Determination of protection provided by two modified live bovine herpesvirus-1 vaccines against bovine herpesvirus-1 conjunctivitis in calves

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Abstract

A study was performed in 2 phases to determine the ability of 2 different modified live (MLV) bovine herpesvirus-1 (BoHV-1) vaccines to protect against conjunctivitis caused by a recent field isolate of BoHV-1. In phase I, BoHV-1 isolates were used to determine the ability of the isolates to cause conjunctivitis and to establish a challenge model for phase II. Fifteen calves were randomly assigned to 1 of 3 groups: 5 calves received 5 mL containing 10^7 tissue culture infectious dose $(TCID)_{50}$/mL of BoHV-1 by the intraocular route (topical administration) for 2 consecutive days, 5 calves received 5 mL of inoculum consisting of 10^7 $TCID_{50}$/mL of BoHV-1, administered intranasally for 2 days, and 5 calves served as unchallenged sentinels and received phosphate buffered saline, 5 mL administered by the intranasal and 5 mL by the intraocular route for 2 days. All 15 calves developed pyrexia as defined by a rectal temperature greater than 103.5°F (39.7°C), were BoHV-1 virus isolation positive from ocular swabs and 10/15 calves had increased ocular discharge and conjunctivitis. In phase II, 10 calves were intranasally vaccinated with a MLV BoHV-1, 10 calves were parenterally vaccinated with a MLV BoHV-1, and 5 calves were unvaccinated. All 25 calves were challenged by receiving 5 mL by the intraocular route of inoculum consisting of 10^7 $TCID_{50}$/mL of BoHV-1 for 2 consecutive days. Following challenge, calves were examined daily, and blood (serum), nasal swabs, and conjunctival swab samples were obtained on study days 0, 3-7, 10, 14 and 28. Both vaccines significantly decreased post challenge ocular lesions, clinical scores, and quantity of virus isolated.

Key words: bovine herpesvirus-1, conjunctivitis, vaccination

Introduction

Bovine herpes virus type 1 (BoHV-1) belongs to the subfamily of alphaherpes-virinae. It has a size of 150-200 nm and contains a double-stranded DNA genome and is surrounded by an envelope containing lipids. BoHV-1 can be differentiated on the DNA level into 3 subtypes by restriction endonuclease methods.[3,4,6,10]

BoHV-1 causes a variety of clinical signs, e.g., rhinotracheitis, fever, conjunctivitis, drop in milk production, abortion, reproductive losses, encephalitis and lesions of the mucous membranes of the genital tract.[6,8,11,12] While many studies have been published on the respiratory and reproductive forms of BoHV-1, little has been published on the impact of BoHV-1 on the bovine eye. While BoHV-1 has been shown to cause conjunctivitis,[2,10,12,13] the involvement of BoHV-1 in infectious bovine keratoconjunctivits (IBK) is still unknown even though bovine herpesviruses are often isolated from cases of IBK.[2,7,13,15,16]

This is also true for BoHV-1 vaccination. The research and challenge models have all focused on BoHV-1 associated respiratory and/or reproductive diseases of BoHV-1 for which there are vaccine label indications. However, no BoHV-1 vaccine has an indication for protection against conjunctivitis caused by BoHV-1 even though BoHV-1 vaccination is often recommended for preventing BoHV-1 conjunctivitis.[12] This is in part because there is no established challenge model for BoHV-1 conjunctivitis.

This set of studies assessed the ability of BoHV-1 isolates from recent natural cases of IBK to cause conjunctivitis and to establish a potential challenge model. The challenge model was then used to assess the ability of 2 different modified live BoHV-1 vaccines to provide protection against BoHV-1 induced conjunctivitis.

Material and methods

These studies were segregated into 2 separate phases. The objective of phase I was to establish ocular infectivity of BoHV-1 isolates collected from natural IBK outbreaks and to assess spread from those challenge inoculated calves to unchallenged, at-risk sentinel calves. Phase II of the study was designed to assess protection afforded by 2 separate modified live BoHV-1 vaccines in the challenge model described in Phase I.

Phase I

All study procedures were approved by the Auburn University Animal Care and Use Committee (IACUC, PRN 2020-3792). The objective of phase I was to establish infectivity of the pooled inoculum in this challenge model of BoHV-1 isolates collected from natural IBK cases and to assess spread in uninoculated at-risk calves, prior to Phase II which was to assess efficacy of vaccine protection.

Animals

Fifteen Angus-Simmental crossbred beef steers (179-214 days of age) were enrolled in the study. Each steer was individually identified using a unique numbered ear tag. Weights at

enrollment ranged from 249-315 pounds (113-143 kg). Since phase I of this study was designed to see if conjunctivitis could be caused by the virus and spread among calves, no power calculation was necessary. Prior to study initiation, all calves were examined and were healthy, seronegative for BoHV-1 and BVDV, and were BVDV-virus negative. They had not been vaccinated against BoHV-1. Calves were weighed to ensure they were of similar weights and randomly assigned to 1 of 3 groups: The 5 calves in Group A received 5 mL of inoculum consisting of 10^7 $TCID_{50}$/mL of BoHV-1 by the intraocular route (topical) for 2 consecutive days, Group B (n = 5) received 5 mL of inoculum consisting of 10^7 $TCID_{50}$/mL of BoHV-1, administered intranasally for 2 days, and Group C (n = 5) served as unchallenged sentinels and received phosphate buffered saline, 5 mL administered by the intranasal and 5 mL by the intraocular route for 2 days.

All study cattle were housed at the North Auburn Beef Unit on the campus of Auburn University and were housed together. The unchallenged calves served as sentinels and were used to assess transmission of the virus from challenged calves. Calves showing signs of systemic illness were eligible for treatment with an antibiotic at the discretion of the investigator. All steers had nasal and conjunctival swabs collected 30 days after the completion of the study for BoHV-1 PCR testing. Once all animals had negative results on both samples they were sold.

Challenge inoculum

The viral inoculum consisted of a pool of 5 individual isolates obtained from the Wisconsin Veterinary Diagnostic Laboratory that had been propagated in Madin-Darby bovine kidney (MDBK) cell cultures, titered and frozen prior to use. These isolates were from recent clinical cases of conjunctivitis/IBK in dairy heifers.

Samples collected

Following inoculation, calves were examined daily for ocular and nasal discharge and inflamed conjunctiva, and blood (serum), nasal swabs, and conjunctival swab samples were obtained on study days 0, 3-7, 10, 14 and 28. Any calves with increased ocular discharge and/or inflamed conjunctiva had fluorescein dye applied to the affected eye(s) to check for corneal lesions.

Phase II

All study procedures were approved by the Auburn University Animal Care and Use Committee (IACUC PRN 2020-3792).

Animals

Twenty-five Angus-Simmental crossbred beef steers (179-214 days of age) were enrolled in the study. Each steer was individually identified using a unique numbered ear tag and weights ranged from 249-315 pounds (113-143 kg). All calves were unvaccinated and sampled prior to the initiation of the study. Calves were determined to be seronegative for BoHV-1 and BVDV and were BVDV-virus negative. The culture/PCR results from study 1 showed 100% infected eyes. Since no previous study was found to determine vaccine efficacy against BoHV-1 induced conjunctivitis, a decrease in infection of 50% was used in sample size determination using an alpha = 0.10 and 80% power. These parameters resulted in a sample size of 10 animals per treatment group and 5 animals in the negative control group.

Steers were weighted to ensure similar weights and randomly assigned to 1 of 3 treatment groups: Group IN (n = 10) received 2 mL of a modified live (MLV) BoHV-1, bovine respiratory syncytial virus (BRSV) and parainfluenza-3 (PI3) vaccine intranasally in a single naris via cannula, Group SQ (n = 10) received 2 mL of a MLV bovine viral diarrhea virus (BVDV) types 1 and 2, BoHV-1, BRSV, PI3 and a *Mannheimia haemolytica* leukotoxoid by subcutaneous injection, and Group CON calves were administered saline subcutaneously. Calves were housed in separate groups for 21 days according to their treatment group to avoid shed of vaccine virus between treatment cohorts. After BoHV-1 challenge the calves were housed together in a single pen.

On day 21 rectal temperatures were obtained from each calf, blood was drawn and centrifuged to collect serum for BoHV-1 serology. On days 21 and 22, all calves were challenged intraocularly in both eyes with live field strain BoHV-1. All calves were challenged on 2 consecutive days (day 21 and 22) with 10^7 $TCID_{50}$ of BoHV-1 isolate (from phase I) diluted in 5 mL of culture media. An aerosol was generated by an intranasal mucosal atomization device to deliver 2.5 mL of inoculum misted onto the corneal and conjunctival surfaces of each eye.

Challenge strain

The viral inoculum used for phase II was a BoHV-1 isolated from a calf with post-challenge severe conjunctivitis from the phase I study (see phase I results below). This isolate was propagated in MDBK cell cultures, titered and frozen prior to use.

