# Effect of intranasal vaccination against bovine enteric coronavirus on the occurrence of respiratory tract disease in a commercial backgrounding feedlot

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**Objective**—To measure antibody titers against bovine coronavirus (BCV), determine frequency of BCV in nasal swab specimens, and compare calves treated for bovine respiratory tract disease (BRD) between those given an intranasally administered vaccine and control calves.

**Design**—Randomized clinical trial.

**Animals**—414 heifer calves.

**Procedure**—Intranasal BCV antigen concentration and antibody titer against BCV were measured on entry to a feedlot. Calves were randomly assigned to receive 3.0 mL of a modified-live virus vaccine against bovine enteric coronavirus and rotavirus or 3.0 mL of saline (0.9% NaCl) solution. Calves were confined to 1 of 2 pens, depending on vaccination status, for a minimum of 17 days of observation (range, 17 to 99). Selection of calves for treatment of BRD and scoring for severity of disease were done by veterinarians unaware of treatment status.

**Results**—Intranasal BCV (125/407 [31%]) and serum antibody titers ≥ 20 against BCV (246/396 [62%]) were identified in calves entering the feedlot. Vaccination was associated with significant decrease in risk of treatment for BRD; intranasal BCV on entry to the feedlot was associated with increased risk of treatment. Univariate analysis revealed that control calves with intranasal BRD on entry to the feedlot and those with antibody titer < 20 were significantly more likely to be treated for BRD.

**Conclusions and Clinical Relevance**—These data provide further evidence of an association between BCV and respiratory tract disease in feedlot calves. An intranasally administered vaccine appeared to reduce risk of treatment for BRD. (*J Am Vet Med Assoc* 2004;225:726–731)

RUMINANTS

Coronaviruses have trophism for the respiratory and gastrointestinal tracts in humans, as well as a variety of other animal species.[1,2] **Bovine coronavirus (BCV)**, a member of the family Coronaviridae, order Nidovirales, is composed of a single strand of nonsegmented, positive-sense RNA.[3] It was first recognized as the cause of a fatal enteric disease of calves in 1972 and later shown to be a cause of winter dysentery in adult cattle.[4] Since 1972, BCV has been reported from numerous countries and is probably distributed worldwide.[5] The first report associating coronavirus with **bovine respiratory tract disease (BRD)** was in 1982, when coronavirus particles were found in a lung lavage specimen during a search for microorganisms associated with outbreaks of pneumonia in recently weaned calves.[6] More recently, isolation of BCV from the nasal passages of cattle entering a feedlot was reported to increase the risk of BRD.[7] In a separate study, deaths from pneumonia were associated with intranasal isolation of BCV prior to an outbreak of shipping fever pneumonia in feedlot calves.[8]

There is conflicting information in the literature regarding the role of BCV in BRD. At least 2 studies[9,10] failed to detect an association between shedding of BCV via the respiratory tract or changes in antibody titer against BCV and the occurrence of BRD. It was reported that Koch's postulates were fulfilled for the role of BCV in BRD in 1995; however, this was later disputed.[8,11] Subsequently, the criteria for establishing BCV as a cause of BRD, based on Thomson's modification of Evans' criteria, were fulfilled.[8,12]

Early references[13,14] suggested antigenic and genomic similarity between isolates of BCV from the respiratory and enteric tracts of cattle. Later studies[15-18] detected differences in antigenic, genomic, and culture characteristics between the respiratory and enteric isolates. Presently, it is still unclear whether bovine respiratory and enteric coronavirus isolates differ in their virulence and trophism for the respiratory and digestive tracts.[3,15]

The cause of BRD is multifactorial, and vaccination against specific viral and bacterial agents reduces the incidence of disease. An intranasally administered vaccine against infectious bovine rhinotracheitis virus induces a rapid IgA response and effective immunity against the virus.[19,20] A commercial, modified-live vaccine,[a] administered PO to calves at birth or IM to adult cattle, is available for the prevention of enteric disease caused by BCV and rotavirus in neonatal calves. The effect of intranasal vaccination against BCV on the occurrence of BRD in recently weaned calves entering a commercial feedlot may provide additional evidence of an association of BCV with BRD and stimulate interest in development of a vaccine specific for BRD.

