field strain of BHV-1 from an outbreak attributed to vaccine failure in Saskatchewan (Sask 30407), and a BHV-5 strain (B5) were propagated to similar titers and tested for the aforementioned extraneous agents before being aliquoted and frozen at –80°C for subsequent use in cross-neutralization or genetic analyses.

Experimental challenge—On day 30 after vaccination in the first replicate and day 97 after vaccination in the second replicate, all calves were shipped in 2 loads in 7.3-m stock trailers to the Western College of Veterinary Medicine (total distance, approx 113 km; total duration, approx 90 minutes). This was done to simulate the distress associated with shipping and environmental change.

On arrival, calves were exposed to an aerosol of challenge inoculum by placing 16 calves at a time into a stock trailer measuring 7.3 X 2.4 X 2.4 m (42 m³ of air space). The openings in the trailer were sealed with pieces of clear acrylic glass. Eighty milliliters of inoculum (P3 or P1, depending on the replicate) was rapidly thawed and divided into 3 ultrasonic nebulizers placed off-center on each side of the trailer (2 on one side and the third on the opposite side). The inoculum was nebulized into the trailer airspace for approximately 40 minutes, and the calves were retained in the trailer for a total of 45 minutes before they were released back into the same large drylot pen for the remainder of the study period. This resulted in a calculated total dose of approximately 1.77 mL/min/group.

Analysis of BHV-1 proteins and DNA— Cultures of Madin-Darby bovine kidney cells were infected with each of the various BHV-1 strains (multiplicity of infection, 1). At 24 hours after infection, when posttranslational modification (eg, glycosylation) of proteins would be complete, kidney cells were collected and lysed in 500 μL of 1X SDS sample buffer (50mM Tris-HCl [pH, 6.8], 10% glycerol, 2% SDS, and 5% β-mercaptoethanol). The cell extract was boiled for 5 minutes, and the supernatant was used for SDS-PAGE. Detection of viral proteins on immunoblots was performed with a caprine primary antibody directed against BHV-1 viral proteins.

Similarly, viral DNA was prepared from groups of Madin-Darby bovine kidney cells that had been exposed to each of the various BHV-1 strains (multiplicity of infection, 0.01) for 1 hour. Cell cultures were subsequently rinsed twice with calcium and magnesium–free PBS solution, and new tissue culture medium was added. At 24 to 36 hours after infection, when cytopathic effect was approximately 80%, the supernatant was collected and clarified by ultracentrifugation (3,500 g at 4°C for 20 minutes). Virus was subsequently pelleted through a buffer cushion of 30% sucrose solution in 10mM Tris (pH, 7.4) and 1mM EDTA (25 mL of virus/5 mL of sucrose solution) by means of ultracentrifugation (12,500 X g at 4°C for 2 hours). The pellet was suspended in 623 μL of buffer (10mM Tris-HCl [pH, 7.4], 10mM KCl, and 1.5mM MgCl₂) and then treated with 6.3 μL of deoxyribonuclease I (105 U/mL) at 37°C for 2

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rate (breaths/min) was also determined on the basis of measurement for 15 seconds. Values obtained on days 26 through 28 were used to establish a baseline respiratory rate for each calf. A score of 0 (normal) was assigned when the respiratory rate after challenge differed from the baseline rate by ≤ 12 breaths/min, and a score of 1 (abnormal) was assigned when the rate differed by ≥ 12 breaths/min. All calves were weighed on days 1 and 7 after challenge.

Any calves with severe clinical signs and deemed unlikely to survive were euthanatized. All calves that died before the end of the study underwent a postmortem examination; gross findings were recorded, and a cause of death was determined whenever feasible. In addition, postmortem tissue samples were collected as necessary and submitted for additional testing to enable a diagnosis. All calves that required euthanasia during the study or were alive on day 10 after challenge were euthanatized by IV barbiturate administration, and samples of tracheal tissue were collected for histologic examination.