Samples collected

Calves were monitored for signs of respiratory disease, fever, nasal and ocular lesions (conjunctivitis, keratitis). Calves showing signs of systemic illness were eligible for treatment with an antibiotic[a] at the discretion of the investigator. On study days 21, 24-28, 31, 35 and 42 all calves had nasal and conjunctival swabs collected for BoHV-1 virus isolation and BoHV-1 polymerase chain reaction (PCR). These sample days corresponded to days 0, 3-7, 10, 14 and 28 post-challenge like phase I. Final ocular and nasal swabs were collected 30 days after completion of the trial for BoHV-1 PCR analysis. Serum for BoHV-1 virus neutralization titers was obtained on day 0 (pre vaccination), day 21 (pre-challenge) and day 42.

Clinical scores

Calves were observed daily during feeding time. Rectal temperatures and clinical scores were collected on study days 21, 22, 24-28, 31, 35 and 42. Clinical scores were evaluated using a clinical scoring system (Table 1) utilized in a previous study and approved by IACUC. A cumulative clinical score was calculated based on rectal temperature, attitude and breathing characteristics.

Ocular scores

Based upon the observations in phase I, an ocular scoring system was developed for phase II. Ocular lesion scores were classified 0-3 according to the adapted Clinical Scoring method in Table 1, consisting of normal serous secretions (0), bilateral mucous discharge without corneal or conjunctival lesions (1), bilateral mucopurulent discharge with conjunctival erythema but no corneal lesion (2), bilateral mucopurulent discharge with corneal lesion (3).

Table 1: Clinical scoring standards used throughout both phases of a study evaluating whether a vaccination could prevent conjunctivitis after inoculation with BoHV-1 (10^7 $TCID_{50}$/mL).

Clinical outcome	Score
1) Rectal temperature (degrees F)	
100 – 100.9	0
101 – 101.9	1
102 – 102.9	2
≥ 103	3
2) Attitude	
Bright and alert (head up and responsive)	0
Quiet and alert (head up but slow to respond)	1
Lethargic (head down but standing slow to respond)	2
Obtunded (head down and recumbent no response)	3
3) Breathing	
Normal breathing (respiratory rate < 30 rpm)	0
Rapid breathing (respiratory rate 30-60 rpm)	1
Tachypnea (respiratory rate 61-100 rpm)	2
Difficult breathing (respiratory rate > 100 rpm or open mouth breathing)	3
4) Cough	
None	0
Induce single cough	1
Induce repeated coughs or occasional spontaneous cough	2
Repeated spontaneous cough	3
5) Nasal secretions	
Normal serous discharge	0
Bilateral mucous discharge	1
Bilateral mucopurulent discharge	2
Bilateral thick purulent discharge	3

Total score interpretation

Total score	Description
0-5	None/Mild
6-10	Moderate
> 10	Severe

6) Ocular secretions	
Normal serous discharge	0
Bilateral mucopurulent discharge without conjunctival or corneal lesion	1
Bilateral mucopurulent discharge with conjunctival erythema but no corneal lesion	2
Bilateral mucopurulent discharge with corneal lesion*	3

* Measure size of corneal lesion +/- fluorescein stain

Laboratory testing (both phases)

Virus isolation

All samples for BoHV-1 virus isolation underwent co-cultivation in 24-well plates containing monolayers of MDBK cells. The 24-well plates were incubated for 4 days at 37° C and 5% CO2. Daily observations were made to examine for the characteristic BoHV-1 cytopathic effect. Upon the detection of widespread lysis of cells or after 4 days of culture, the plates were frozen. The assessment of cytopathic effect (CPE) characteristic of BoHV-1 was confirmed by PCR. For any samples where CPE was observed but uncharacteristic of BoHV-1, these samples were classified as "suspect" and PCR analysis was also performed on those samples.

Virus neutralization titers

A standard virus neutralization microtiter assay was used to detect antibodies to BoHV-1 in serum. Sera were tested for neutralizing antibodies against BoHV-1 (Colorado strain). Following heat inactivation of serum samples at 56° C for 30 minutes, serial 2-fold dilutions were made in 50 µL of culture medium. For each dilution, 3 wells of a 96-well plate were inoculated with an equal volume (50 µL) of culture medium containing 100-500 $TCID_{50}$ of the test strain. After inoculation, the plate was incubated at 38.5° C in a humidified atmosphere of 5% CO2 and room temperature for 1 hour. After this, 2,500 MDBK cells in 50 µL of culture medium were added to each well. Plates were incubated for 96 hours and evaluated visually for cytopathic effect. The geometric mean of antibody titers was calculated from the endpoint log2 titers of the animals in each group.

Polymerase chain reaction

The real-time qPCR amplification was conducted using Bio-Rad SSO Advanced Universal SYBR Green Supermix (Catalog Number 1725271) using a Bio-Rad CFX-96 thermocycler. Specifically, the master mix consisted of 10 µl 2X SYBR Green Supermix, 0.5 µL Bio-Rad PrimePCR Assay, and 4.5 µL nuclease-free water. When the PCR was developed, it was tested by using the Colorado reference isolate. Dilutions were made to test the PCR against multiple quantities of virus. The PCR was then run against isolates obtained from previous studies.

To this, 5 µL template DNA was added for a final volume of 20 µL. The final concentration for the primers and probe were 187.5 nm and 125 nm, respectively. The primers and probe were selected from Wang et al. 2007, with modifications made to the probe to use Cy5 instead of FAM as to not compete with SYBR Green. Primers and probe included 5' – GTAAGGGTATATTATTGATTGC – 3' (forward), 5' – GACAGTGAGTATGAGGAC – 3' (reverse), and 5' Cy5-AGGACCGAGAGTTCATTGCCGC-Iowa Black RQ-Sp (probe). The PCR cycle used an initial activation at 95°C for 3 minutes, followed by 45 replication cycles which consisted of 10 seconds 95°C and 30 seconds 60°C. Negative Control and No Template Control (negative control) samples always gave results greater than 36, therefore, samples with cycle time values less than 36 were considered positive. All steers had nasal and conjunctival swabs collected 30 days after the completion of the study for BHV-1 PCR testing. Once all animals had negative results on both samples, they were sold.

Statistical analyses

Both phase I and phase II were conducted as completely randomized designs where calves were randomly assigned to 1 of 3 treatment groups. All dependent variables were analyzed using mixed-model methodology (SAS, v 9.4, Cary, NC). Serum neutralization, BoHV-1 titers and temperature were analyzed using PROC MIXED for continuous data with repeated measures. The model included fixed treatment group effect and a group by day interaction, and random effect of day. Calf was nested within day with a block diagonal residual covariance structure and covariance parameters were estimated within treatment group with calf considered as the subject. For virus isolation (0, 1) and variables recorded as discrete scores PROC GLIMMIX was used for categorical data. The model included treatment group as a fixed variable, a group by treatment day interaction and as a random variable, with calf considered as the subject within groups for covariance estimation. Significance for treatment effect for both continuous and categorical variables was considered at $P < 0.50$. All categorical variables (cough, nasal and ocular secretion scores) were analyzed as binary outcomes (0 = not observed, 1 = observed). Multiple test comparisons (PROC MULTTEST) were used to compare scores across treatments.

Results

Phase I

Clinical outcomes

All 15 calves in the study developed pyrexia as defined by rectal temperature greater than 103.5° F (39.7°C, Figure 1). The highest temperature, 107.1°F (41.7°C), was in a sentinel calf on day 7. Ocular discharges were present in all 5 calves in Group A (intraocular inoculation) as compared to 2/5 and 3/5 calves in Groups B (intranasal inoculation) and C (non-inoculated in contact sentinels), respectively. Two calves from Group A (intraocular) still had ocular discharge 21 days after the challenge. However, no corneal lesions were detected with fluorescein dye (Figure 12 A and B). Three calves from both inoculated groups (Group A and B) presented with a cough on at least 1 day, while only 1 calf from the sentinel group (Group C) had a cough. Four calves from the inoculated groups (both A and B), and 3 calves from the sentinel group (C) had a nasal discharge for at least 1 day (Figure 13). No calves required antibiotic treatment.

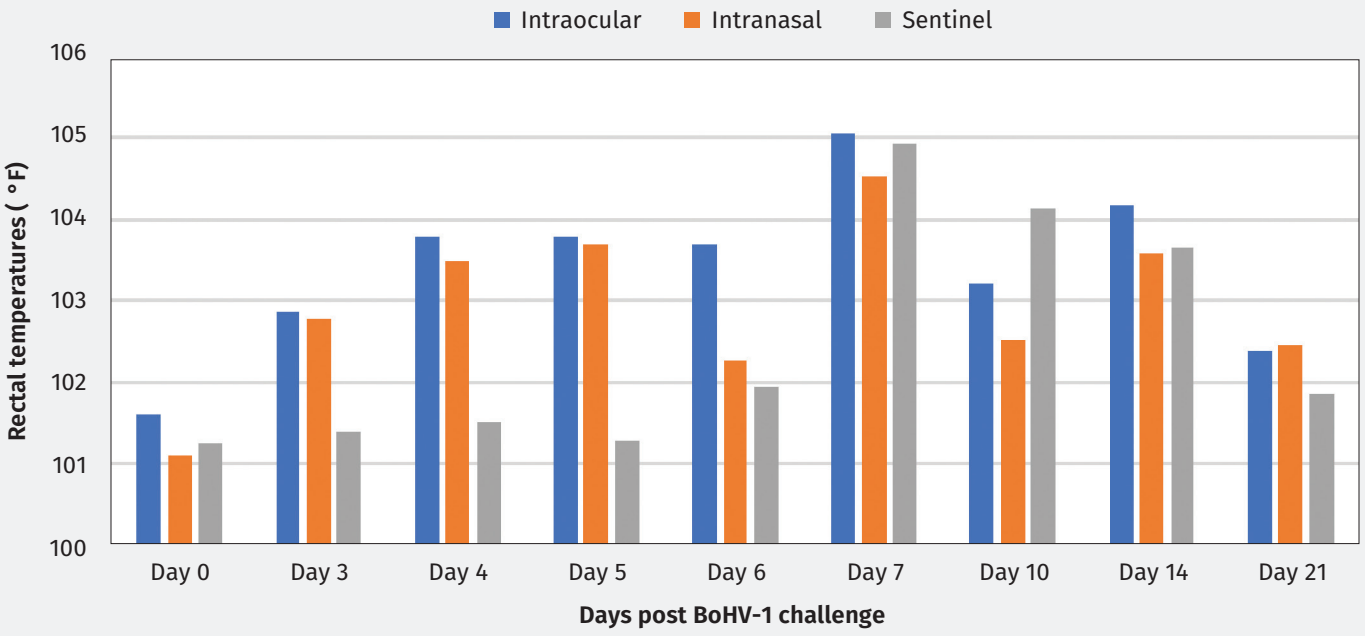
BoHV-1 virus neutralizing antibody results

