

SYSTEMATIC REVIEW OF CATTLE RESPONSES TO VIRAL AND BACTERIAL
BOVINE RESPIRATORY DISEASE PATHOGENS AND EFFECT OF HIGH AMBIENT
TEMPERAURE ON VIRAL REPLICATION AND SEROLOGY TO AN INTRANASAL
MODIFIED-LIVE (BOVINE RHINOTRACHEITIS-PARAINFLUENZA-3) VIRAL VACCINE
IN BEEF CATTLE

by

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Abstract

Objective- To compare serologic response and viral replication following intranasal administration of a modified-live bovine rhinotracheitis (IBR) parainfluenza-3 (PI-3) vaccine in high (32°C) and moderate (21°C) ambient temperatures.

Animals- 28 heifers (mean body weight, 206.8 kg)

Procedures- Heifers randomly allocated to treatment groups: High Ambient Temperature (HAT, n=10): received vaccine, housed outdoors, Moderate Ambient Temperature (MAT, n=10): received vaccine, housed indoors, High Ambient Control (HAC, n=4): no vaccine, housed outdoors, Moderate Ambient Control (MAC, n=4): no vaccine, housed indoors. Rectal and nasal mucosal temperatures were recorded every 2 hours from 8am to 8pm on trial days 0 and 1. Nasal swabs were collected on trial days 0 through 7 for virus isolation. Serum samples were collected for serology on trial days 0, 7, 14, and 28.

Results- Rectal temperatures did not differ among treatment groups over the study period, but nasal temperatures were higher in the HAT calves compared to MAT group at study hours: 6, 24, 30, 32, and 38. Two weeks post-vaccination, IBR titers were significantly greater in vaccinates (HAT, MAT) relative to non-vaccinates (HAC, LAC), but no differences were identified among HAT and MAT. Viable IBR virus was recovered via virus isolation from all vaccinated calves (HAT, MAT) on trial days 1 through 6.

Conclusions and Clinical Relevance- The ability to isolate IBR and stimulate the calf immune response following administration of a modified-live IBR-PI3 intranasal vaccine did not differ in calves housed in temperature-controlled and high ambient temperature environments.

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Chapter 1 - Thesis Introduction

Bovine respiratory disease complex (BRD) remains one of the most significant economic diseases in the cattle industry. Bovine respiratory disease is a multi-factorial disease involving the host, environment, and pathogens. Due to the complexity and importance of BRD, significant emphasis is placed on research and prevention.

Bovine respiratory disease remains an intensively researched disease focused on disease course, biomarkers of disease, prevention, and treatment. Despite this vast knowledge, no single source exists summarizing the expected disease course and duration. Chapter 2 is a systemic review of viral and bacterial pathogens. This review evaluated BRD challenge models with specific criteria. Additionally, chapter 2 offers insight into duration of clinical disease, duration of pathogen shedding, and time until seroconversion. Knowledge of clinical sign duration for each pathogen involved in the BRD complex would assist with disease model studies, as well as, potentially directing antibiotic treatment, and expectations of the duration of production loss during illness. Pathogen shedding is important knowledge for the amount of time an individual may be infective to the herd after exposure to the disease and may assist with quarantine procedures.

Prevention of BRD is a core component of management. Numerous vaccines are available on the market for the major BRD pathogens including: modified-live vaccines, killed vaccines, and temperature-sensitive vaccines. Proper administration of these vaccines is crucial to ensuring appropriate efficacy. It is common knowledge that vaccines must be properly stored and not exposed to extreme temperatures. Otherwise, inactivation of the vaccine or decreased

vaccine efficacy occurs. Chapter 3 is a study that evaluated the effects of high ambient temperatures on an intranasal modified-live vaccine. Production practices sometimes result in vaccine administration during excessive ambient temperatures, particularly the summer, which is not optimal for maximal vaccine efficacy. This thesis demonstrates whether vaccine administration during times of excessive outdoor temperatures may be detrimental for one intranasal modified-live vaccine product success.

