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Clinical Immune Response to Experimental BHV-1 Challenge of Cattle Treated with Florfenicol at the Time of BHV-1 Vaccination

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Abstract

The purpose of this study was to determine the clinical immune response of cattle experimentally challenged with BHV-1 following treatment with florfenicol at the time of BHV-1 vaccination.

Forty unvaccinated beef-type, crossbred steers were randomly assigned to one of four treatment groups: a) florfenicol-treated and BHV-1-vaccinated (FF+,VX+), b) florfenicol-untreated and BHV-1 vaccinated (FF-, VX+), c) florfenicol-treated and BHV-1 unvaccinated (FF+,VX-), and d) florfenicol-untreated and BHV-1 unvaccinated (FF-,VX-). Florfenicol was administered day -24 and day -22 as per label. BHV-1 vaccine was administered both intranasally and intramuscularly on day -22 to cattle in both vaccinated groups. BHV-1 experimental challenge was done 21 days post-vaccination (day 0).

Post-challenge rectal temperature was significantly lower ($P<.05$) in vaccinated groups (FF+,VX+ and FF-,VX+) as compared to unvaccinated groups (FF+,VX- and FF-,VX-). Post-challenge cumulative clinical score, composed of cough, nasal and ocular discharge, and respiration character, was significantly lower ($P<.05$) in vaccinated groups (FF+,VX+ and FF-,VX+), as compared to unvaccinated groups (FF+,VX- and FF-,VX-). Post-challenge BHV-1 shedding was significantly reduced ($P<.05$) in vaccinated groups (FF+,VX+ and FF-,VX+) as compared to unvaccinated groups (FF+,VX- and FF-, VX-). Rectal temperature and clinical illness scores following vaccination and challenge were not different between florfenicol-treated (FF+,VX+ and FF+,VX-) and non-treated cattle (FF-,VX+ and FF-,VX-). Serum neutralization titers were significantly greater ($P<.05$) in florfenicol-treated groups (FF+,VX+ and FF+, VX-) as compared to untreated groups (FF-, VX+ and FF-, VX-) 21 days post-vaccination (day 0). Leukograms were within normal limits for all treatment groups at all time points. Vaccinated calves (FF+,VX+ and FF-,VX+) lost 7.95 lb/hd, while unvaccinated calves (FF+,VX- and FF-, VX-) gained 3.15 lbs/hd day -24 to d -20 ($P=.0555$).

In conclusion, BHV-1 vaccinated calves developed clinically protective immune responses. There was no indication of interference by florfenicol with response to vaccination.

Introduction

Some veterinarians and their clients have expressed concerns to the authors regarding use of florfenicol[a] in conjunction with vaccinations routinely given to cattle at arrival to the feedyard and boostered in feedyard hospital programs. These concerns are based on the extrapolation of data from studies which investigated *in vitro* immune function effects of chloramphenicol. Specifically, chloramphenicol is reported to cause suppression of blastogenesis in human lymphocytes[23] and suppressed lymphocyte mitotic index,[19] as well as prolonged rejection of skin homografts in rats and rabbits.[1,14,15,25] Suppression of serum antibody response in mice,[25] rabbits,[5,8] and humans[6,11,20] has also been reported. Duration of chloramphenicol treatment in these studies varied; the shortest duration of treatment was 2 days in one study while another study reported chloramphenicol treatment duration of 21 days.

Clinical relevance of these findings was not supported by results of a Canine Distemper Virus (CDV) challenge study involving beagle pups. Pups were given

long-term oral chloramphenicol treatment (50 mg/kg, tid X 14d) and vaccinated day 7 of treatment prior to CDV challenge 20 days post-vaccination.[17] In this study, normal *in vivo* and *in vitro* immune responses to CDV were reported in the chloramphenicol-treated group, although transient morphologic changes of erythrocytes and granulocytes were noted. Challenged dogs that had been treated with chloramphenicol and vaccinated prior to exposure were not different clinically than vaccinated control dogs.

Producer and veterinarian concerns are not clearly resolved by the existing data in the literature, given the equivocal results of the *in vitro* and *in vivo* reports. Also, studies investigating clinical immune function of florfenicol-treated calves have not been reported. The present study was done to address the issue raised by veterinarians and their clients of potential interference by florfenicol with vaccines in cattle, using protection from challenge as a clinically relevant and externally valid indicator of overall immune function.