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The purpose of the study reported here was to measure antibody titers against BCV, determine frequency of BCV in nasal swab specimens, and compare calves treated for BRD between those given an intranasally administered vaccine and a group of control calves.

## Materials and Methods

**Calves**—Four hundred fourteen heifer calves that weighed 159 to 340 kg (350 to 750 lb) were studied. Calves were purchased weekly from September 2001 until November 2002 in groups of 6 to 44 from various livestock auctions in Virginia, North Carolina, and Tennessee within a 120-mile radius of Knoxville, Tenn.

**Experimental design**—The study was designed as a randomized, single-blind clinical trial. Each group of calves was processed within 24 hours of arrival at the feedlot, and individuals were randomly assigned to experimental (vaccinated) or control (placebo) groups by use of a random number table and blocking on groups of 2 calves. Vaccinated and control groups were separated by allocation to 1 of 2 pens of approximately 5 acres each and observed for periods varying from 17 to 99 days depending on week of purchase. At the end of the observation period, calves were commingled in a third common pasture. When approximately 150 calves were accumulated, purchasing was stopped until the commingled group was shipped to a finishing feedlot. The cycle was repeated 3 times. Pens had no common fence lines, were separated by > 50 meters, and were vacant for at least 30 days prior to receiving a new group of 150 calves. With each new group of calves, assignment of treatment groups to pens was reversed.

**Procedures**—Calves with BRD on arrival at the feedlot were treated at processing, eliminated from the study, and confined in a pasture separate from study calves. At processing, each calf was given a unique identification (ear tag) number, a separate cotton-tipped applicator was inserted approximately 3 inches into each naris to collect a sample for ELISA, a blood sample was obtained, and random assignment was made to the vaccinated or control (placebo vaccinated) group. In addition, all calves were given a 7-way bacterin-toxoid[b] vaccine against clostridial disease and a modified-live virus vaccine[c] against **infectious bovine rhinotracheitis virus (IBR)**, **parainfluenza virus type 3 (PI3)**, and bovine respiratory syncitial virus. Calves received implants with 200 mg of testosterone propionate and 20 mg of estradiol benzoate[d] and were dewormed with oxfendazole[e] (4.5 mg/kg [2.05 mg/lb], intraruminal). Dehorning was performed if needed, and dinoprost tromethamine[f] (25 mg, IM) was given to heifers suspected to be pregnant. In the fall (September to December), metaphylaxis with tilmicosin[g] (10 mg/kg [4.5 mg/lb], SC) was given to all incoming cattle. Vaccine or placebo was administered by 1 of 2 authors (PJP or BWR). Calves in the vaccinated group received 1.5 mL of the commercial, modified-live vaccine against coronavirus and rotavirus in each naris. Control calves received sterile saline (0.9% NaCl) solution in the same manner. All processing procedures were performed by 1 of 2 authors (RAD or RAD). A second blood sample was obtained at the end of the period during which calves were confined to separate pens.

**Outcomes**—Calves were monitored twice daily, and diagnosis of BRD was made by 1 of 2 authors (RAD or RAD) who were unaware of treatment status of calves. Criteria for diagnosis of BRD included obvious signs of respiratory tract disease (eg, dyspnea, cough, and ocular and nasal discharge). In addition, calves were examined if they were separated from the group, the only calf lying down, did not react or retaliate when butted by another calf, or had signs of depression. Final diagnosis was made, and a severity of disease score was assigned. Calves were scored on severity of illness on the basis of a scale (1 = healthy, 2 = slightly ill, 3 = moderately ill, 4 = severely ill, and 5 = moribund). Relapse was defined as a calf treated 3 or more days after a previous treatment. Calves that died were examined via field postmortem to verify the cause of death. Seroconversion was defined as antibody titer < 20 on entry into the feedlot and ≥ 40 at the end of the period of confinement in separate pens. The incidence of BRD in feedlot calves is highest during the first 3 to 4 weeks after arrival; therefore, observations were divided into events that occurred in ≤ 28 days while calves were confined to separate pens and events that occurred at anytime while in separate pens.