Chapter 2 - Systematic Review of Cattle Response to Viral and Bacterial Pathogens for Bovine Respiratory Disease Complex

Bovine respiratory disease (BRD) continues to be an economically significant disease of cattle with losses estimated as \$23.60 per treated calf.(USDA 2011) Bovine respiratory disease is a multi-factorial disease involving infectious agents, compromised host immune system, and environmental factors ultimately resulting in bronchopneumonia. The viral pathogens associated with BRD include: infectious bovine rhinotracheitis (IBR), parainfluenza-3 (PI3), bovine viral diarrhea (BVD), and bovine respiratory syncytial virus (BRSV). Bacterial pathogens associated with BRD include: *Mannheimia haemolytica*, *Mycoplasma bovis*, *Pasteurella multocida*, and *Histophilus somni*.

Viral pathogens are capable of causing primary infection that is generally associated with mild clinical signs (CS) of BRD.(Martin, Bateman et al. 1989; Gilliam, Thackray et al. 1993; Van der Poel, Schrijver et al. 1996; Radostits 2001; Salt, Thevasagayam et al. 2007; Smith 2009) An important role for BRD viral pathogens is causing immune suppression which increases susceptibility to secondary bacterial infections.(Smith 2009) Bovine viral diarrhea can be transmitted horizontally through aerosolization or ingestion. Persistently infected (PI) individuals are the major source of transmission and sustainability of virus.(Smith 2009) Infectious bovine rhinotracheitis is spread via aerosolization and can persist in a latent state in neural tissues and later shed in times of stress.(Gilliam, Thackray et al. 1993; Meyer, Lemaire et al. 2001; Radostits 2001; Smith 2009) Parainfluenza-3 and BRSV are considered to be minor contributors to BRD and are spread via aerosolization.

Similar to the viral pathogens, BRD bacterial pathogens are often present as co-infections. *Mannheimia haemolytica* is considered the most common bacterial pathogen in beef cattle BRD and is a normal inhabitant of the nasopharynx; becoming opportunistic during stress or viral infection.(Purdy, Raleigh et al. 1997; Radostits 2001; Smith 2009) *Mycoplasma bovis* can be a primary pathogen or co-infection, with some studies showing synergism with *M. haemolytica*.(Houghton and Gourlay 1983; Nicholas and Ayling 2003; Smith 2009; Dudek, Bednarek et al. 2013) Like *M. haemolytica*, *Pastuerella multocida* and *Histophilus somni* are also normal flora of the respiratory tract and become opportunistic colonizers of the lung following viral infection of the respiratory tract.(Smith 2009)

As BRD is a syndrome, the specific pathogens involved in individual cases or outbreaks are often unknown. Management and control of BRD outbreaks is influenced by disease risk factors as well as transmission dynamics of the pathogens involved. Understanding the cattle response and infectious period associated with each pathogen can lead to a better understanding of how to mitigate negative impacts of BRD in populations. While there are numerous challenge studies using the common BRD pathogens, a resource summarizing the time from exposure to a viral or bacterial BRD pathogen to exhibition of CS, pathogen shedding, and seroconversion does not exist.

The objective for this study was to perform a systematic review of the published literature and a descriptive analysis of cattle responses (the minimum time to onset of CS, time to peak CS, time to resolution of CS, minimum time to shedding, time to maximum shedding, time to resolution of shedding, time to seroconversion, and time to maximum seroconversion) to challenge with common viral and bacterial BRD pathogens. Our secondary objective was to

provide a resource to veterinarians and researchers to assist in understanding the pathogenicity of BRD pathogens and a resource for induced pathogen model design for future studies.

Material and Methods

A literature search was performed on 3/7/2014 using PubMed, CAB, and Agricola databases to identify studies published in English that reported cattle BRD experimental challenge models for BHV-1, BVDV, PI-3, BRSV, *Histophilus*, *Pasteurella*, *Mycoplasma*, and *Mannheimia*. The search strategies and keywords are listed in Table 2.1. Only challenge models were included as time to onset of CS, shedding, and resolution times were important outcomes and challenge models provide data with specific known time of pathogen exposure. The titles from the combined search outcomes were evaluated for inclusion and exclusion criteria. Then, the abstracts of relevant titles that met the inclusion criteria were evaluated. Of the pertinent abstracts, the material and methods were read and studies were included in or excluded from the systematic review based on study criteria. Table 2.2 shows the number of papers included for each pathogen during each stage of evaluation. A hand search was performed of included studies to ensure no additional valid studies were omitted from the search results. A published manuscript was considered a study while each individual challenge pathogen was considered a trial.