Materials and Methods

Design--A 2 X 2 factorial design was used; BHV-1 vaccination and florfenicol treatment were the factors of interest. Forty mixed-breed, beef-type steers from a single source with a history of no previous BHV-1 vaccination, were used in this trial. The steers weighed an average of 708 lbs [95% CI: 678.05, 738.4] at initiation of the trial. Steers were randomly assigned individually by computer-generated random number code, within blocks of four, based on chute order, to one of four treatment groups, resulting in ten head per treatment group. Treatment groups were a) florfenicol-treated and BHV-1 vaccinated (FF+,VX+), b) florfenicol-untreated and BHV-1 vaccinated (FF-,VX+), c) florfenicol-treated and BHV-1 unvaccinated (FF+,VX-), and d) florfenicol-untreated and BHV-1 unvaccinated (FF-,VX-).

Cattle were housed outdoors on concrete with a shelter-covered feedbunk and penned by treatment group with an empty buffer pen between experimental pens. The diet was the same across all treatments and consisted of 46.74% #2 corn, 8.35% whey and corn steep liquor (70/30), 4.26% finisher protein supplement (urea in molasses), 1.37% monensin, 1.37% tylosin, and 37.91% alfalfa hay. Dry matter was 77.29% and crude protein was 14.80%.

Viral Challenge--All calves in each of the four treatment groups were challenged on day 0 with BHV-1 according to the Animal and Plant Health Inspection Service, Center for Veterinary Biologics protocol.[b] Specifically, BHV-1, Cooper strain was obtained from the Center for Veterinary Biologics Laboratory, Ames, IA and transported on dry ice to the research site. Challenge virus titer was $10^{8.6}$ $TCID_{50}/2$ ml and was second cell passage from nasal secretions obtained from a calf infected with seed virus. Virus was received as 1.2 ml in a 2 ml ampule. Contents of the ampule were diluted to 4 ml with balanced salt solution immediately prior to challenge. Each calf was challenged with 2 ml of challenge solution in each nostril using a DeVilbiss nebulizer.[c]

Procedure--Calves were bled and screened initially for serostatus to BHV-1 at the site of origin and again 1 week post-arrival at the research facility. Serostatus was also assessed on the day of challenge (day 0) and on day 11. Calves were penned separately according to treatment group following vaccination to avoid potential natural aerosol transmission.

FF+,VX+ and FF+,VX- groups were treated with 9.09 mg/lb (20 mg/kg) florfenicol, intramuscularly (IM) in the neck, on day -24 and day -22. On day -22, each calf in the FF+,VX+ and FF-,VX+ groups was vaccinated both IM[d] (2 ml, right neck musculature) and intranasally (IN)[e] (1 ml/nostril) with commercial MLV-BHV-1 vaccines labeled for these routes and dosages. All calves were challenged intranasally with Cooper strain BHV-1 on day 0.

Rectal temperature and clinical signs of respiratory disease were monitored and recorded daily on all calves from day -3 through day 11 by personnel blinded to treatment assignment. Clinical illness scores were comprised of scores for ocular discharge, nasal discharge, character of cough, and character of respiration (Table 1); in addition, a total clinical illness score was determined by summing component scores. A clinical depression score was not used in this study since this seemed to be even more subjective than clinical illness scores and we were concerned about consistency between observations.

Table 1. Clinical Scoring System used for each animal daily from day -3 to day 11 post-challenge.

	Severity			
Clinical Parameter:	0	1	2	3
Ocular Discharge	none	serous	mucopurulent	NA
Nasal Discharge	none	serous	mucopurulent	NA
Cough	none	dry, raspy	moist, productive	NA
Respiration	normal	elevated rate	severe dyspnea	open-mouthed

Nasal swabs for virus titration were collected on days -3, 0, 2, 4, and 7. Blood was collected for leukograms on days -24, -22, -20, 0, and 2 through 7.

All calves were weighed individually on days -24, -22, -20, -14, 0 and 11.

Statistical Analysis--Normality of distribution for continuous outcome variables (rectal temperature) of

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each treatment group was evaluated using the Shapiro-Wilk test.[4] The data were then rank-transformed and ANOVA was done on the ranks.[3] This approach was used since the Kruskal-Wallis test doesn't allow for evaluation of an interaction term (i.e. florfenicol by vaccination). For nonparametric variables (clinical illness scores), ANOVA was done on ranks of total and individual component scores. Ranked data were analyzed by split-plot ANOVA. Main-plot factors of florfenicol, vaccination and florfenicol by vaccination were tested by the main-plot error (animal within florfenicol by vaccination). Trial day by main-plot factor interactions were included in the subplot and tested by residual error.[10] Seroconversion and virus titer were analyzed using ANOVA of geometric means following logarithmic transformation of raw serum neutralization titers.[26] Probability of type I error was established at $\alpha < 0.05$.