**Sample collection**—Deep nasal swab specimens were collected from each calf prior to vaccination, prior to the challenge, and on days 2 through 10 after challenge to determine titers of shed virus and the percentage of calves shedding virus per treatment group. Swab specimens were placed in 1 mL of transport medium consisting of Dulbecco modified Eagle medium,10% fetal bovine serum (γ-irradiated),200 U of sodium penicillin/mL,200 μg of streptomycin sulfate/mL,200 μg of gentamicin/mL, and 1 μg of amphotericin B/mL and frozen at −70°C until analyzed. Jugular venous blood samples were collected from each calf on the days of vaccination and challenge and on day 7 after challenge for determination of antibody titers against BHV-1.

**Antibody assays**—One set of assays was performed for each replicate. An ELISA was used to measure BHV-1–specific IgG titers, as described14; a 1:50 dilution of serum was used. This ELISA and dilution reportedly have good accuracy for detecting infectious bronchitis virus–1 neutralizing antibodies.14 Convalescent serum from a cow with a high titer of antibody against BHV-1 was used as the positive standard; fetal bovine serum was used as the negative standard. Optical density values for test samples were expressed as the percentage of the ODs of the standards as follows: 100 × (sample OD – negative standard OD)/(positive standard OD – negative standard OD).

Virus-neutralization titers were determined by means of tripling serial dilutions of heat-inactivated (56°C for 30 minutes) serum in cell culture medium on 96-well tissue culture plates as described elsewhere.15 Serum samples were tested in duplicate, and the final dilution was 1:162. For each stock strain of BHV-1 (Cooper, Sask 30407, P3, or P1), 100 TCID₅₀ was added to the diluted serum. This virus concentration was verified by conducting a back-titration with each run. The serum-virus mixture was incubated at 37°C for 1 hour, after which a suspension of bovine embryonic lung cells was added. A cytopathic effect was evident after 5 days of incubation, and the results were expressed as the reciprocal of the final dilution of serum that neutralized the virus in 50% of the wells.
**Quantitative virus isolation**—Viral shedding was quantitatively determined by use of a standard microtitration assay. Briefly, each nasal swab specimen was titrated in duplicate wells of a tissue culture–treated 96-well plate, starting at a 1:10 dilution, followed by 3 serial 10-fold dilutions in 100-µL volumes. Afterward, 100 µL of bovine embryonic lung cells was added to each duplicate well. Dilutions of nasal swab material and bovine embryonic lung cells were made in Dulbecco modified Eagle medium containing cell culture medium, 7.5% fetal bovine serum (γ-irradiated), 200 U of sodium penicillin/mL, 200 µg of streptomycin sulfate/mL, 200 µg of gentamicin/mL, and 1 µg of amphotericin B/mL. The plates were incubated at 37°C for 3 days, then assessed for cytopathic effect typical of BHV-1. Endpoint titers were recorded as TCID₅₀/mL, with a maximum calculated sensitivity of 10 TCID₅₀/mL.

**Pathologic assessment**—Samples from mid sections of trachea from each calf were collected at the time of euthanasia, fixed in formalin for 48 hours, trimmed, and embedded in paraffin for routine H&E and immunohistochemical staining for BHV-1. Tracheal tissue sections were examined by a pathologist who was unaware of treatment assignment and were scored from 0 to 3+ (0 = least severe; 3+ = most severe) for tracheitis, considering epithelial hyperplasia or necrosis and degree of inflammatory cell infiltration in the lumen, epithelium, lamina propria, and submucosa. Immunohistochemically stained slides were scored as positive or negative for appreciable evidence of BHV-1 antigen.