Inclusion criteria for each study comprised using cattle confirmed to be pathogen-free prior to challenge, using only single pathogen exposure, having a negative control group and that challenged animals received no other treatment or vaccination for BVD, BHV-1, PI-3, BRSV, *Mannheimia haemolytica*, *Mycoplasma bovis*, *Histophilus somni*, and *Pasteurella multocida*. Outcomes of interest included: minimum time to onset of CS, time to peak CS, time to resolution

of CS, minimum time to rectal temperature exceeding 40°C, time to peak rectal temperature, time to resolution of rectal temperature less than 40°C, minimum time to shedding, time to maximum shedding, time to resolution of shedding, time to seroconversion, and time to maximum antibody titers. Other data collected included: study length, challenge inoculum route, number of calves in the trial, frequency of sample collection, and blinding status. Bovine viral diarrhea type 1 and 2 were analyzed together and not separated into two separate categories. Trial day 0 was defined as the day the pathogen challenge was administered for all included studies. Trials were included for analysis regardless of completion of all outcomes of interest. If CS, high rectal temperature, or shedding had not resolved by conclusion of the trial, then the trial was included for analysis with resolution fields being omitted from analysis. Seroconversion data only included trials utilizing serum neutralization to test for antibody response. For the viral pathogens, shedding was determined by trials utilizing virus isolation from nasal swabs. For the bacterial pathogens, trials that utilized PCR for determination of shedding from nasal swabs were included in the systematic review.

A systematic review and analysis were performed using descriptive statistics for the trials within each pathogen evaluated. Box and whisker plots were produced summarizing the data for each pathogen.

Results

After evaluation of article titles, abstracts, and then complete review of subsequent manuscripts, a total of 35 studies and 64 trials were included in the descriptive analysis. No additional study was included after a hand search of references cited in included articles. All included studies were in the PubMed and CAB databases.

Bovine Viral Diarrhea

We identified 12 BVD trials from 8 studies for inclusion in the analysis.(Broderson 1998; Ganheim, Hulten et al. 2003; Peters, Thevasagayam et al. 2004; Galav, Mishra et al. 2007; Kelling, Hunsaker et al. 2007; Salt, Thevasagayam et al. 2007; Xue, Ellis et al. 2010; Xue, Mattick et al. 2011) Blinding was reported for 9 of the trials. The mean trial length was 15.5 days with a mean of 10.2 calves included in each trial. Type 1 and type 2 BVD were included in the same category for analysis. Bovine viral diarrhea type 1 was used as the challenge pathogen for 6 trials. Two trials utilized BVD type 2 and 4 trials did not specify the BVD type. Eight trials challenged the calves intranasal with the BVD challenge pathogen. Three trials challenged via aerosolized administration and 1 trial used a combination of intranasal and aerosolized for challenge exposure. No single trial contained all the desired areas of interest for systematic review. Table 2.3 summarizes the number of trials (n) present for each analyzed area of interest.

The median for the minimum number of days until BVD shedding was 2 days (Figure 2.1). The median peak of BVD shedding occurred at 7 days post challenge with resolution at 12 days post challenge. Median rectal temperatures began exceeding 40°C four days post-challenge. Rectal temperature peaks occurred approximately 7 days post challenge with resolution occurring approximately 10 days after challenge. Median onset of CS occurred at 2 days post-challenge. Median peak CS occurred 8 days post-challenge with median days to resolution being 15 days. Median time to seroconversion occurred 17 days post-challenge with peak seroconversion occurring at 27 days post-BVD challenge in one study.

Infectious Bovine Rhinotracheitis

Infectious bovine rhinotracheitis had 9 trials and 7 studies included for systematic review.(Gilliam, Thackray et al. 1993; Castrucci, Ferrari et al. 1995; Castrucci, Ferrari et al.

1996; Castrucci, Frigeri et al. 1998; Meyer, Lemaire et al. 2001; Peters, Thevasagayam et al. 2004; Xue, Ellis et al. 2010) Of the 9 trials, 3 reported using blinding with the other 6 trials either not being blinded or blinding status was not reported. The mean study length was 33.1 days with a mean of 6.2 study calves for each trial. All trials utilized BHV-1 for the IBR challenge model. Five trials utilized intranasal administration for the IBR challenge and 4 trails challenged with aerosolization. Table 2.4 summarizes the number of trials (n) present for each analyzed area of interest.