Statistical power to detect the potential interaction (vaccine modification) of florfenicol by vaccination was calculated for each day in which there was a significant difference in clinical scores or rectal temperature between vaccinated (FF+,VX+ and FF-,VX+) and unvaccinated groups (FF+,VX- and FF-,VX-). The mean square of the main-plot error was used as the variance in power calculations. The measure of vaccine efficacy was the difference in clinical scores or rectal temperature between vaccinated (FF+,VX+ and FF-,VX+) and unvaccinated groups (FF+,VX- and FF-,VX-). Therefore, it was necessary to determine the specific level of reduction in vaccine efficacy, if it existed, which could be detected with the statistical power inherent in this experimental design. Maximum treatment difference suggestive of no effect on vaccine efficacy was the difference between vaccinated and unvaccinated groups at each trial day in which there was a significant vaccine effect. Maximum probability of committing Type II error was established at $\beta \leq 0.20$.

Results

In the initial analysis, trial day was included as a main effect; this was found to be significant ($P<0.0001$). Since a significant interaction was found between trial day and vaccination for each of the outcomes measured, the data was analyzed for vaccination effect at each level of trial day.

Rectal temperature was significantly lower ($P<.05$) in vaccinated groups (FF+,VX+ and FF-,VX+) as compared to unvaccinated control groups (FF+,VX- and FF-,VX-) on days 2 through 5 post-challenge, and days 8, 10 and 11 post-challenge; this trend ($P<.10$) was also seen on days 1, 7 and 9 post-challenge (Fig 1). Statistically significant differences in rectal temperature were not found between florfenicol-treated (FF+,VX+ and FF+,VX-) and non-treated controls (FF-,VX+ and FF-,

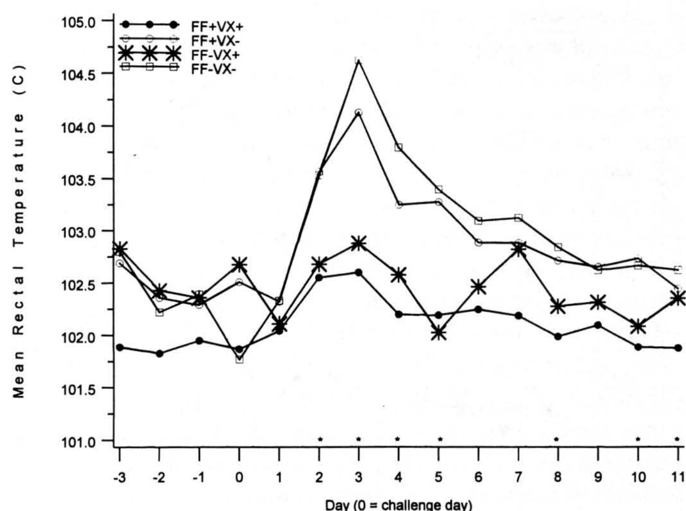

Figure 1. Mean rectal temperature of calves taken 3 days pre-challenge to 11 days post-challenge. (*$P<0.05$)

VX-) on any day measured following challenge. Significant florfenicol by BHV-1 vaccination interactions ($P<0.05$) were detected on days -3, -2 and day of challenge (day 0). On each of these days, the florfenicol-treated and BHV-1 vaccinated group (FF+,VX+) had lower mean rectal temperature than all florfenicol untreated groups (FF-,VX+ and FF-,VX-).

As mentioned, total clinical scores were the sum of individual scores for ocular discharge, nasal discharge, character of cough, and character of respiration. Total clinical scores were significantly lower ($P<.05$) in vaccinated groups (FF+,VX+ and FF-,VX+) as compared to unvaccinated control groups (FF+,VX- and FF-,VX-) on days -1 and 2 through 8 post-challenge (Fig 2). Significant difference in total clinical score was found between florfenicol-treated (FF+,VX+ and FF+,VX-) and non-treated groups (FF-,VX+ and FF-,VX-) only on day 3 post-challenge; in this case, florfenicol-treated groups (FF+,VX+ and FF+,VX-) had significantly lower ($P<.05$) total clinical illness scores than non-treated groups (FF-, VX+ and FF-,VX-).