**Statistical analysis**—For each calf, the number of abnormal clinical signs (depression, coughing, nasal discharge, ocular change, and respiratory effort) was calculated at each time point and transformed prior to analysis by means of a square-root transformation. The transformed data were analyzed by use of a general linear repeated-measures mixed model that included fixed effects of treatment, time point, and interaction between treatment and time point and random effects of block and calf. Back-transformed least squares means and 95% confidence intervals were calculated from least squares estimates obtained from the analysis and are reported with the range of block and calf. Additionally, linear functions of the least squares means for body weights were used to calculate estimates of weight gain and contrasts were constructed to compare treatment groups for differences in weight gain. Linear combinations of variable estimates (obtained from the model) were used in a priori contrasts to compare treatment groups at each time point when the overall treatment effect or the interaction between treatment and time point effect was significant. Least squares means, SEs, 95% confidence intervals of the means, and ranges were calculated (back-transformed for transformed data) for each treatment at each time point. A general linear repeated-measures mixed model that included random effects of block and calf and fixed effects of treatment, time point, and interaction between treatment and time point was used to evaluate associations with body weight, rectal temperature, serum antibody titer against BHV-1, and nasal swab–specimen titer of BHV-1. An appropriate logarithmic transformation was applied to titer values prior to analysis. Titer values preceded by a less-than (<) symbol were divided by 2 prior to transformation.
linear mixed model that included the random effect of block and the fixed effect of treatment was used to analyze virus-neutralization titer data. An appropriate log transformation was applied to titers prior to analysis. Titer values preceded by a < symbol were divided by 2 prior to transformation. Linear combinations of the parameter estimates (obtained from the model) were used in a priori contrasts to compare between and within treatment groups. Least squares means, SEs, 95% confidence intervals, and ranges were calculated (back-transformed for transformed data) for each treatment. The proportion of calves that became pyrexic after challenge was calculated for each treatment group in accordance with US federal governmental criteria, and values were compared by use of a Fisher exact test. A value of $P \leq 0.05$ was considered significant for all analyses.

**Results**

**Animals**—In the first replicate, in which calves were vaccinated 8 days after weaning and challenged with BHV-1 strain P3 30 days after vaccination, 1 control calf was euthanatized prior to challenge because of deteriorating health. A diagnosis of bacterial endocarditis was made on postmortem examination; the condition was considered unrelated to the study. In the second replicate, in which calves were vaccinated 59 days after weaning and challenged with BHV-1 strain P1 97 days after vaccination, the data for 1 calf were removed because it could not be determined which treatment the calf had received. Another calf, which had been anorexic and losing body condition, died prior to challenge. A postmortem examination was performed, revealing gross lesions suggestive of a diffuse omentitis and splenitis likely associated with inapparent or healed traumatic reticulitis. Removal of any data associated with these 2 calves resulted in data from 21 (instead of 23) calves being used in analyses for the second replicate.

**Clinical response to challenge**—In the first replicate, infection with BHV-1 strain P3 resulted in 1 or more clinical signs consistent with infectious tracheobronchitis in all calves from both treatment groups (vaccination with combination modified-live vaccine with or without BHV-1) during the 10-day postchallenge period. However, there was a significantly ($P = 0.001$) lower proportion of calves vaccinated against BHV-1 that developed pyrexia (rectal temperature $\geq 40.3°C$; $1/20$) than control calves ($6/9$), and vaccinated calves had a significantly ($P \leq 0.01$) lower mean rectal temperature than control calves on 7 of 10 days after challenge (Figure 1). Vaccinated calves also had significantly ($P < 0.05$) fewer clinical signs of disease on days 4, 5, and 6 after challenge than did control calves (Figure 2). One calf in the control group was euthanatized prior to the end of the study (day 8 after challenge) for humane reasons and on the basis of the severity of clinical signs consistent with BHV-1 infection. Postmortem examination revealed necrotizing tracheitis consistent with BHV-1 infection. There was a significant ($P < 0.001$) difference in body-weight change in the postchallenge period, during which control calves lost a mean of 3.8 kg/d (8.6 lb/d) whereas vaccinated calves gained a mean of 0.4 kg/d (0.88 lb/d).

In the second replicate, the proportion of vaccinated calves that developed pyrexia after the viral challenge was also significantly ($P < 0.001$) lower than that of control calves. Seven of 10 control calves had a rectal temperature $\geq 40.3°C$. None of the vaccinated calves became pyrexic in the postchallenge period (Figure 3). Vaccinated calves also had significantly ($P < 0.05$) fewer clinical signs of disease on days 4, 5, and 6 after challenge than did control calves (Figure 4).
6 after challenge (Figure 4). As was evident in the first replicate, there was a significant (P = 0.006) change in body weight in the postchallenge period, during which calves in the negative control group lost a mean of 2.3 kg/d (5.1 lb/d), compared with a slight daily loss for vaccinated calves of 0.6 kg/d (1.3 lb/d).