**Treatment for BRD**—All injections were given in the cervical area. If no metaphylaxis was given (January to August), initial treatment consisted of a single injection of tilmicosin. If there was no improvement in 24 hours or relapse occurred, a single dose of florfenicol[h] (20 mg/kg [9 mg/lb], SC) was given. If there was no response in 24 hours or relapse occurred, enrofloxacin[i] (10 mg/kg, SC, q 24 h) was given for 2 to 7 days. If again there was no response or relapse occurred, ceftiofur hydrochloride[j] (2.2 mg/kg [1 mg/lb], SC, q 24 h) was given. The treatment program for calves receiving metaphylaxis was the same, with the exception that the initial treatment with tilmicosin was eliminated from the protocol. In addition to the antimicrobial protocol, a single injection of flunixin meglumine[k] (1.5 mg/kg [0.7 mg/lb], IM) was given to calves with severity of disease score ≥ 3, and an electrolyte and *Lactobacillus* sp combination product[l] (0.63 g/kg [0.29 g/lb], PO) was given to calves that relapsed.

**ELISA for detection of BCV antigen**—Nasal swab specimens from both nares of a single calf were combined and suspended in 2 mL of lactated Ringer's solution and frozen at –20°C prior to processing. Detection of BCV antigen was performed by use of a commercially available antigen test kit.[m] The kit uses 2 BCV antigen-specific monoclonal antibodies as capture antibodies, guinea pig polycolonal anti-BCV antibody as the detector, and horseradish peroxidase anti-guinea pig IgG conjugate. Samples were thawed and vigorously vortexed, and the liquid was tested for BCV antigen. Results were obtained via measurement of **optical density (OD)** at 450 nm, and sample readings were normalized against negative and positive controls. Optical density values < 0.30 were considered to indicate negative results, and values > 0.39 were considered to indicate positive results. Samples with values from 0.30 to 0.39 were reassayed with a modified detector reagent that contained no detector antibodies, and OD values were again normalized by use of negative and positive controls. Samples with OD values < 0.30 were considered to have negative results, and samples with OD values ≥ 0.30 were considered to have positive results.

**Indirect fluorescent antibody test for BCV antibody**—Serum antibody titers were measured by use of indirect immunofluorescence, as described.[21] Briefly, BCV was propagated in human rectal tumor cells. The virus-infected cells were applied and fixed to glass slides for use as capture antigens.[22] Two-fold serial dilutions of the sample sera were made starting at 1:80 dilution and proceeding to a maximum of 1:320 for initial screening. Antibody was detected with anti-bovine IgG conjugated to fluorescein isothiocyanate.[n] Antibody titer was reported as the reciprocal of the highest dilution in which fluorescence was still detected. Samples with titers > 320 or < 80 were retitrated to determine the end point. Titers were not determined beyond 5,120. Antibody titers of < 20 were considered to indicate negative results.

**Data collection**—Each time a calf was treated for BRD, the identification number, date, diagnosis, severity of disease score, rectal temperature, and treatment (drug, route, and amount given) were recorded.

**Statistical analyses**—Univariate tests for significance of categoric variables among treatment groups were performed by use of $\chi^2$ or a Fisher exact test,[o] depending on the expected cell counts. For statistical comparison, serum titers against BCV were transformed by adding 1 to the titer dilution and use of log base 10 to normalize the distribution of the data. After statistical manipulation, the log values were back-transformed to calculate **geometric mean titers (GMTs)**. Comparison of GMTs among groups was performed by use of ANOVA.[p] A multivariate logistic regression model[q] was used to evaluate the effects of vaccination, presence of BCV, and antibody titer ≥ 20 against BCV on entry to the feedlot on treatment for BRD. Vaccination, pen, presence of BCV, antibody titer ≥ 20, and all 2- and 3-way interactions were included as independent variables in the model. Independent variables were removed from the model on the basis of $P \geq 0.25$ and the $r^2$ value indicating the fit of the model to the data. For all final analyses, values of $P \leq 0.05$ were considered significant.

## Results

Thirty-one serum samples were not included in the data analysis because of lost ear tags or missing or unreadable labels. In addition, 8 calves died prior to collection of convalescent serum samples, and therefore convalescent serum was not available for analysis (**Table 1**). A similar number of acute and convalescent sera were lost from each of the treatment groups. Seven nasal swab specimens were lost because of labeling errors or unreadable labels: 4 from vaccinated calves and 3 from controls. Days of observation, serum antibody titers against BCV, and proportion of calves with intranasal BCV were similar among vaccinated and control calves.