All calves were BoHV-1 seronegative (< 1) on day-0 sampling and developed titers by 21 days after challenge. While no significant treatment differences were detected between groups ($P = 0.1421$), the highest average titers on days 21 and 42 were seen in the sentinel calves (Figure 2). In most calves, titers were beginning to decline by day 42 after challenge.

BoHV-1 virus isolation and PCR results

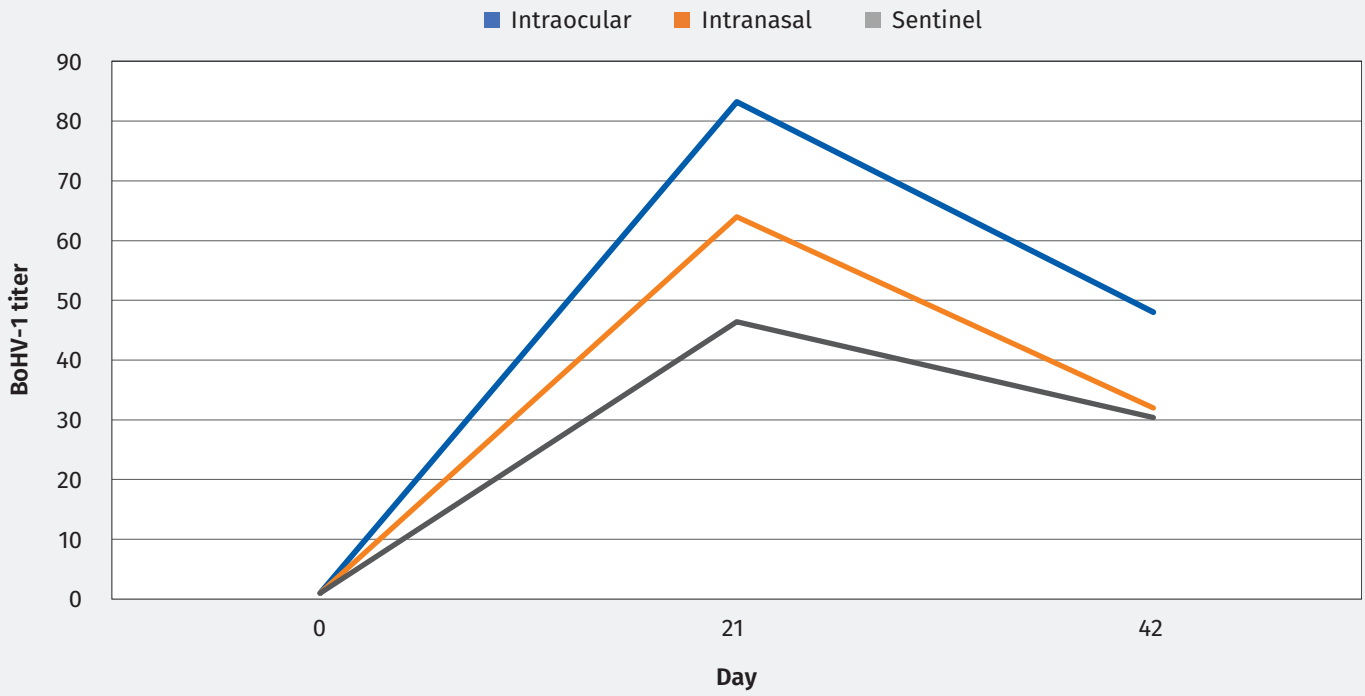
The sentinel group of calves (group C) all became infected (Figures 3-5). The calves that were inoculated by the intraocular route (group A) became BoHV-1-positive in nasal swabs, and all the calves that were inoculated by intranasal route (group B) became BoHV-1-positive on conjunctival swabs. The challenge strain was not detected in the serum of the calves after challenge. Four calves were still BoHV-1 virus isolation and PCR positive from ocular swabs at 21 days after challenge (Figures 3-5). All steers had BoHV-1 negative nasal and conjunctival swabs 30 days after the completion of the study and were sold.

Figure 1: Phase I - Mean rectal temperatures for 3 groups of steers (n = 5 each) in a study after inoculation with BoHV-1 (10^7 $TCID_{50}$/mL) to determine if it would induce conjunctivitis.



Intraocular challenge (group A), Intranasal challenge (group B), sentinels (group C)

Figure 2: Phase I – Least square geometric mean BoHV-1 serum titers for 3 groups of steers (n = 5 each) in a study after inoculation with BoHV-1 (10^7 $TCID_{50}$/mL) to determine if it would induce conjunctivitis.



Group A intraocular challenge, Group B intranasal challenge, Group C sentinel calves

Figure 3: Phase I - Group A (ocular challenge, n = 5): Proportion of animals with positive nasal and conjunctival virus isolation results to BoHV-1 after inoculation with BoHV-1 (10^7 $TCID_{50}$/mL).

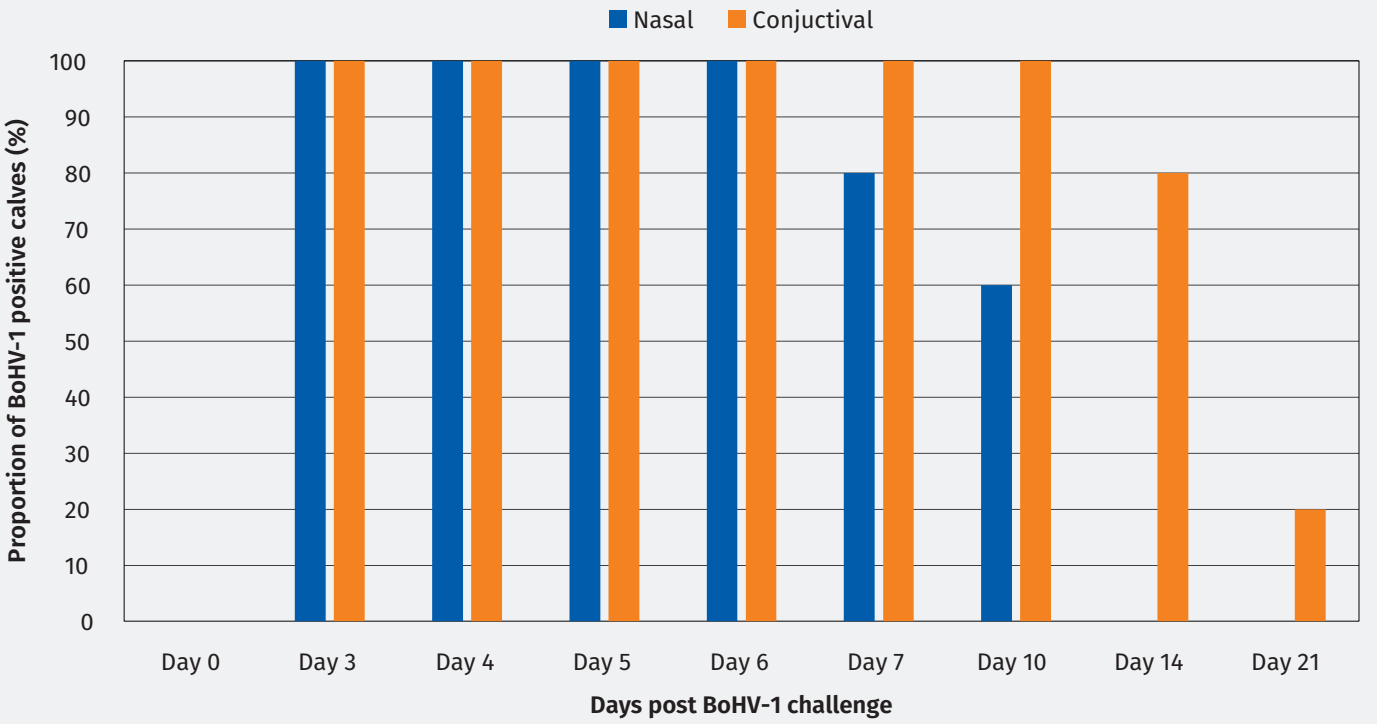


Figure 4: Phase I - Group B (nasal challenge, n = 5): Proportion of animals with positive nasal and conjunctival virus isolation results to BoHV-1 after inoculation with BoHV-1 (10^7 $TCID_{50}$/mL).