Individual component clinical score results are shown in Table 2. No significant differences in clinical illness scores for any clinical component measured, or total clinical score, were found between florfenicol-treated (FF+,VX+ and FF+,VX-) and non-treated groups (FF-,VX+ and FF-,VX-) on any day measured post-challenge except for significantly lower nasal discharge scores and total clinical illness scores on day 3 post-challenge in florfenicol-treated calves (FF+,VX+ and FF+,VX-).

Virus isolation attempts for BHV-1 were negative from nasal secretions collected on all animals of each group on days -3 and 0 (day of challenge). Animals from each group were shedding BHV-1 virus in nasal secre-

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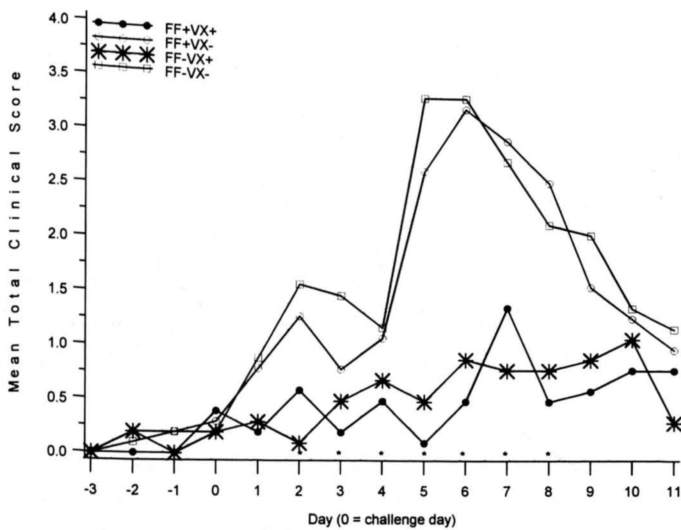

Figure 2. Total clinical illness score. Sum of individual component scores (nasal discharge, ocular discharge, cough, respiratory character) for each treatment group. (*$P<0.05$)

Table 2. Individual component clinical score results of statistical significance ($P<0.05$) by trial day.

	(FF+,VX+ and FF-,VX+) vs. (FF+,VX- and FF-,VX-)	(FF+,VX+ and FF+,VX-) vs. (FF-,VX+ and FF-,VX-)
Ocular Discharge	days 2, 4-9	NS
Nasal Discharge	days 2, 3, 5-8	3
Cough Character	days 1, 5, 6, 8, 9	NS
Respiration Character	days 2, 5-8, 11	NS

tions on days 2, 4, and 7. BHV-1 titers ranged from 50 $TCID_{50}$/ml to 5 X 10^5 $TCID_{50}$/ml on day 2, from <10 to 5 X 10^6 $TCID_{50}$/ml on day 4 and from <10 to 5 X 10^4 $TCID_{50}$/ ml on day 7. Geometric mean titers of BHV-1 shedding in BHV-1-vaccinated calves (FF+,VX+ and FF-VX+) were significantly less ($p<.05$) than in unvaccinated groups (FF+,VX- and FF-,VX-) on day 2 and day 4 post-challenge, but were not significantly different on day 7 post-challenge (Fig 3). Geometric mean titer of BHV-1 shedding was not significantly different between florfenicol-treated calves and non-treated control groups. In addition, no significant florfenicol by BHV-1 vaccination interaction was found at any day measured.

Serum neutralization assays on day -24 revealed that 18 of 40 animals had serum antibody titers to BHV-1 ≥ 1:2; one of 40 had a serum antibody titer of 1:16 and one of 40 was 1:32. Serum from each collection day were assayed simultaneously at the end of the trial after all samples had been collected. These two animals, by chance at randomization, were assigned to the florfenicol-treated, unvaccinated group (FF+,VX-). Since trial day by florfenicol interaction was significant

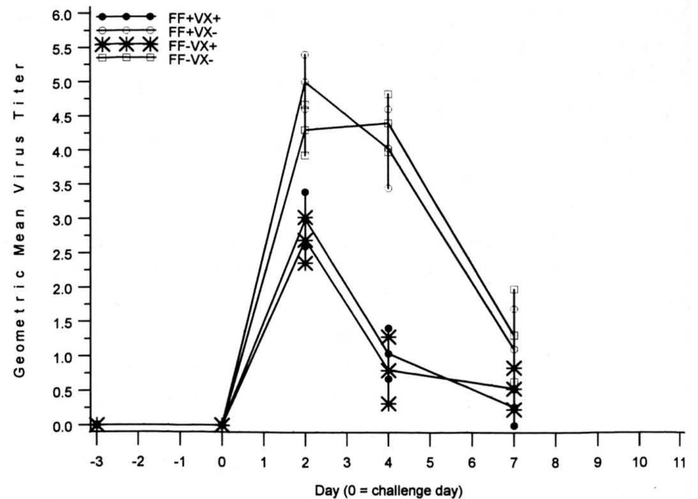

Figure 3. Geometric mean virus isolation titer (± standard error).