During the entire period while confined to separate pens, 2 (1%) calves from the vaccinated group died from BRD pneumonia, compared with 6 (3%) in the control group (**Table 2**). Although the difference was not significant via univariate analysis, fewer calves in the vaccinated group were treated 1 or more times for BRD than controls. Total relapses and severity of disease scores were similar among vaccinated and control calves.

Antibody titers < 20 on entry to the feedlot were observed in 148 of 396 (37%) calves entering the feedlot. Seroconversion from an initial titer of < 20 to ≥ 40 occurred in 136 of 140 (97%) calves for which acute and convalescent samples were available. Among vaccinated calves, 99% seroconverted, compared with 95% of control calves. Among calves that seroconverted, 42 of 136 (31%) were treated for BRD, compared with 1 of 4 calves that did not seroconvert. None of the differences were significant.

While calves were confined in separate pens, the incidence of BRD in calves with intranasal BCV on entry to the feedlot was higher in both groups, compared with calves from which intranasal BCV was not detected (**Table 3**). Although the proportion of calves with posi-

Table 1—Comparison of observations for control calves and calves given a vaccine against bovine coronavirus (BCV) intranasally.

| Observation | Vaccinated | Control |
|---|---|---|
| Enrolled (No. calves) | 208 | 206 |
| Days of observation (≤ 28) in separate pens* | 28 (17–28) | 28 (17–28) |
| Total days of observation in separate pens | 42 (17–99) | 42 (17–99) |
| Antibody titer ≥ 20† | | |
| Acute | 122/197 (62) | 126/199 (63) |
| Convalescent | 196/198 (99) | 192/195 (98) |
| Nasal swab specimen positive results† | 63/204 (31) | 62/203 (31) |

*Median (range). †No. calves/total calves (%).

Table 2—Comparison of outcomes between control calves and calves given a vaccine against BVC intranasally.

| Variable | Vaccinated | Control | *P* value* |
|---|---|---|---|
| During first period (≤ 28 d) while in separate pens | | | |
| Died (No. [%]) | 2 (1) | 5 (2.4) | 0.28 |
| One or more treatments for BRD | 42 (20) | 52 (25) | 0.22 |
| No. relapses | 9 | 10 | 0.80 |
| Severity of disease score (median [range]) | 3 (1–4) | 3 (1–5) | 0.85 |
| During entire period in separate pens | | | |
| Died | 2 (1) | 6 (3) | 0.17 |
| One or more treatments for BRD | 48 (23) | 64 (31) | 0.07 |
| No. relapses | 15 | 16 | 0.83 |
| Severity of disease score (median [range]) | 3 (1–4) | 3 (1–5) | 0.99 |
| Seroconversion from < 20 to ≥ 40† | 73/74 (99) | 63/66 (95) | 0.25 |

*Univariate analysis. †No. calves/total calves (%).
BRD = Bovine respiratory disease.

Table 3—Effects of vaccination, BCV antibody titer, and isolation of BCV from the nares on BRD in recently weaned control calves and calves given a vaccine against BVC intranasally.

| Variable | Vaccinated | Control |
|---|---|---|
| BRD during first period (≤ 28 d) while in separate pens | | |
| Nasal coronavirus* | | |
| Pos | 15/63 (24) | 21/62 (34) |
| Neg | 25/141 (18) | 30/141 (21) |
| Antibody titer against BCV* | | |
| ≥ 20 | 24/122 (20) | 29/126 (23) |
| < 20 | 16/75 (21) | 21/73 (29) |
| BRD during entire period in separate pens | | |
| Nasal coronavirus* | | |
| Pos | 17/63 (27) | 26/62 (42)[a] |
| Neg | 29/141 (21) | 37/141 (26)[b] |
| Antibody titer against BCV* | | |
| ≥ 20 | 28/122 (23) | 32/126 (25)[a] |
| < 20 | 18/75 (24)[1] | 30/73 (41)[b,2] |

*No. calves/total calves (%).[1,2,a,b]Different numbers indicate significant ($P \leq 0.05$) difference between treatment groups in the same row; different letters indicate significant ($P \leq 0.05$) difference between results within a treatment group in the same column. *P* values determined via univariate analysis.
Pos = Positive results. Neg = Negative results.