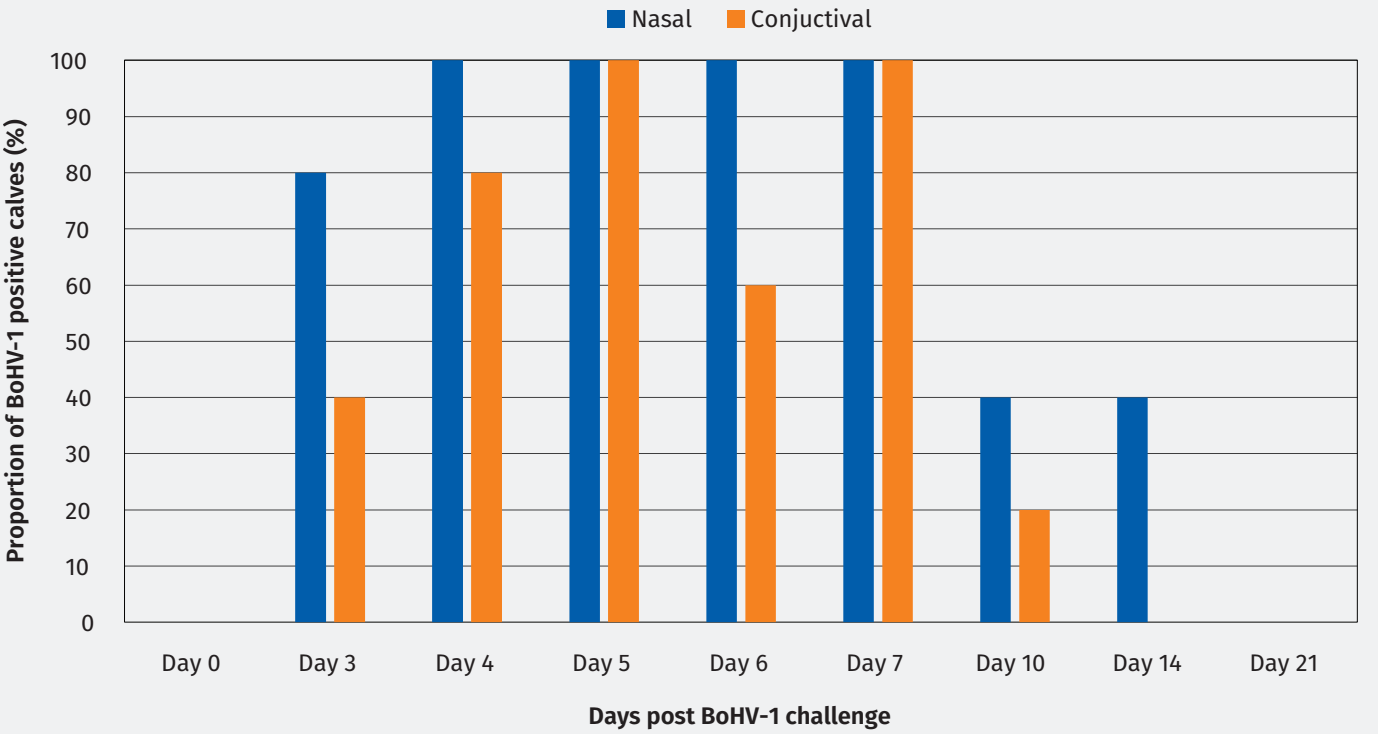
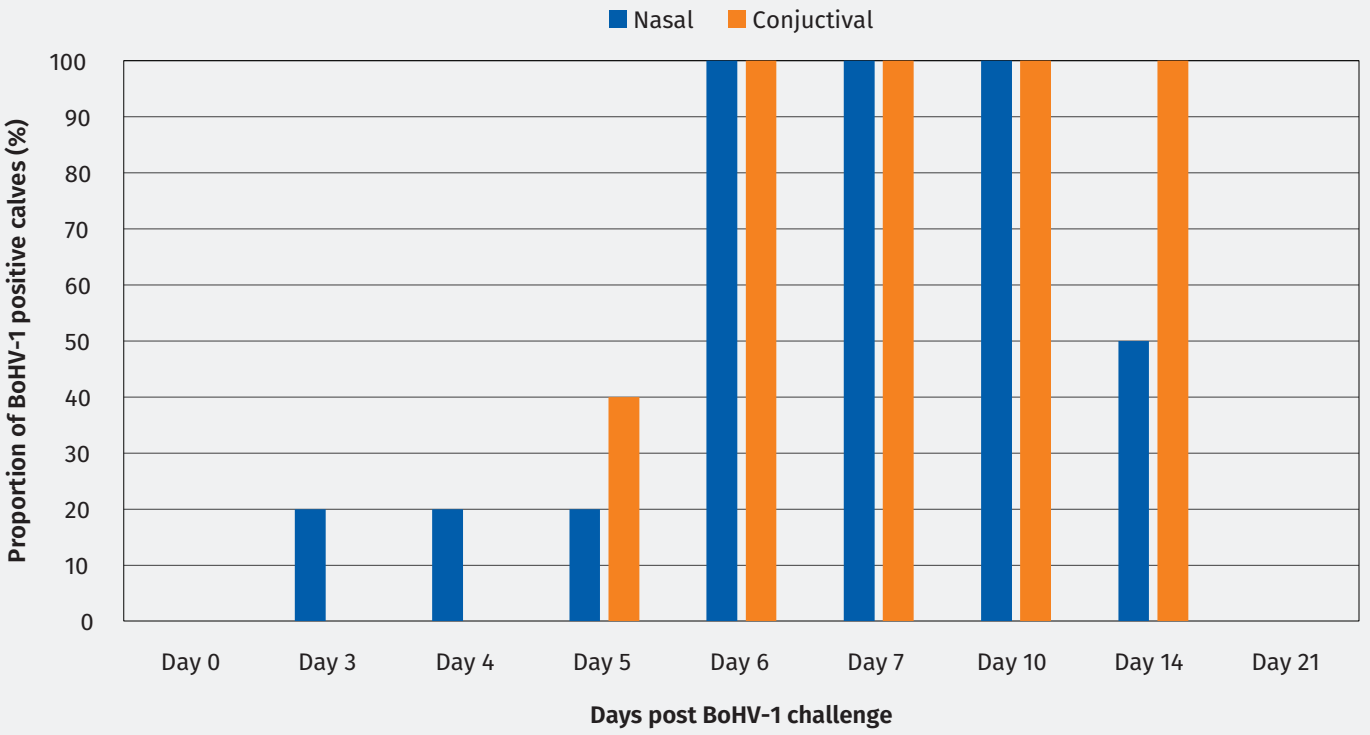


Figure 5: Phase I - Group C (nonchallenged sentinels in contact with challenged animals, n = 5): Number of animals with positive nasal and conjunctival virus isolation results to BoHV-1 after inoculation with BoHV-1 (10^7 $TCID_{50}$/mL).



Single nucleotide polymorphisms (SNP) genotyping

The BoHV-1 challenge inoculum and the virus isolated from a conjunctival swab sample from calf 9670 (day-7 post challenge) were sent to the diagnostic laboratory at South Dakota State University for sequencing and SNP genotyping utilizing published primers designed to detect BoHV-1 strains present in commercial vaccines and differentiate those sequences from wild type BoHV-1 strains.[5] Results from SNP genotyping for both the inoculum and samples recovered after challenge were determined to be group 4 BoHV-1 isolates, which are different from any BoHV-1 strains used in currently licensed vaccines in the United States.

Phase II

Clinical outcomes

All the CON calves 5/5 (100%) had bilateral ocular discharge and conjunctivitis for at least 1 day after challenge (ocular score 2). In contrast, only 2/10 (20%) group IN calves and none (0%) in the group SQ calves had ocular scores of 2. However, 7/10 (70%) group IN calves and 3/10 (30%) group SQ calves had an ocular secretion score of 1 for at least 1 day. Proportions of animals with observed ocular secretions are plotted in Figure 6 by treatment and day. A similar pattern was seen for nasal secretions (Figure 7). Both vaccinated groups had lower proportion of animals with nasal secretions compared to CON calves ($P = 0.0227$ and $P < 0.0001$, for IN group and SQ group, respectively). Temperature measurements from day 21 to day 42 (3 weeks post challenge) were lower in the SQ group compared to the IN group ($P = 0.0454$), and compared to the CON group ($P = 0.0236$), but not significantly different between the IN group and the CON group (Table 2). Temperature changes post-challenge are plotted in Figure 8. No significant differences were detected between treatments for cough scores across the groups and no calves required antibiotic intervention.