Viral shedding—Results of analysis of nasal swab specimens indicated that, in the first replicate, vaccination with Cooper strain–derived modified-live BHV-1 significantly (P < 0.001) reduced nasal shedding of virus during the entire postchallenge observation period, compared with shedding in control calves (Figure 5). There were no significant (P = 0.96) differences between treatment groups in proportions of calves shedding virus.

In the second replicate, vaccinated calves shed significantly (P ≤ 0.003) less virus than control calves during the entire study period after challenge (Figure 6). Compared with control calves, vaccinated calves had a significant (P = 0.001) reduction in the percentage of days after challenge in which nasal shedding of BHV-1 was detected (control calves, 90.24%; vaccinated calves, 80.06%).

BHV-1–specific antibody—Results of ELISA testing for antibody against BHV-1 indicated that all calves were seronegative at the time of weaning and at the time of vaccination. In the first replicate, vaccinated calves had a significant (P < 0.001) increase in serum BHV-1 ELISA titer after vaccination with the Cooper strain–derived BHV-1 vaccine, compared with control calves, both 30 days after vaccination, and at 7 days after challenge (Figure 7). In addition, vaccination stimulated significant increases in virus-neutralizing antibody. Serum samples obtained from vaccinated calves 30 days after vaccination effectively neutralized the challenge BHV-1 strain P3 (geometric mean titer, 15.62) as well as the Cooper strain (standard reference challenge strain for USDA testing13 and progenitor of vaccine strain; geometric mean titer, 25.09) and the recent field strain Sask 30407 (geometric mean titer, 24.59). There were no significant (overall test of treatment effect, P = 0.14) differences in the vaccine-stimulated virus-neutralization titers among the 3 strains tested.

Similar to findings in the first replicate, vaccinated calves in the second replicate had a significantly (P = 0.001) higher serum titer of antibody against BHV-1 than control calves, both 97 days after vaccination and at 7 days after challenge (Figure 8). Vaccination stimulated significant increases in virus-neutralizing antibody to the challenge virus (P1; geometric mean titer on day 97 after vaccination, 11.27; value on day 10 after challenge, 169.22) and the Cooper strain (geometric mean titer on day 97 after vaccination, 14.24; value on day 10 after challenge, 219.81). There were significant (overall test of treatment effect, P = 0.001) differences in vaccine-stimulated virus-neutralization titers between treatment groups. These differences did not exist prior to vaccination (P = 1.00). The vaccine-stimulated virus-neutralization titers at 97 days after vaccination (prior to challenge) and 10 days after the challenge differed for the P1 and Cooper strains (P = 0.011 and P = 0.005 for the 2 time points, respectively).
Pathologic assessment—Scores for tracheal lesions typical of BHV-1 infection (epithelial hyperplasia, often with erosion or ulceration and inflammation) were tabulated but not statistically analyzed. In the first replicate, tracheal lesions were scored as moderate to severe in 6 of 9 control calves; in vaccinated calves, tracheal lesions were scored as mild in 18 of 20 calves. Reflecting the detection of no or minimal nasally shed virus on day 10 after infection, no immunohistochemical evidence of BHV-1 antigen was detected in situ in the trachea. In the second replicate, tracheal lesions were scored as moderate to severe in 6 of 10 of the control calves; in vaccinated calves, tracheal lesions were scored as moderate in 4 of 21 and none were scored as severe. In contrast, 17 of 21 vaccinated calves had mild tracheitis, compared with only 4 of 10 of the control calves. Immunohistochemical staining for BHV-1 antigen was not performed because BHV-1 had not been detected in tracheal tissues from the first replicate.