tive results for intranasal BCV and treated for BRD was higher in the control than in the vaccinated calves during the entire period of confinement, a univariate statistical test of these subgroups did not reveal significance (relative risk, 1.6; $P = 0.08$). However, the incidence of BRD among calves in the control group with intranasal BCV was significantly (relative risk, 1.6; univariate $P = 0.03$) greater than among control calves without intranasal BCV (37/141; 26%). Similarly, the incidence of BRD in control calves with antibody titers < 20 against BCV was significantly (relative risk, 1.6; univariate $P = 0.02$) greater than in control calves with antibody titers ≥ 20. Among all calves with serum antibody titers < 20 against BCV, control calves were 1.7 times more likely to develop BRD, compared with vaccinated calves (univariate $P = 0.03$); however, this association did not achieve statistical significance in the multivariate analysis. Among calves with serum BCV antibody titers ≥ 20, the incidence of BRD was similar for vaccinated (28/122; 23%) and control (32/126; 25%) groups.

In a multivariable model that included vaccination status, the presence of intranasal BCV on entry to feedlot, serum BCV antibody titer ≥ 20, 2- and 3-way interactions as independent variables, the effect of vaccine ($P = 0.008$), presence of intranasal BCV ($P = 0.009$), and the 3-way interaction between vaccination, pen, and BCV ($P = 0.017$) had a significant effect on the occurrence of BRD (**Table 4**). The effect of vaccination was most noticeable during the first 3 weeks of observation (**Figure 1**). Serum antibody titer ≥ 20 was found in 209 of 270 (77%) calves with negative results for intranasal BCV and only 39 of 121 (32%) calves in which intranasal BCV was detected (univariate $P < 0.001$).

Table 4—Multivariate model to assess the effects of vaccination, pen, BCV in nasal swab specimen, and antibody titer on occurrence of BRD in control calves and calves given a vaccine against BVC intranasally.

| Effect | *P* value |
|---|---|
| Vaccination | 0.008 |
| Pen (lot) | 0.10 |
| BCV in nasal swab specimen | 0.009 |
| Vaccination • titer ≥ 20 | 0.132 |
| Vaccination • pen (lot) • BCV | 0.017 |
| BCV • titer ≥ 20 | 0.006 |

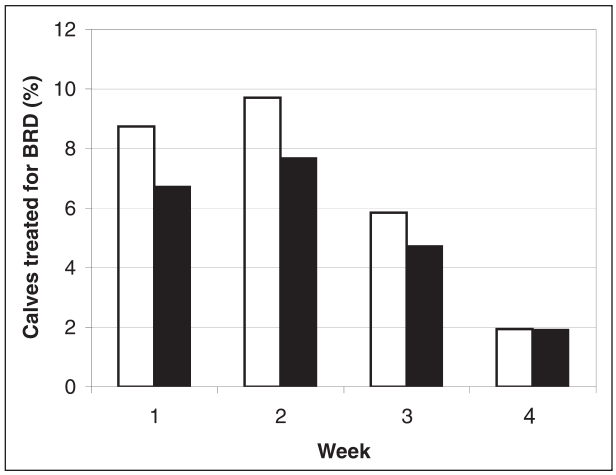


Figure 1—Proportions of control calves (open bars) and calves given a vaccine against bovine coronavirus intranasally (solid bars) that were subsequently treated for bovine respiratory tract disease (BRD) after arrival (weeks) at a feedlot.

## Discussion

Intranasal vaccination of calves against BCV on entry to the feedlot was protective, and the presence of intranasal BCV increased the risk of treatment for BRD. Vaccination had the greatest effect on calves with intranasal BCV and those with antibody titers < 20, on entry to the study. Vaccinated calves with intranasal BCV on entry to the study had 36% reduction in treatment 1 or more times for BRD, and those with antibody titers < 20 had 42% reduction, compared with control calves. Serum antibody titers ≥ 20 against BRD appeared to nullify the effect of vaccine because there were similar proportions of calves among the vaccinated (23%) and control (25%) calves treated for BRD.