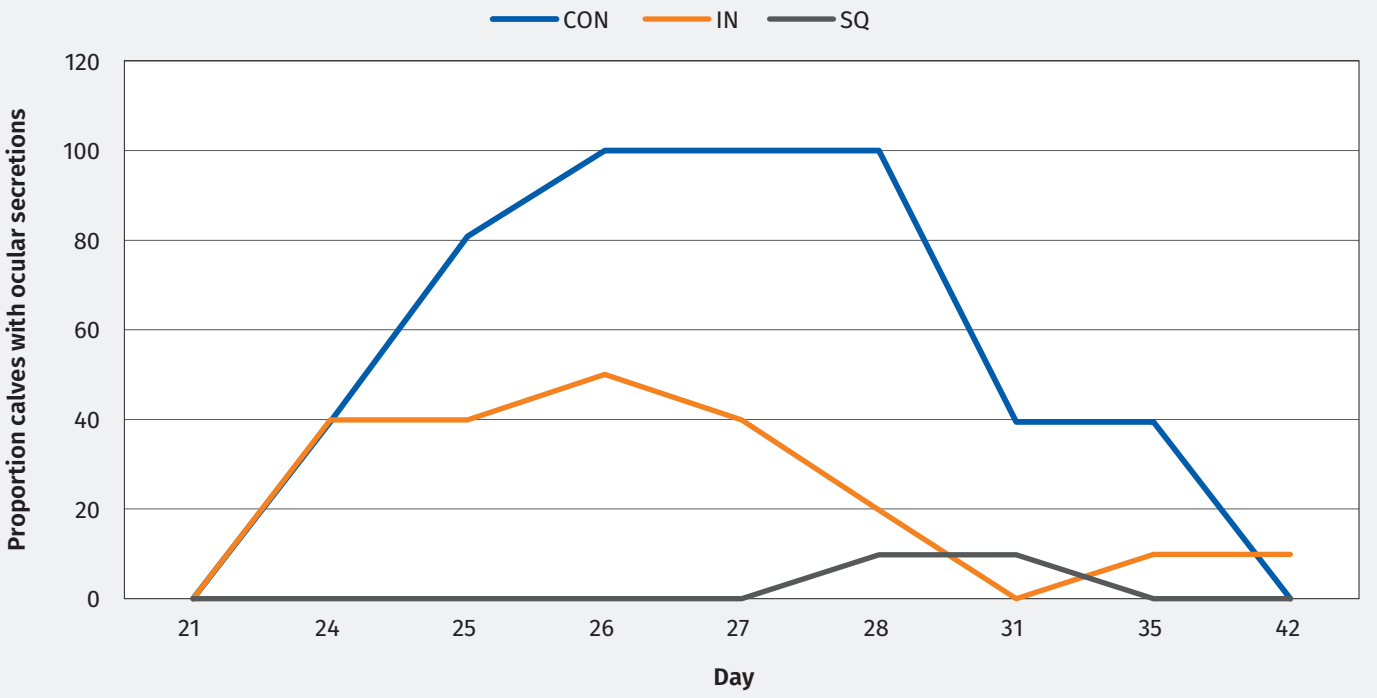
BoHV-1 virus neutralizing antibody results

Back-transformed geometric least square means of serum neutralization titers (BoHV-1 SN) were different between treatment groups (Group IN = 1.56, Group SQ = 2.73, Group CON = 1.67, $P = 0.0001$). Group SQ was higher than both Group IN ($P < 0.0001$) and CON ($P < 0.0003$) and there was no difference between IN and CON groups ($P = 0.7173$). On day 21, Group SQ BoHV-1 SN titers were significantly higher than Group IN ($P < 0.0001$) and CON ($P < 0.0001$) and on day 42 Group IN was significantly lower than Group SQ ($P < 0.0700$, Figure 9).

Virus isolation and PCR results

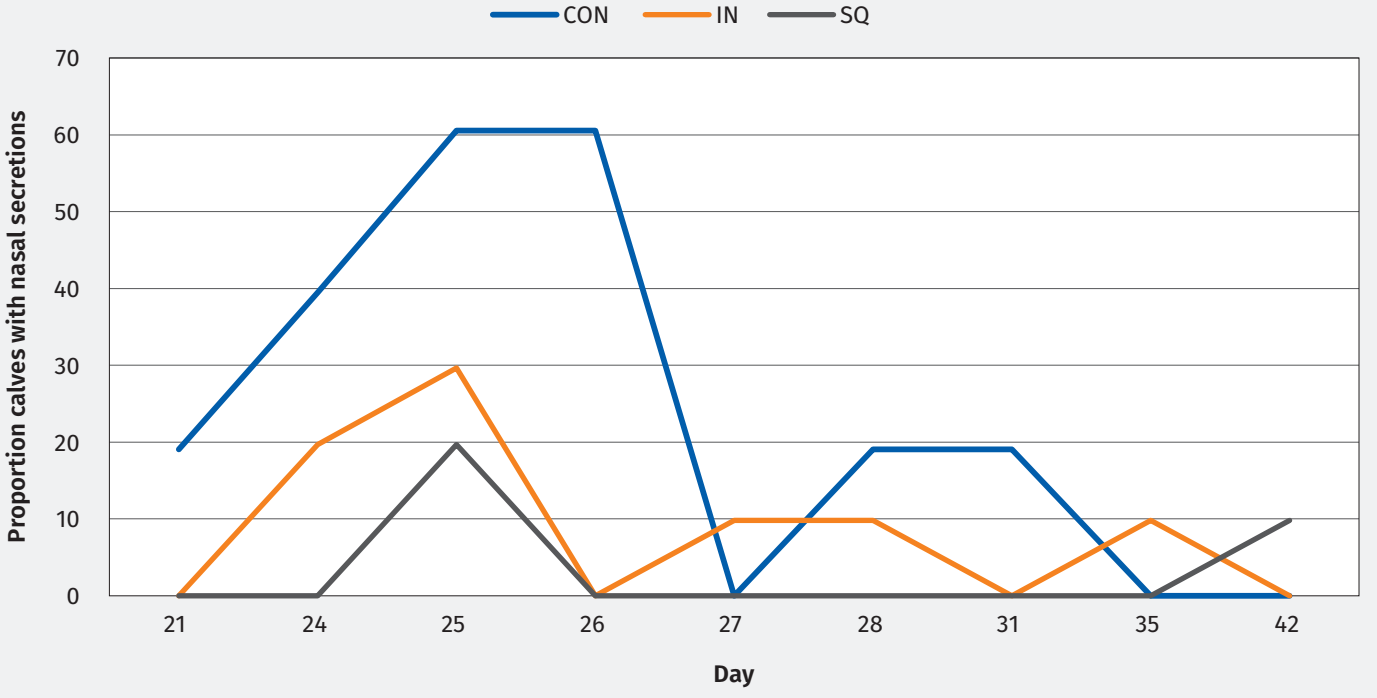
Ocular swabs from all calves were BoHV-1 virus isolation positive on day 3 (Figure 10) after challenge and 21/25 nasal swabs were also BoHV-1 positive (Figure 11). Serum samples were negative for virus on all sample days. Virus isolation rates from nasal and ocular swabs as well as clinical scores for nasal secretions and ocular lesions and secretions were consistently lower for both vaccinated groups compared to unvaccinated controls (shown in Table 2). Additionally, BoHV-1 virus isolation rates were lower for Treatment Group SQ than for Treatment Group IN for both ocular and nasal swabs ($P < 0.0001$ and $P = 0.0290$ respectively, Table 2).

Figure 6: Phase II - Proportion of animals with ocular secretions (scores 1 to 3) in a study with 3 groups of steers (group IN [n = 10], group SQ [n = 10], and group CON [n = 5]) after inoculation with BoHV-1 (10^7 $TCID_{50}$/mL) to determine if vaccination could prevent conjunctivitis.



CON – Unvaccinated control, IN – Intranasal vaccinate, SQ – Subcutaneous vaccinate

Figure 7: Phase II - Proportion of animals with nasal secretions (from scoring method) in a study with 3 groups of steers (group IN [n = 10], group SQ [n = 10], and group CON [n = 5]) after inoculation with BoHV-1 (10^7 $TCID_{50}$/mL) to determine if vaccination could prevent conjunctivitis.



CON – Unvaccinated control, IN – Intranasal vaccinate, SQ – Subcutaneous vaccinate

Table 2: Phase II – Proportion of animals with positive virus isolation (VI) and least squares means for temperature (± standard errors) in a study with 3 groups of steers (group IN for intranasal vaccination [n = 10], group SQ for subcutaneous vaccination [n = 10], and group CON for unvaccinated controls [n = 5]) after inoculation with BoHV-1 (10^7 $TCID_{50}$/mL) to determine if vaccination could prevent conjunctivitis.