Analysis of BHV-1 proteins and DNA—Analysis of BHV-1 proteins on immunoblots revealed only subtle differences among all the BHV-1 strains tested (Figure 9). The wild-type Cooper strain, temperature-sensitive vaccine strain, and P1 and P2 field strains had 2 distinct protein bands in the 40-kDa range. On the other hand, the P3 field strain and the attenuated C-13 vaccine strain that was derived from the wild-type Cooper strain had 1 distinct band in this area. No other differences in proteins were detected among the 6 BHV-1 strains tested. In contrast, all of the proteins with a molecular weight < 70 kDa were missing in the BHV-5 isolate, indicating that the 3 field strains, the wild-type Cooper strain, and the vaccine strains were distinct from BHV-5.

Differences were detected in the DNA profiles of the BHV-1 strains produced by cutting with the re-
restriction enzymes HindIII and PstI, which yielded low molecular weight fragments, all of which were distinct from BHV-5 (Figures 10 and 11). The modified-live vaccine strain (C-13) contained a 2.5-kb HindIII restriction enzyme fragment that was absent in the temperature-sensitive and wild-type strains. This 2.5-kb band was present in the P1 and P3 field strains but not in the P2 field strain. The P2 strain had a 2.4-kb band that appeared to comigrate with the band evident in the temperature-sensitive strain. The C-13 strain and all of the field strains except P3 contained a PstI fragment that migrated between 4 and 5 kb (approx. 4.8 kb). The P3, temperature-sensitive, and wild-type strains contained 2 PstI fragments that migrated in this same region. With respect to bands that migrated between 2 and 3 kb, it was clear that the wild-type and P3 strains had the same banding pattern. The P1 and P2 bands did not match those of the vaccine or wild-type strains. Although the banding pattern of PstI restriction fragments that migrated between 1.2 and 1.5 kb was faint, the wild-type, C-13, and P2 strains had a similar banding pattern. The P1, P3, and the temperature-sensitive strain also had similar banding patterns, which were distinct from those of the other viral strains.

These results indicated that none of the field strains tested (P1, P2, or P3) were similar to BHV-5. There were subtle differences between the C-13 strain and the temperature-sensitive strain, compared with the wild-type Cooper strain from which the former vaccinal isolates were derived; strains P1 and P2 were similar, if not identical, to the C-13 strain contained in the vaccine used in both challenge studies. Field strain P3 had a novel absence of a protein that the 2 vaccines and the wild-type Cooper strains contained. The P3 strain also had a novel restriction enzyme profile. Collectively, these results suggested that P3 might be a novel field strain.

**Discussion**

The results of the study reported here supported and extended findings from other studies that indicated there are expected detectable differences in the genome and viral proteins of bovine herpesviruses over time; however, there has not been, as yet, a report of mutants that escape the polyclonal bovine immune responses engendered by vaccines derived from some of the original strains of the virus. This conservation of epitopes critical to the induction of protective immune responses was evident in the significant sparing of disease in calves vaccinated with a derivative of the Cooper strain of BHV-1 and subsequently infected with 2 strains of BHV-1 from recent outbreaks of disease in vaccinated cattle.
If viruses can, in the broadest sense, be considered living entities, then they would be expected to vary over time because of natural mutation or selection. Differences in the mutability and resultant phenotypic variation among different families of viruses is largely dependent on their genetic constitution. Double-stranded DNA viruses such as herpesviruses are much less variable than single-stranded RNA viruses such as bovine viral diarrhea virus, which are not subject to proofreading mechanisms that scrutinize the fidelity of DNA replication in cells. The degrees of difference in viral DNA and protein detected in the present study were consistent with the degrees of difference that have been reported for BHV-1 strains.

Differences in virulence among BHV-1 strains have been reported; however, the viral factors that confer variation in virulence are largely unknown. Given the variation in cofactors that contribute to clinical disease following BHV-1 infection, it is difficult to compare results of studies of experimental infection, even among various replicates, when single-source calves are used. However, the 2 field strains used in our study were apparently more virulent than the laboratory-passaged Cooper strain and another field strain that had been used in another study to infect calves from the same commercial beef herd from which the calves in the present study were obtained. Moreover, it appeared that the clinical disease that resulted from infection with the P3 strain was somewhat more severe than that associated with the P1 strain; the resulting disease was so severe that 1 unvaccinated, P3-infected calf required euthanasia for humane reasons. Such differences in virulence among BHV-1 strains reported here and elsewhere could contribute to the perception that vaccine failure or biologically important antigenic change of BHV-1 (antibody escape mutation) is involved.