($P<0.0001$) for serum antibody, florfenicol effects were analyzed at each level of trial day. Florfenicol-treated groups had significantly higher ($P<.05$) geometric meantiters than non-treated groups 21 days following BHV-1 vaccination (Fig 4). No significant difference in titer was seen between florfenicol-treated (FF+,VX+ and FF+,VX-) and non-treated groups (FF-,VX+ and FF-,VX-) on day -24 or day 11. No significant difference in geometric mean titer was seen between BHV-1 vaccinated (FF+,VX+ and FF-,VX+) and unvaccinated groups (FF+,VX- and FF-,VX-) on any day measured, although a trend ($P=.06$) towards higher titers in vaccinated groups was seen 21 days following vaccination.

Significant treatment group differences ($P<.05$) in leukograms (WBC) were found on days 5, 6, and 7 (Table 3). Average WBC counts across all study days for BHV-1 vaccinated groups was 8.99 X 10^3/μl as compared to

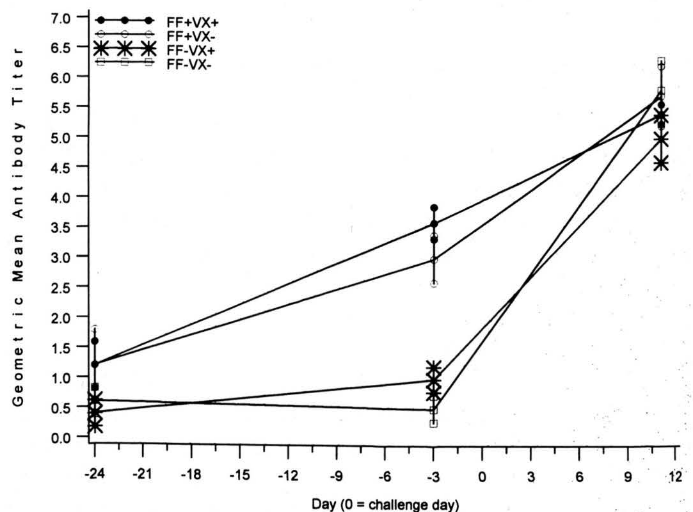

Figure 4. Geometric mean serum neutralizing titer (± standard error). Vaccination was day -24.

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7.98 X 10^3/µl for unvaccinated groups, which was statistically significant ($P<0.05$). Average WBC counts across all study days for florfenicol-treated groups was 8.68 X 10^3/µl and 8.29 X 10^3/µl for non-treated groups ($P<0.05$).

Table 3. Mean White Blood Cell counts X 103 (± standard error X 103) by treatment group over trial day.

	FF+,VX+	FF-,VX+	FF+,VX-	FF-,VX-
Day -24	10.33 (0.838)	8.28 (0.501)	8.71 (0.582)	8.54 (0.530)
Day -22	10.28 (0.841)	9.59 (0.635)	9.59 (0.621)	7.77 (0.803)
Day -20	9.83 (.838)	8.48 (0.798)	9.21 (0.528)	8.16 (0.398)
Day 0	8.70 (1.171)	9.12 (0.752)	7.63 (0.440)	8.62 (0.588)
Day 2	8.48 (1.128)	8.60 (0.521)	7.37 (0.379)	8.12 (0.574)
Day 3	7.98 (1.060)	8.51 (0.462)	7.85 (0.412)	8.07 (0.571)
Day 4	8.53 (0.647)	8.11 (0.385)	7.19 (0.410)	7.69 (0.394)
Day 5	9.25 (0.692)[a]	8.02 (0.424)[ab]	7.36 (0.398)[b]	7.49 (0.371)[b]
Day 6	9.58 (0.864)[a]	8.38 (0.394)[ab]	7.60 (0.338)[b]	7.22 (0.373)[b]
Day 7	10.45 (0.832)[a]	9.41 (0.665)[ab]	7.70 (0.468)[b]	7.71 (0.644)[b]

[a, b] values with different superscripts differ significantly, $P<0.05$.