Variable day 21 – 42 (post challenge)	Vaccine group IN[a]	Vaccine group SQ[b]	Group CON[c]	Overall treatment *P*-value	Day *P*- value	Treatment x day *P*-value
VI from nasal swab	0.463 ± 0.0190	0.375 ± 0.0350	0.600 ± 0.0400	$P = 0.0002$[1]	$P < 0.0001$	$P = 0.0006$
VI from ocular swab	0.550 ± 0.0280	0.300 ± 0.0380	0.700 ± 0.5900	$P < 0.0001$[2]	$P < 0.0001$	$P = 0.0003$
Temperature (degrees F)	103.0 ± 0.1000	102.7 ± 0.1000	103.0 ± 0.1000	$P = 0.0424$[3]	$P < 0.0001$	$P = 0.0011$

[a] Modified live BoHV-1 intranasal Inforce 3®, Zoetis, Parsippany, NJ 07054

[b] Modified live BoHV-1 subcutaneous Bovi-Shield Gold OneShot®, Zoetis, Parsippany, NJ 07054

[c] Unvaccinated BoHV-1 challenged calves

[1] Treatment pair-wise comparisons: IN – SQ, $P = 0.0290$; IN – CON, $P = 0.0020$; SQ – CON, $P < 0.0001$

[2] Treatment pair-wise comparisons: IN – SQ, $P < 0.0001$; IN – CON, $P = 0.0224$; SQ – CON, $P < 0.0001$

[3] Treatment pair-wise comparisons: IN – SQ, $P = 0.0454$; IN – CON, $P = 0.6079$; SQ – CON, $P = 0.0236$

Discussion

Multiple infectious agents are implicated in infectious keratoconjunctivitis (IBK) of cattle including BoHV-1. During the past several years, isolation of BoHV-1 from cases of conjunctivitis and IBK appears to be on the rise.[d] Ocular cases involving BoHV-1 have been reported in the United States (Texas, Kansas, Nebraska, Idaho, Wisconsin, New York, California), South Africa and Canada, often in calves previously vaccinated against BoHV-1. While most cases have been seen in the winter, cases have been seen (with lower numbers) throughout the year. SNP genotype sequencing (when performed) have shown isolates in the group 2 and 4 strains of BoHV-1. The apparent increase in BoHV-1 detection in these field cases may be a consequence of improved surveillance and improved diagnostic tests. Another possible explanation is that these current BoHV-1 field isolates, while causing conjunctivitis, may increase infectivity and/or severity of other endemic ocular pathogens commonly associated with IBK. This study attempted to address the efficacy of 2 different vaccine approaches in reducing ocular lesions and reducing viral presence following a BoHV-1 ocular challenge.

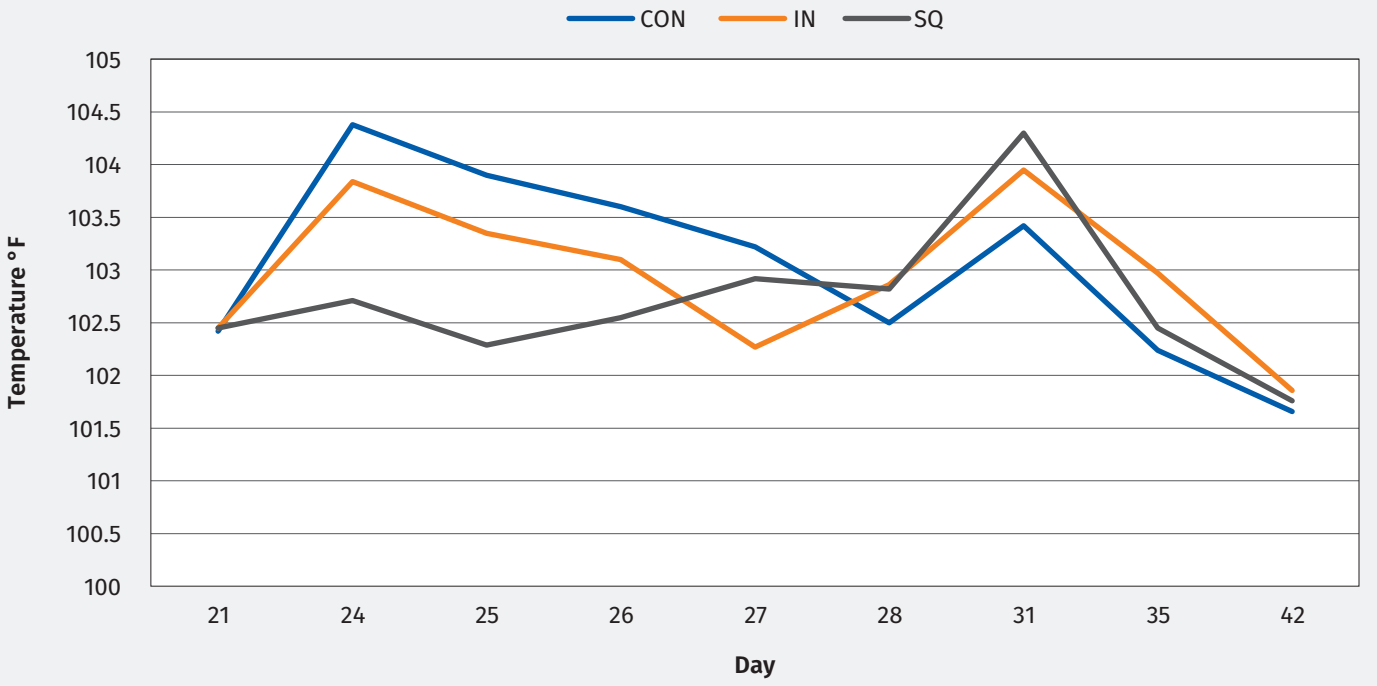
The isolates used in phase I were obtained from clinical cases of IBK in dairy calves in Texas and 6-8 month-old heifers in Kansas. The pooled isolates were able to spread easily to sentinel animals in close contact and cause conjunctivitis, mild upper respiratory disease, and elevated temperatures. Conjunctivitis, without corneal lesions, as seen in the phase I calves, has been previously reported with BoHV-1 ocular disease.[1,2,6,11,12] These field strains showed the ability to cause both respiratory and ocular lesions and spread rapidly, which was consistent with the clinical signs seen in many of the reported cases. The prolonged viral presence demonstrated in phase I could complicate disease dynamics in comingled calves as calves infected 3-4 weeks previously could still be infectious to pen mates. Prolonged infectivity could lead to ocular disease persistence in facilities with frequent or continuous additions of susceptible cattle. The SNP genotype analysis indicating that the isolates were from the BoHV-1 group 4, which is not present in any of the commercially licensed vaccines in the United States.[5] The ability of these field isolates to predictably create ocular disease and respiratory disease was confirmed in phase I, which justified use of this challenge model to assess vaccine protection in phase II.

Both an intranasal and systemic modified live BoHV-1 vaccine were used in phase II. While local immune responses and ocular immunoglobulin type A (IgA) are seen after an infection, it is not clear whether local and/or systemic immune responses are needed to protect against ocular infections.[1,8,9,12,16] By using the 2 different routes of vaccine administration, the ability of both local and systemic immune responses to protect against BoHV-1 conjunctivitis was assessed. Overall, both vaccines demonstrated effectiveness compared to non-vaccinates against this heterologous field strain of BoHV-1 as measured by levels of virus isolation from nasal and ocular swabs and reduced clinical manifestations of ocular and nasal lesion scores after ocular challenge. However, the fact that some vaccinated calves displayed conjunctivitis is consistent with reports that cases of conjunctivitis have been seen in BoHV-1-vaccinated calves. The systemically administered MLV BoHV-1 vaccine demonstrated reduced ocular and nasal BoHV-1 virus isolation as well as improved ocular lesion scores and improved temperature response compared to intranasal vaccinates. This is consistent with 1 study that showed general (systemic) immunity was more important than local immunity for protection against *Moraxella bovis*.[9] It is not clear whether a homologous strain of BoHV-1 in a vaccine would give higher levels of protection or if the difficulty in achieving high levels of ocular protection previously reported for *Moraxella bovis* is also true for BoHV-1 vaccines.

Conclusion

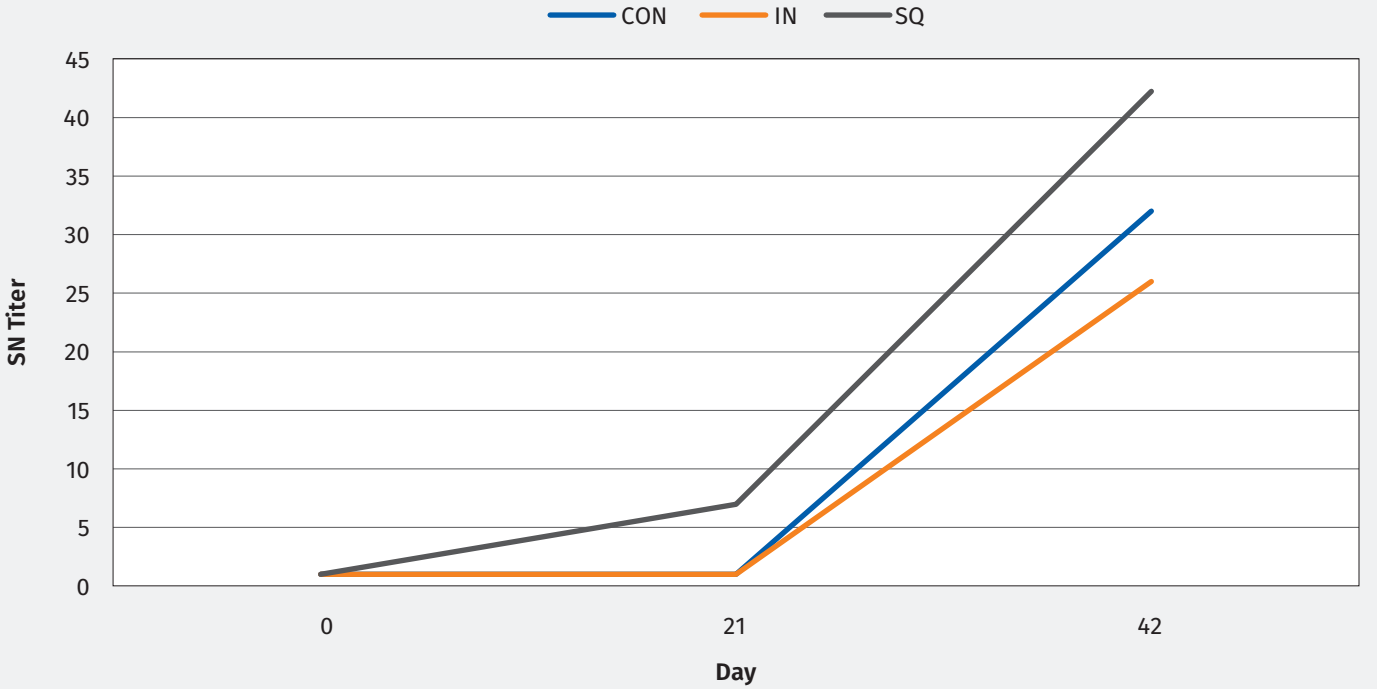
These studies established the ability of BoHV-1 isolates from field cases of IBK to cause conjunctivitis in an ocular challenge. Vaccination decreased the severity of lesions and virus amounts. However, vaccination did not provide complete protection as some vaccinated calves had ocular discharge and all calves were BoHV-1 virus positive post-challenge. These studies show that, while vaccination can decrease severity of post-challenge conjunctivitis, separation of infected calves and good handling techniques to diminish spread of the virus will also be necessary to decrease transmission of the virus to susceptible populations.