The median time until IBR began shedding was approximately 2 days (Figure 2.2). Peak median shedding occurred on 4 days post-challenge for IBR. The median time until shedding of IBR ceased was 14 days, but spanned a time frame of as early as 7 days and as long as 17 days. Median rectal temperatures exceeding 40°C on day 2 post-challenge with median time to maximal rectal temperature on day 4 post-challenge. Rectal temperatures returning to less than 40°C occurred on median on day 8 post challenge. Median IBR seroconversion occurred on day 17.5 post-challenge with peak antibody response on day 40. Median CS began on day 2 after IBR exposure with a range extending from 2 to 5 days after challenge. The median peak CS occurred on day 7 with mean resolution of CS on day 14.

Parainfluenza-3

Three trials from three studies investigating PI-3 were included for analysis.(Peters, Thevasagayam et al. 2004; Salt, Thevasagayam et al. 2007; Xue, Ellis et al. 2010) The average study length was 14 days with all 3 trials being blinded. The average number of calves included for each trial was 14.6. One trial had the PI-3 challenge administered via aerosolization and the other two trials had both intranasal and intratracheal administration. Table 2.5 summarizes the number of trials (n) present for each analyzed area of interest.

Median shedding of PI-3 began 1 day after challenge (Figure 2.3). Median peak nasal shedding occurred 4 days after challenge and shedding resolved on day 11. Systematic review data for CS onset was only available for the minimum (when CS first appeared after challenge) which the median occurred 2 days post-challenge. Only one trial had resolution of CS by the end of the trial (day 14). The other two trials did not have resolution of CS by the end of the trial and the study length was 14 days after PI-3 challenge for both trials. Rectal temperature and serum neutralization data was only available for one trial; therefore, these outcomes were excluded for the systematic review for PI-3.

Bovine Respiratory Syncytial Virus

Investigations of BRSV for this review included 22 trials with 15 studies.(Ciszewski, Baker et al. 1991; LeBlanc, Baker et al. 1991; Otto, Elschner et al. 1996; Van der Poel, Schrijver et al. 1996; Elvander, Baule et al. 1998; Woolums, Anderson et al. 1999; Gershwin, Gunther et al. 2000; West, Petrie et al. 2000; Ellis, West et al. 2001; Peters, Thevasagayam et al. 2004; Mawhinney and Burrows 2005; Ellis, Gow et al. 2007; Salt, Thevasagayam et al. 2007; Vangeel, Antonis et al. 2007; Xue, Ellis et al. 2010) The average study length was 15 days with 12 trials being blinded and 10 trials either not blinded or blinding was not reported. The average number of calves in each trial was 8. Nine calves were challenged with BRSV via aerosolization, 7 with intranasal challenge, and 6 with a combined intranasal and intratracheal method. Table 2.6 summarizes the number of trials (n) present for each analyzed area of interest.

Bovine respiratory syncytial virus CS began on average day 3 post-inoculation with the median peak CS occurring on day 6. Median resolution did not occur until day 12.1 post-inoculation. Rectal temperatures exceeded 40°C on day 5 and median maximum rectal temperature occurred on day 6. Median rectal temperatures returned to less than 40°C on trial

day 8 post-challenge. Seroconversion to BRSV occurred on day 9 (median) post challenge using serum neutralization. Median maximum antibody response occurred on post challenge day 23. Bovine respiratory syncytial virus median shedding began 3 days after challenge with median peak shedding on day 5 and median resolution on day 9. Figure 2.4 summarizes this data.

Mannheimia haemolytica

Five trials from five studies investigating *Mannheimia haemolytica* met inclusion criteria for this systematic review with the average trial length being 23 days.(Olchoway, TerHune et al. 2000; Fajt, Apley et al. 2003; Hewson, Viel et al. 2011; Theurer, Anderson et al. 2013; Amrine, White et al. 2014) Of the trials, four used *Mannheimia haemolytica* type A1 for challenge induction. One trial used *Mannheimia haemolytica*, the type was not reported. Two of the trials were blinded, one trial was not blinded and one trial did not report if the study was blinded. Four trials had the *Mannheimia haemolytica* challenge administered endoscopically and one trial administered the challenge intratracheally. The average number of calves in each trial was 10.4. Area of interest data was only present for CS and rectal temperatures. Only one trial reported seroconversion and no trials reported data for shedding of bacteria. Table 2.7 summarizes the number of trials (n) present for each analyzed area of interest.