No significant differences in weight gain between florfenicol-treated (FF+,VX+ and FF+,VX-) and untreated calves (FF-,VX+ and FF-,VX-) were found between day -24 and days -22, -20, -18, 0, or 11 (Table 4). BHV-1 vaccinated calves (FF+,VX+ and FF-,VX+) lost 7.95 lb/hd and unvaccinated calves (FF+,VX- and FF-,VX-) gained 3.15 lb/hd between day -24 and day -20; this difference approached statistical significance ($P=0.0555$). No statistically significant differences in weight gain were found between BHV-1 vaccinated and unvaccinated groups between day -24 and days -22, -14, 0, or 11.

Discussion

Concerns expressed to the authors by veterinarians in the field regarding potential negative effects of florfenicol treatment on clinical immune function in cattle have not been adequately addressed by existing data in the literature. These concerns have been extrapolated from reports of studies using various chloramphenicol treatment duration ranging from 2 days to 21 days in length. Confusion has arisen since results of *in vitro* studies are inconsistent with the results of *in vivo* challenge studies. Additionally, extrapolating results of these studies to expected response of cattle in feedlots is questionable because of species differences, drug differences, and treatment-period differences. In this study, we used the labeled period of treatment and dose of florfenicol for intramuscular use in cattle. Results of this study showed no significant positive or negative effects on clinical response to experimental BHV-1 challenge in florfenicol-treated calves as compared to untreated controls. Specifically, no negative effects of florfenicol on the clinical immune response to BHV-1 challenge following BHV-1 vaccination were found; clinical illness scores were significantly higher in unvaccinated groups as compared to BHV-1 vaccinated calves, without significant florfenicol by vaccination interaction. In addition, no differences in weight gain was found between florfenicol-treated and untreated calves. Conversely, unvaccinated controls gained approximately 11 lbs/hd more than BHV-1 vaccinated groups over a 4 day period between d -24 and d -20 ($P<.05$). This effect equilibrated and was not found at the next weight measurement taken 6 days later.

Table 4. Mean weight change, lbs (± standard error, lbs) over various time periods post-vaccination.

trial day	FF+,VX+	FF-,VX+	FF+,VX-	FF-,VX-
-24 to -22	6.00 (4.82)	8.00 (5.00)	-0.40 (4.97)	8.3 (6.13)
-24 to -20	-10.10 (6.04)[a]	-5.80 (5.24)[a]	5.80 (6.14)[b]	0.50 (4.92)[b]
-24 to -14	24.00 (8.90)	5.00 (10.71)	14.70 (7.19)	23.20 (9.11)
-24 to 0	52.00 (8.18)	41.90 (10.44)	42.20 (9.25)	45.40 (12.57)
-24 to 11	110.20 (14.22)	106.00 (11.21)	89.90 (13.10)	106.70 (18.61)

[a, b] values with different superscripts differ significantly, $P<0.05$.

In laboratory studies involving humans, mice, rabbits, and rats[1,5,6,8,11,14,15,19,23,25] treated with chloramphenicol for various periods, outcome variables examined include antibody response, lymphoproliferation, and homograft rejection. The responses measured were to non-replicating antigens such as bovine serum albumin (BSA) or sheep red blood cells (SRBC), whereas in this study, the immune response was to modified-live BHV-1 virus. Protection against experimental challenge is a clinically relevant immune function outcome of interest to practitioners and producers. Changes in therapy and procedures are likely to be based on clinically relevant and economically important outcomes such as morbidity, mortality, performance, and cost of gain rather than serum antibody titer, lymphoproliferation assays, or interferon levels. Immune function outcomes such as serum antibody titer, lymphoproliferation assays, and interferon levels, are substitution indicators[18,21] for the issue of concern to practitioners, which is the health status of the animal or population.

Serum antibody titers were significantly greater in florfenicol-treated cattle 21 days following vaccination. This differs from antibody suppression reported in human patients and laboratory animals treated with chloramphenicol for various periods. Florfenicol and chloramphenicol inhibit bacterial protein synthesis by binding 50s ribosomes.[24,27] Conversely, antibody synthesis is mediated through 80s ribosome function.[17] Therefore, it is unlikely that the mechanism of antibody suppression reported for chloramphenicol is due to interference at the level of the ribosome.