Although the main effect of antibody titer ≥ 20 in a multivariate analysis was not significantly associated with BRD, fewer unvaccinated calves with antibody titers ≥ 20 were treated for BRD. The reduction in risk was significant when evaluated over the entire period of confinement to separate pens, based on a univariate $\chi^2$ test. Control calves without measurable antibody against BCV were at significantly greater risk to develop BRD, compared with control calves with serum antibody titers ≥ 20. Vaccination appeared to have no effect on calves with antibody titers ≥ 20 against BCV. This evidence suggests that serum antibody titers ≥ 20 against BCV may provide a measure of protection against BRD and supports a causal association of BCV with BRD. This finding is consistent with 2 prior studies[23,24] of Canadian feedlot cattle. Calves with virus neutralization antibody titers against BCV on arrival appeared to be protected for the first 28 days, and each increase in titer unit on arrival decreased the risk of BRD by approximately 0.8 (odds ratio).[23] In another study[25] of an epidemic of shipping fever in 6- to 8-month-old feeder cattle, significantly higher concentrations of serum neutralizing antibodies at the beginning of the epizootic were associated with protection against shipping fever pneumonia. In contrast, of 753 cattle arriving at 3 Ontario feedlots, 90% were seropositive for BCV, and it was concluded that BCV was not causally related to BRD in that instance.[9] This may have been caused by a protective effect of serum antibody titer combined with too few susceptible calves to detect a significant effect. In our study, only 60% of calves had antibody titers ≥ 20 against BCV; therefore, they represented a more susceptible population.

Seroconversion from a titer of < 20 to ≥ 40 occurred in 136 of 140 (97%) calves. Calves with antibody titers < 20 (82/143; 57%) were significantly more likely to be shedding intranasal BCV than calves with antibody titers ≥ 20 (39/247; 16%). Because these calves were also more likely to be treated for BRD, this suggests that prior exposure to BCV provides some protection against BRD. Among control calves, those with antibody titers ≥ 20 had 38% reduction in BRD, compared with calves in which serum antibody titers were < 20. In contrast, among calves in the vaccinated group, the incidence of BRD among those with serum

BRD antibody titers < 20 was similar to those with titers ≥ 20. These findings suggest that the effectiveness of vaccination depends on prior exposure to BCV and, therefore, will vary among populations of calves entering the feedlot.

We were able to detect BCV from the nasal passages of 31% of study calves. In other studies,[7,10,16] reported rates of nasal BCV isolation have ranged from 8.1% to 21.5%. In a multivariable analysis controlling for the main effects of vaccination, pen, antibody titer, and 2- and 3-way interactions, the presence of intranasal BCV was significantly associated with treatment for BRD. The greatest effect was observed in nonvaccinated calves, in which those with intranasal BCV were significantly more likely to develop BRD. In a previous study,[7] 44 of 68 (65%) cattle shed BCV from the respiratory tract and were treated for BRD, compared with 404 of 760 (53%) cattle that were not shedding BCV. Calves in both vaccinated and control groups at 28 days and for duration of time in separate pens were more likely to be treated for BRD if there was nasal shedding of BCV on entry in the study. Vaccinated calves with intranasal BCV were less likely to be treated for BRD than controls with intranasal BCV at 28 days and during the entire time the calves were in separate pens; however, the differences were not significant. These observations add to a mounting body of evidence linking BCV with respiratory tract disease in calves and are consistent with results of several published studies,[6,8,11] beginning with the early isolation of BCV from a calf during an outbreak of calf pneumonia in 1982.

In a multivariable analysis, vaccinated calves were significantly less likely to be treated for BRD while confined to separate pens. Univariate subgroup analyses revealed a significant effect of vaccine in calves with antibody titers < 20; however, a nonsignificant ($P$ = 0.08) but clinically important (36%) reduction in BCV was also observed among vaccinated calves with intranasal BCV on entry to the feedlot, compared with control calves. Although, to our knowledge, there are no published studies on the use of intranasally administered BCV vaccine, an intranasally administered vaccine against IBR is superior to inactivated and modified-live vaccines given by the IM route. Intranasal vaccination against IBR decreases the incidence of clinical signs and excretion of wild-type virus as early as 3 days after vaccination.[26] In another study,[27] calves were protected against IBR infection when vaccinated by the intranasal route 48 hours prior to exposure to experimentally infected calves. The serum antibody response to intranasally administered IBR vaccine was 100%, compared with only 60% in calves vaccinated IM with a modified-live vaccine.[19] When intranasal vaccination was compared with IM administration of a modified-live IBR vaccine, there was no difference in the systemic response; however, local antibody response and the ability of nasal leukocytes to inhibit viral cytopathic effect were greater after nasal administration.[28] Results of these studies suggest that the development of an intranasally administered vaccine against BCV, either separate or preferably combined with IBR, may have an added or synergistic effect to reduce treatments for BRD in feedlot calves. The observed effect of vaccination was to reduce the number of calves treated for BRD by 26%, from 31% in nonvaccinated calves to 23% in vaccinated calves. Although a univariate test did not detect significance ($P$ = 0.08) when controlling for the effects of antibody titer, intranasal BCV, and pen, the effect of vaccination was highly significant ($P$ = 0.01). The significant 3-way interaction indicates that the effect of vaccination was not consistent across all levels of pen (location) and intranasal BCV status.