It is unclear whether antigenicity of available vaccines is a factor in determining whether vaccine failure happens and whether reconfiguring these vaccines with the inclusion of contemporary field strains of BHV-1 would improve efficacy. Despite some expected minor differences in the restriction fragment length polymorphism profiles and viral proteins, there were no significant differences in the ability of the Cooper strain–derived combination BHV-1 vaccine we used to stimulate the production of antibody that cross-neutralized field BHV-1 strains, indicating that there are most likely no biologically important antigenic differences between the Cooper strain and the more recently isolated BHV-1 strains that were tested. The concept that historical BHV-1 strains such as the Cooper strain induce broadly cross-protective responses against contemporary BHV-1 strains is supported by the finding that BHV-1 vaccines confer considerable disease-sparing effects against infection with BHV-5 (formerly designated as a BHV-1 variant), which is a virus that is genetically and antigenically distinct from BHV-1 strains.

It is possible that the differences in virulence among the respective BHV-1 strains result from antigenically silent mutations, as has been reported. Such changes could improve the “fitness” of strains and may affect efficiency of transcription of viral mRNA, translation of viral proteins, or overall rate of replication or shedding. Examination of emerging neurovirulent strains of equine herpesvirus-1, another alphaherpesvirus that is biologically similar to BHV-1, has revealed a consistent point mutation that results in a single amino acid change in a viral polymerase. This mutation has been associated with more prolonged and higher degrees of cell-associated viremia in infected horses than are characteristic of other strains but not with detectable genetic (on the basis of restriction fragment length polymorphisms) or antigenic changes in mutated viruses.

In such situations, it is highly unlikely that inclusion of contemporary strains would improve efficacy of a vaccine that is based on the induction of specific immune responses to antigenically different viruses. Moreover, differential virulence based on antigenically silent mutations underscores the limitations of the immune response in dealing with some variants of antigenically similar infectious agents.

Although in situations of apparent vaccine failures the tendency has been to assume available BHV-1 vaccines are not efficacious in inducing antigenically relevant and protective responses against contemporary BHV-1 strains, other factors could explain incidents of vaccine failure in feedlot cattle. Foremost is the not uncommon failure of the vaccine to adequately immunize vaccinated cattle. Despite available information concerning vaccine efficacy in feedlots and the role of neural regulation (in response to distress), changes in nutrition, and other management factors on immune responses, in general, the industry standard continues to be to vaccinate cattle when they arrive at the feedlot. Exposure to infectious agents shortly after vaccination and prior to the development of an immune response or concurrent infection such as persistent infection with bovine viral diarrhea virus in calves in commingled groups may also contribute to apparent vaccine failures in feedlots. As well, there is the possibility that effective protective immunity against herpesvirus infection is relatively short and does not extend through the feedlot stage of production. In fact, a recent study in which a combination viral vaccine similar in configuration to the one used in the present study revealed exactly this effect; the degree of clinical immunity to BHV-1 was directly related to the interval between vaccination and challenge, which again points out the limitations of some protective immune responses.

The results of the study reported here indicated that vaccination with a combination viral vaccine containing a Cooper strain–derived strain of BHV-1 induced cross-reactive antibody responses that neutralized contemporary strains of BHV-1 in vitro and, importantly, conferred significant disease-sparing effects that were manifested as reductions in pyrexia, clinical signs, weight loss, and nasal shedding of the virus. These effects persisted for at least 3 months after experimental challenge with virulent strains of BHV-1 that had been obtained during outbreaks of BHV-1 infection attributed to vaccine failure. These and previous data do not support the supposition that BHV-1 vaccine failures are primarily attributable to mutant strains of BHV-1 that escape immune responses in cattle properly vaccinated with contemporary BHV-1 vaccines.
References