The association between BHV-1 serum antibody levels and protection against clinical disease has been

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equivocal and inconsistent.[2,9,12,13,16] This is further supported by the results of our study. Despite vaccination, there were no differences in serum neutralizing antibody titers between vaccinates and unvaccinated control calves when these titers were measured after vaccination, but prior to challenge (day 0). Also, post-vaccination and pre-challenge serum neutralizing antibody titers were not associated with clinical protection against challenge, since vaccinated calves showed significant levels of clinical protection, despite widely varying serum neutralizing antibody titers. As mentioned, there was no statistically significant association between serum neutralizing antibody levels and florfenicol treatment on day -24 or day 11.

It is uncertain why approximately one-half (18/40) of the cattle had serum antibody titers of >1:2 on day -24. As mentioned, the two animals which had serum antibody titers to BHV-1 of 1:16 and 1:32 were randomly assigned, by chance, to the FF+,VX- group. Intuitively, this would tend to reduce the ability to detect clinical differences between vaccinated and unvaccinated control groups. However, differences in clinically relevant outcomes were detected between these groups despite seropositive animals in non-vaccinated groups at trial entry. It is possible that these cattle were previously subclinically exposed to BHV-1 through natural field exposure. However, no significant association was found between day -24 serostatus and vaccination treatment group assignment (P=0.75; Yates corrected), florfenicol treatment assignment (P=0.756), or trial day (P=0.54). Therefore, the method of randomization used was effective in distributing seropositive animals across both florfenicol and vaccination treatment groups. Also, results of this study were not significantly (P>0.05) biased based on initial serostatus.

The leukogram data was mostly unrevealing. Differences in WBC between vaccinates and non-vaccinates and between florfenicol-treated groups and non-treated groups were statistically significant (P<.05) when measured across all trial days. However, this finding is likely not clinically important since all values fall nearly mid-range within published normal limits.[7] This reflects non-significant effects of vaccination, challenge, and florfenicol treatment. This is interesting in light of leukopenia and lymphopenia[14] reported for chloramphenicol and leukopenia for thiamphenicol.[22] This difference may be due to differences in duration of treatment, species, and/or drugs.

Differences in rectal temperature, nasal discharge, ocular discharge, respiration, cough, and total clinical score were dependent on level of vaccination status with no significant association between level of florfenicol treatment and clinical outcome. This addresses the primary objectives of the study, i.e. to determine the specific level of clinical response to experimental BHV-1 challenge in florfenicol-treated calves.

Type II error is the probability of failing to find existing differences between treatment groups. One minus the probability of type II error is the definition of statistical power. Statistical power should always be assessed in cases of failing to find treatment differences. This study reports that florfenicol treatment did not significantly modify BHV-1 vaccination. No significant vaccine by florfenicol interaction was found on days that there was a statistically significant vaccine effect. However, in order to demonstrate this non-effect with confidence, the power, or ability to detect treatment group differences, was calculated at each day that there was a significant vaccination effect for total clinical score or for rectal temperature. Not surprisingly, the statistical power curve followed that of morbidity. This is due to the fact that as cattle become more sick following challenge, the ability to identify vaccinates as compared to non-vaccinates is enhanced. Therefore, if there had been reduced vaccine efficacy induced by florfenicol, we had sufficient power to detect 29.3% to 100% of vaccine effect modification at mid-ranges in the morbidity curve, i.e. days 2 through 8 post-challenge, when effects of the challenge were most severe. This pattern was more evident with total clinical score, but was also found with rectal temperature. As veterinarians and pen riders observe cattle for clinical respiratory disease, it seems reasonable that they would be able to detect differences between pens of cattle or treatment groups if there were 30% to 100% difference in severity of clinical illness between pens or treatments. Therefore, we feel we had reasonable statistical power from a practical standpoint.

The challenge model used in this study was successful in producing clinical signs of cough, serous ocular and copious ropy serous nasal discharge, increased respiration rate and some cases of open-mouthed breathing in unvaccinated groups. This was interesting since nearly one-half (9/20) of these calves were seropositive (≥ 1:2) at trial initiation. In the feedyard setting, cattle with signs similar to those seen in the unvaccinated groups of this study would likely be pulled for treatment based on the clinical signs of nasal and ocular discharges and cough as outlined above. However, response to treatment would be expected to be unsatisfactory since the etiology was predominately viral, based on clinical signs. We concede that lung or nasal discharges were not sampled for bacterial culture; however, character of discharges and cough were most suggestive of uncomplicated viral infection, where bacterial pathogens, if present, were at least not playing a primary role.