Figure 8: Phase II - Least squares means of rectal temperature in a study with 3 groups of steers (group IN [n = 10], group SQ [n = 10], and group CON [n = 5]) after inoculation with BoHV-1 (10^7 $TCID_{50}$/mL) to determine if vaccination could prevent conjunctivitis.



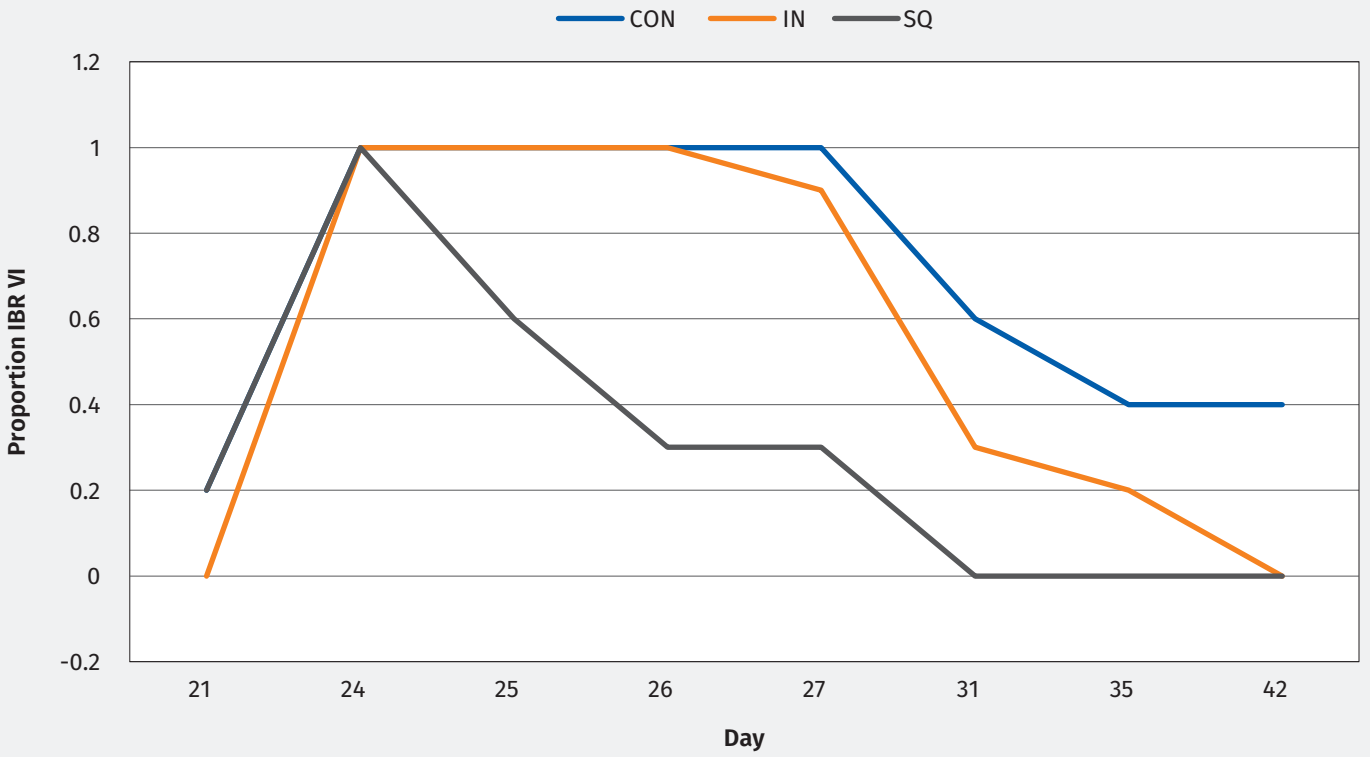
CON – Unvaccinated control, IN - Intranasal vaccinate, SQ – Subcutaneous vaccinate

Figure 9: Phase II - Back transformed geometric least squares means of BoHV-1 serum neutralization titers in a study with 3 groups of steers (group IN [n = 10], group SQ [n = 10], and group CON [n = 5]) after inoculation with BoHV-1 (10^7 $TCID_{50}$/mL) to determine if vaccination could prevent conjunctivitis.



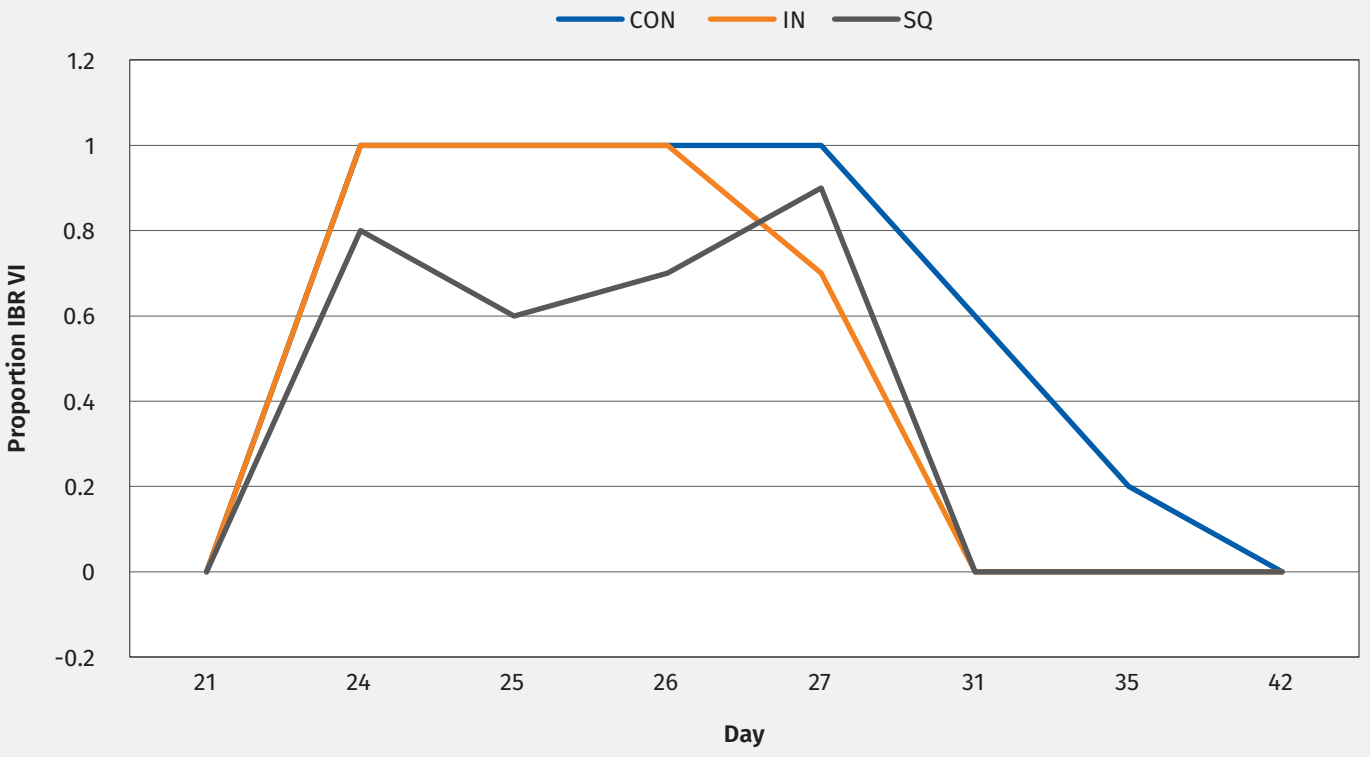
CON – Unvaccinated control, IN - Intranasal vaccinate, SQ – Subcutaneous vaccinate

Figure 10: Phase II - Least squares means of BoHV-1 virus isolation from conjunctival swabs in a study with 3 groups of steers (group IN [n = 10], group SQ [n = 10], and group CON [n = 5]) after inoculation with BoHV-1 (10^7 $TCID_{50}$/mL) to determine if vaccination could prevent conjunctivitis.