The median onset of CS occurred 1 day after challenge inoculation. Median peak CS occurred 1 day after challenge with median resolution on 8 days after inoculation. The median minimum time until rectal temperatures exceeded 40°C was 1 day after challenge. Median peak rectal temperatures occurred 1 day after challenge with the median time until rectal temperatures returned to less than 40°C on day-2 post-challenge. Figure 2.5 summarizes these results.

Mycoplasma bovis

Investigations of *Mycoplasma bovis* for this review included eight trials and four studies.(Howard and Gourlay 1983; Godinho, Wolf et al. 2005; White, Anderson et al. 2012; Dudek, Bednarek et al. 2013) The average number of calves included in each trial was 15.6 with an average study length of 23.8 days. Two studies were blinded, one study was not blinded, and five did not state blinding status. Seven trials performed intratracheal inoculation and one trial challenged intranasally. Table 2.8 summarizes the number of trials (n) present for each analyzed area of interest.

The median onset of CS was 2 days post-challenge with peak CS occurring on day 2. All trials either still had ongoing CS at the end of the trial or the time to resolution of CS was not reported. Median rectal temperatures exceeding 40°C occurred on day 1 after challenge with median peak on 4.5 days. Rectal temperature resolving to less than 40°C occurred on median day 8. Median seroconversion occurred 21 days post challenge with median peak antibody titers on day 26.6. Figure 2.6 summarizes these results.

Pasteurella multocida

One study reporting four trials investigating *Pasteurella multocida* met inclusion criteria for the systematic review.(Dowling, Hodgson et al. 2002) The average number of calves in the trial was 4 and all calves were challenged intratracheally. Blinding was not reported for the study. The study length was 4 days for all trials. Table 2.9 summarizes the number of trials (n) present for each analyzed area of interest.

Median onset of CS for *Pasteurella multocida* occurred 1 day after challenge. Median peak CS occurred 1 day after challenge with median resolution on day 2. Rectal temperatures

also exceeded 40°C on day 1 post challenge. Maximum rectal temperatures also occurred on day 1 post challenge with resolution on day 2 post challenge. Figure 2.7 summarizes these results.

Histophilus somni was excluded from the systematic review because no trials were identified that coincided with our inclusion criteria.

Discussion

This systematic review serves as a resource and summary for the common BRD pathogens with regards to expected times for CS, high rectal temperature, shedding, and seroconversion after pathogen exposure. For the viruses, the relationship between resolution of CS and shedding is interesting. For BVD, CS persisted an average of 3 days after the resolution of shedding on day 15. However, if you look at the range, one trial didn't have resolution of CS until 23 days post challenge. Unfortunately, this trial did not report virus isolation data. Infectious bovine rhinotracheitis average resolution of shedding and CS occurred around the same time with shedding resolving around 12.6 days and CS at 12.8 days. These results correlate with other summaries reporting IBR shedding resolution between 10-17 days and peak CS occurring between 4-6 days with our study showing peak at 5 days post-inoculation.(Straub 1990) For BRSV, CS resolved 2.1 days after shedding ceased on day 12.1. Sacco summarized that viral detection is expected until 7 to 10 days after infection with viral detection beginning at day 2 to 3 which correlates with our results of resolution at 10 days and shedding beginning at day 3.(Sacco, McGill et al. 2014) Besides the outlier for BVD, CS for the viral pathogens (BVD, IBR, BRSV) resolved near the time of shedding cessation or up to 3 days after shedding ceased. This information could be vital to know in regards to instituting proper quarantine periods in association with onset of BRD CS. Unfortunately, investigations of PI-3, *M. haemolytica*, *M.*

bovis, and *P. multocida* did not report complete data sets to make comparisons between shedding and CS.