When these findings are extrapolated to the feedyard setting, there is an obvious risk of abandoning therapeutic programs that are potentially successful in treatment of bacterial infections. However, therapeutic programs may be changed by the veterinarian or feedlot management over time, with subsequent programs

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or antibiotics appearing to be effective based on the point in the infection and disease process in which they are used. In reality, it is possible that programs used in later stages of viral infection benefit from good timing rather than true efficacy in some cases.

Vaccination in this study was by both intranasal and intramuscular routes; however, many feedlot vaccination programs are parenteral or mucosal only. In this study, in order to maximize the probability of clinically apparent treatment group differences, both mucosal and systemic immunity were targeted. This may have increased resistance against experimental challenge and obliterated subtle negative immune function effects. We concede that this may not accurately reflect some feedyard vaccination programs which administer BHV-1 vaccination by one route only. However, it must also be noted that vaccination was administered on day 2 of florfenicol treatment. This is also not representative of field conditions, but was done to allow for maximum florfenicol effect on immune function if this effect existed under conditions of the product label. These deviations from "real world" applications in our experimental design were intended to increase the probability of clinically detectable differences between treatment groups. In other words, we wanted to provide optimal opportunity for protection in vaccinated groups and for negative immune function effects, if they existed as per product label, in florfenicol-treated groups.

Conclusions

Cattle treated with florfenicol and simultaneously vaccinated against BHV-1 are capable of developing a clinically protective immune response as determined by clinical response to experimental BHV-1 challenge. No significant differences in weight gain or BHV-1 shedding were found between florfenicol-treated and untreated cattle.

Acknowledgments

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Footnotes

[a] Nuflor®, 20 mg/kg, IM q 48 hr, Schering-Plough Animal Health, Union, NJ 07083.
[b] Reagent data sheet, BHV-1 Cooper strain, Lot 96-18, Animal and Plant Health Inspection Service, Center for Veterinary Biologics, Ames, IA 50010.
[c] DeVilbiss Model #163 Atomizer, DeVilbiss Health Care, Inc., Somerset, PA 15501.
[d] Bovine Rhinotracheitis Vaccine, Sanofi Animal Health, Inc., Overland Park, KS 66210.
[e] Nasalgen IP, Coopers Animal Health Inc., Mundelein, IL 60060.

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Abstract

Comparison of danofloxacin with baquiloprim/sulphadimidine for the treatment of experimentally induced *Escherichia coli* diarrhoea in calves

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Thirty-eight, one- to two-week-old calves with experimentally induced *Escherichia coli* diarrhoea were randomly assigned to three treatment groups. Two groups of 15 calves were treated intramuscularly once daily for three days with either danofloxacin mesylate at 1·25 mg/kg bodyweight, or with baquiloprim/sulphadimidine as a positive control (10 mg of combined active ingredient/kg); eight calves were treated with 0·9 per cent sodium chloride solution as a negative control (1 ml/20 kg). Faecal consistency, demeanour, hydration status, appetite and bodyweight were monitored before, during, and for four days after treatment by an investigator unaware of the animals' treatment. Before treatment, the clinical, biochemical, and faecal indices were similar among the groups. By 24 hours after treatment began, the proportion of observations of faeces recorded as of normal consistency was highest in the danofloxacin-treated group (26 of 60), compared with 16 of 60 in the baquiloprim/sulphadimidine treated groups and four of 32 in the control group. The proportion of calves with a normal demeanour was highest in the danofloxacin-treated group at all the evaluations and these calves gained significantly ($P<0{\cdot}05$) more weight (1·6 [0·27] kg) than the calves treated with baquiloprim/sulphadimidine (0·67 [0·36] kg). The calves in the danofloxacin-treated group maintained relatively normal blood pH values, whereas the calves in the control group became progressively acidotic. By the end of treatment, the mean bicarbonate concentration was significantly ($P<0{\cdot}05$) higher in the danofloxacin-treated calves than in the control group. The pH of the calves in the baquiloprim/sulphadimidine-treated group changed little during treatment, but by three days after the last treatment their mean pH had dropped to the level of the calves in the control group. The mean bicarbonate concentration of the baquiloprim/sulphadimidine-treated calves, like that of the danofloxacin-treated calves, was significantly ($P<0{\cdot}05$) higher than that of the calves in the control group.