CON – Unvaccinated control, IN - Intranasal vaccinate, SQ – Subcutaneous vaccinate

Figure 11: Phase II - Least squares means of BoHV-1 virus isolation from nasal swabs in a study with 3 groups of steers (group IN [n = 10], group SQ [n = 10], and group CON [n = 5]) after inoculation with BoHV-1 (10^7 $TCID_{50}$/mL) to determine if vaccination could prevent conjunctivitis.



CON – Unvaccinated control, IN - Intranasal vaccinate, SQ – Subcutaneous vaccinate.

Figure 12A and B: Phase I - Conjunctivitis and ocular discharge induced after intraocular challenge with BoHV-1 (10^7 $TCID_{50}$/mL) in calves. Note the lack of corneal lesions (examples of a ocular secretion score = 2).

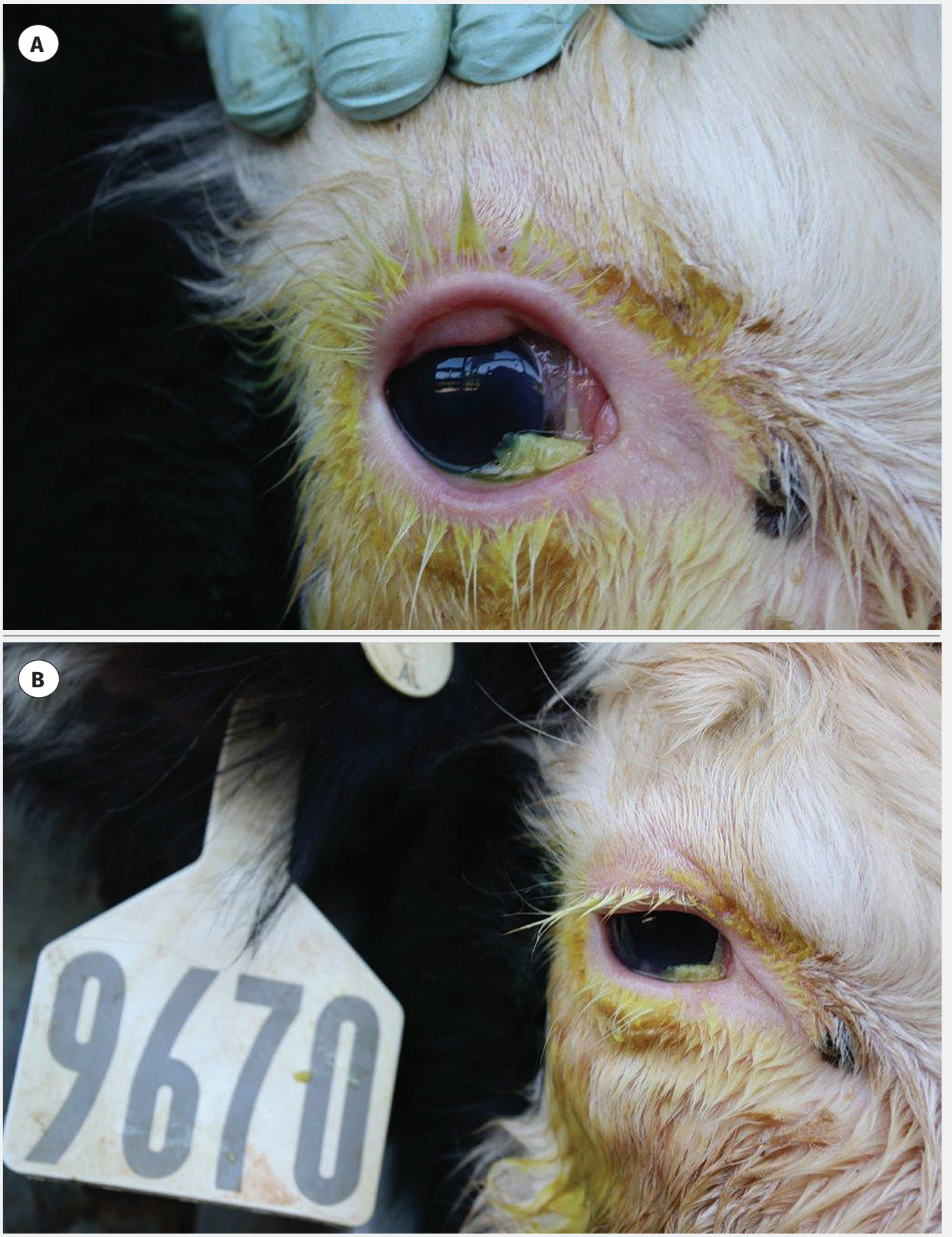
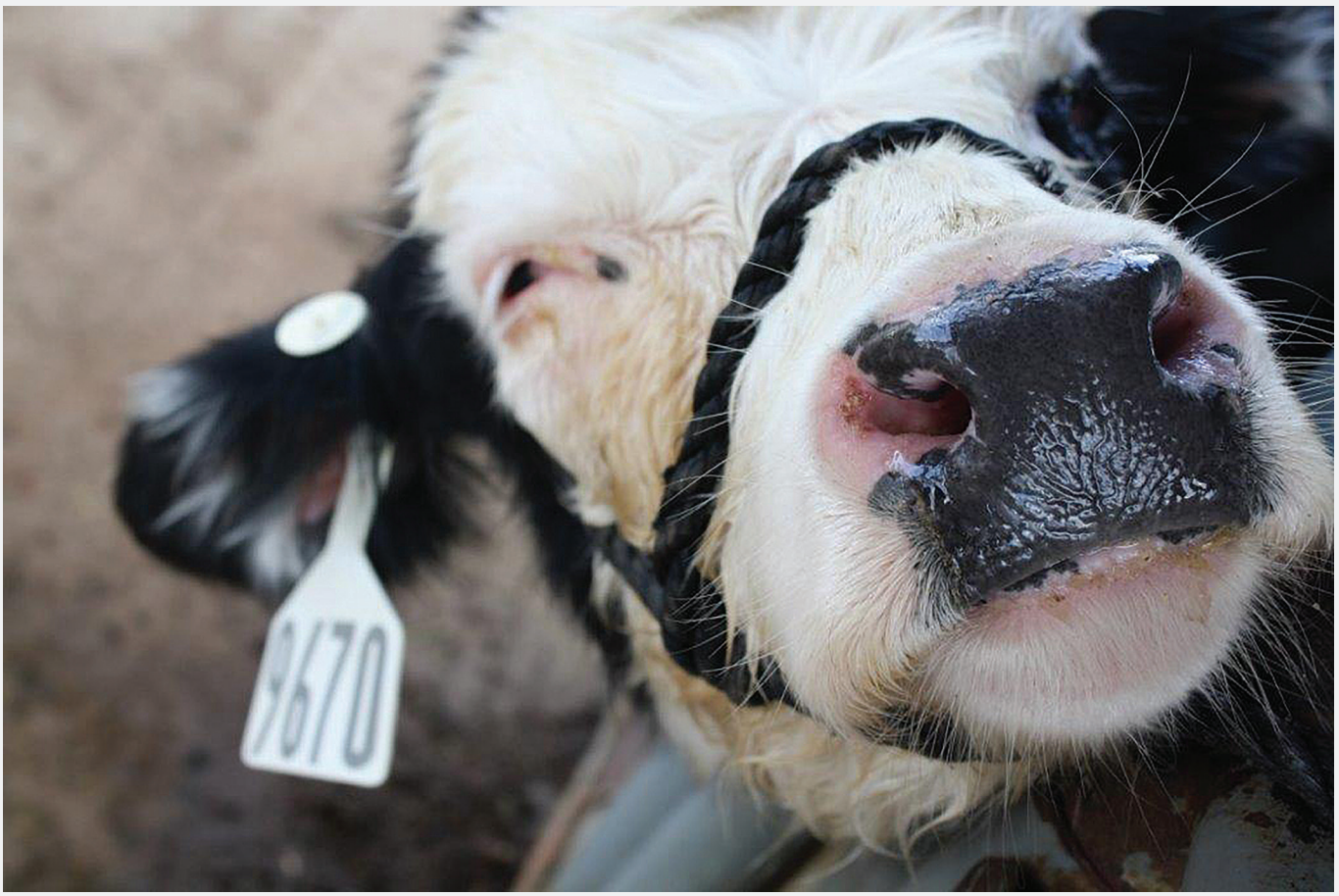


Figure 13: Phase I – Evidence of nasal lesions in a calf day-10 after intraocular challenge with BoHV-1 (10^7 $TCID_{50}$/mL).



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Three of the authors were Zoetis employees during the trial.

Endnotes

[a] Draxxin®, Zoetis, Parsippany, NJ 07054

[b] Inforce 3®, Zoetis, Parsippany, NJ 07054

[c] Bovi-Shield Gold OneShot®, Zoetis, Parsippany, NJ 07054

[d] Personal communication Zoetis Veterinary Medicine Investigation and Support Group, Exton, PA 19341

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