A randomized, blind clinical trial is the strongest design for evaluation of risk. Calves in each of the treatment groups were similar with regard to serum BCV antibody titers < or ≥ 20 on entry to the study and presence of intranasal BCV, which provided evidence that the randomization scheme was effective. Potential for bias in selecting calves to be treated for BRD was addressed by masking the observers to the treatment status of calves. Sample size was a weakness in the study, as indicated by several subgroup comparisons that did not achieve significance when clinically important effects were observed. There was an overall reduction of 26% among vaccinated calves treated for BRD and a reduction of 36% among vaccinated calves with intranasal BCV on entry to the study, compared with control calves. The differences in both instances are clinically important; however, univariate statistical analysis did not reveal significant differences. Therefore, these observations need to be validated in future studies. Another issue is the possible effect of interferon production by respiratory tract cells infected with vaccine BCV to induce resistance in uninfected cells. We cannot separate the effect of interferon from the immune response, particularly surface IgA antibody production in response to the intranasal BCV vaccination. Results of previous studies[29,30] of calves exposed to intranasal IBR vaccine indicated that interferon production peaked at 3 to 4 days and disappeared from serum and nasal secretions at 10 days. Although the greatest effect of the vaccine is seen in the first 2 weeks, the effect appeared to persist for the entire period that calves were confined to separate pens. Although both groups of calves received an IM injection of modified-live vaccine containing IBR, PI3, and BRSV, in a previous report[29] it was stated that interferon was not detected in serum or nasal secretions of calves vaccinated IM with a modified-live IBR vaccine. We assume that the rotavirus component in the BCV vaccine had no effect on outcomes measured among vaccinated calves in our study; however, there is no published evidence to support this assumption.

These data add to a growing body of evidence in support of a causal relationship between BCV and BRD. In addition, intranasal vaccination with a modified-live product against BCV appears to reduce the risk of treatment for BRD in calves entering the feedlot. Development of a vaccine from strains of BCV isolated from the respiratory tract of cattle with BRD and evaluation of cost effectiveness of vaccination under field conditions are needed.

[a]Calf-Guard, Pfizer Animal Health, New York, NY.
[b]Electroid-7, Schering-Plough, Union, NJ.

[c]Bovi-Shield, IBR-PI$_3$-BRSV, Pfizer Animal Health, New York, NY.
[d]Synovex-H, Fort Dodge Animal Health, Fort Dodge, Iowa.
[e]Synanthic, Fort Dodge Animal Health, Fort Dodge, Iowa.
[f]Lutalyse, Pharmacia & Upjohn, Kalamazoo, Mich.
[g]Micotil 300, Elanco Animal Health, Indianapolis, Ind.
[h]NuFlor, Schering-Plough, Union, NJ.
[i]Baytril, Bayer Healthcare LLC, Shawnee Mission, Kan.
[j]Excenel, Pharmacia & Upjohn, Kalamazoo, Mich.
[k]Banamine, Schering-Plough, Union, NJ.
[l]Power Punch, Animal Health & Nutrition, Knapp, Wis.
[m]Syracuse Bioanalytical Inc, Ithaca, NY.
[n]Kirkegaard & Perry Laboratories Inc, Gaithersburg, Md.
[o]Proc Freq, SAS, version 9, SAS Institute Inc, Cary, NC.
[p]Proc Mixed, SAS, version 9, SAS Institute Inc, Cary, NC.
[q]Proc Logistic, SAS, version 9, SAS Institute Inc, Cary, NC.

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