Generally, most induced infections whether viral or bacterial in origin reported resolution of pyrexia before all CS resolve. For BVD, high rectal temperature resolution occurred 5 days before the cessation of CS on day 15. Infectious bovine rhinotracheitis and BRSV on average resolved high rectal temperatures 4 days before the resolution of CS. *M. haemolytica* on average resolved high rectal temperature on day 3 which was 5 days before resolution of CS. *M. bovis* had resolution of high rectal temperatures on average of day 8 post challenge. However, we have no data regarding time to resolution of CS since all the trials in the systematic review concluded before resolution of CS. This could be a result of short trial durations or it could be in conjunction with the known long, often chronic disease course associated with *M. bovis*. Parainfluenza-3 did not have data for time to resolution of CS and rectal temperatures. *P. multocida* was the only outlier of the common BRD pathogens with high rectal temperatures and CS resolving around the same time (high rectal temperature resolution on day 2, clinical sign resolution on day 2.5). Knowledge of high rectal temperature resolution with regards to time to resolution of CS could be an important disease progression indicator for producers, veterinarians, and researchers. For the most BRD pathogens, we can expect clinical sign resolution 4 to 5 days after rectal temperatures have returned to less than 40°C.

Seroconversion is defined as the time at which antibodies are first detected in the serum. Seroconversion occurred between 10.8 days and 21 days for the pathogens in this study, on average occurring on day 17 for BVD, day 17.5 for IBR, day 10.8 for BRSV, and day 21 for *M. bovis*. Data were not available for PI-3 and *M. haemolytica*. Peak seroconversion occurred on day 27 for BVD, day 41 for IBR, day 21 for BRSV, and day 26 for *M. bovis*. For *M. bovis*, this

partially concurs with one study evaluating response of naïve calves being exposed to a herd endemically infected with *M. bovis* with antibodies first detected by day 29-35; however, peak antibody response did not occur until day 60 post-introduction.(Nagatomo, Shimizu et al. 1996)

The time to seroconversion or peak seroconversion could have been affected simply by the animals' ability to respond to the antigen and produce appropriate antibody or simply confounded by the sampling time selected by the researchers for each trial. For BVD, IBR, BRSV, and *M. bovis*, seroconversion can be expected to occur in a range of 10 days to 21 with peaks between 21 days to 41.

There are certainly limitations associated with this systematic review and descriptive analysis. The biggest limitation would be the low number of trials for each pathogen. Ideally, the systematic review would have been limited to studies that were blinded. Non-blinded studies were included because the number of trials included would have been too severely limited. Additionally, non-blinding is not as likely to affect our objective areas of interest such as: rectal temperature, seroconversion, and viral shedding. Another limitation is the lack of shedding data for the bacterial pathogens. Careful extrapolation of this data needs to be performed before applying to clinical scenarios due to poor external validity. However, the information in this systematic review provides a resource when designing clinical trials for the specific pathogens of interest.

This systematic review serves as a valuable summary and resource for veterinary researchers, veterinarians, and producers interested in the length of time between exposure to common BRD pathogens until expected time to resolution of CS, high rectal temperature, shedding, and seroconversion. Important take home points are that CS resolved near the time of shedding cessation or up to 3 days after shedding ceased for BVD, IBR, and BRSV; and high

rectal temperatures resolved approximately 4-5 days before resolution of CS for BVD, IBR, BRSV, and *M. haemolytica*.

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Tables and Figures

Table 2.1 Structured literature search results by database. English only results are listed

Search Terms	Pubmed	CAB	Agricola
<i>Baseline searches for individual search terms</i>			
Bovine OR cattle OR calves	369,041	611,304	171,371
Respirator* OR BRD*	461,954	115,725	19,267
Mannheimia	1,024	855	853
Pasteurella	7,886	12,515	2,932
Histophilus	23,889	268	63
Mycoplasma	21,499	18,379	5,967
Infectious Bovine Rhinotracheitis* OR IBR* OR BHV-1*	14,375	7,758	4,329
Parainfluenza-3* OR PI-3*	7,300	2,666	1,674
Bovine Respiratory Syncytial Virus OR BRSV*	841	1,502	411
Bovine Viral Diarrhea* OR BVD*	3,598	6,953	1,671
<i>Searches for articles on individual pathogens for analyses</i>			
(Bovine OR cattle OR calves)+(Respirator* OR BRD*))+Mannheimia	1,024	855	853
(Bovine OR cattle OR calves)+(Respirator* OR BRD*))+Pasteurella	424	1,411	169
(Bovine OR cattle OR calves)+(Respirator* OR BRD*))+Histophilus	433	94	13
(Bovine OR cattle OR calves)+(Respirator* OR BRD*))+Mycoplasma	259	862	29
(Bovine OR cattle OR calves)+(Respirator* OR BRD*))+Infectious Bovine Rhinotracheitis* OR IBR* OR BHV-1*)	381	1,202	47
(Bovine OR cattle OR calves)+(Respirator* OR BRD*))+Parainfluenza-3* OR PI-3*)	193	662	65

(Bovine OR cattle OR calves)+(Respirator* OR BRD*)+(Bovine Respiratory Syncytial Virus OR BRSV*)	828	1,488	403
(Bovine OR cattle OR calves)+(Respirator* OR BRD*)+(Bovine Viral Diarrhea* OR BVD*)	330	865	117
Total number of articles (from articles on individual pathogens; some articles are present for multiple pathogens)	3,113	6,858	987

Table 2.2 Number of papers present for each pathogen during each stage of evaluation

	BVD	IBR	PI-3	BRSV	<i>M. haemolytica</i>	<i>M. bovis</i>	<i>P. multocida</i>	<i>H. somni</i>
Number of relevant abstracts	17	22	7	44	16	20	19	9
Number papers read for analysis inclusion	8	9	5	31	13	10	12	5
Number of papers (studies) included in systematic review	8	7	3	15	5	4	1	0
Number of trials included in analysis	12	9	3	22	5	8	4 (all from same study)	0

Table 2.3 Number of trials that reported data for each outcome of interest included for systematic review and descriptive analysis for BVD.

	Minimum	Peak	Resolve
Clinical Signs	8	7	2
Antibody titers	2	1	NA
Rectal Temperature	10	8	8
Virus Isolation	10	9	2

Table 2.4 Number of trials that reported data for each outcome of interest included for systematic review and descriptive analysis for IBR.

	Minimum	Peak	Resolve
Clinical Signs	9	5	8
Antibody titers	4	3	NA
Rectal Temperature	8	6	8
Virus Isolation	9	9	6

Table 2.5 Number of trials that reported data for each outcome of interest included for systematic review and descriptive analysis for PI-3.

	Minimum	Peak	Resolve
Clinical Signs	3	0	0
Virus Isolation	3	3	3

Table 2.6 Number of trials that reported data for each outcome of interest included for systematic review and descriptive analysis for BRSV.

	Minimum	Peak	Resolve
Clinical Signs	17	15	7
Serum Neutralization	8	4	NA
Temperature	8	6	4

Virus Isolation	17	14	12
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Table 2.7 Number of trials that reported data for each outcome of interest included for systematic review and descriptive analysis for *Mannheimia haemolytica*.

	Minimum	Peak	Resolve
Clinical Signs	4	4	1
Rectal Temperature	4	4	4

Table 2.8 Number of trials that reported data for each outcome of interest included for systematic review and descriptive analysis for *Mycoplasma bovis*.

	Minimum	Peak	Resolve
Clinical Signs	3	3	NA
Serum Neutralization	3	3	NA
Temperature	5	2	5

Table 2.9 Number of trials that reported data for each outcome of interest included for systematic review and descriptive analysis for *Pasteurella multocida*.

	Minimum	Peak	Resolve
Clinical Signs	4	4	4
Temperature	2	2	2

Figure 2.1 Summary of BVD trials.

For virus isolation (VI), minimum is defined as the day when shedding was first detected, peak is when shedding was at the maximum, and resolution when shedding ceased. For rectal temperature (Temp), minimum is defined as the day when rectal temperature first exceeded 40°C, peak when rectal temperature was highest, and resolution defined as when rectal temperature was less than 40°C. For serum neutralization (Serum), minimum is defined as the day when seroconversion was first detected and peak when serum neutralization was highest. For clinical signs (CS), minimum is defined as the day when clinical signs were first detected, peak being when clinical signs were the most severe, and resolution when clinical signs resumed normal limits.

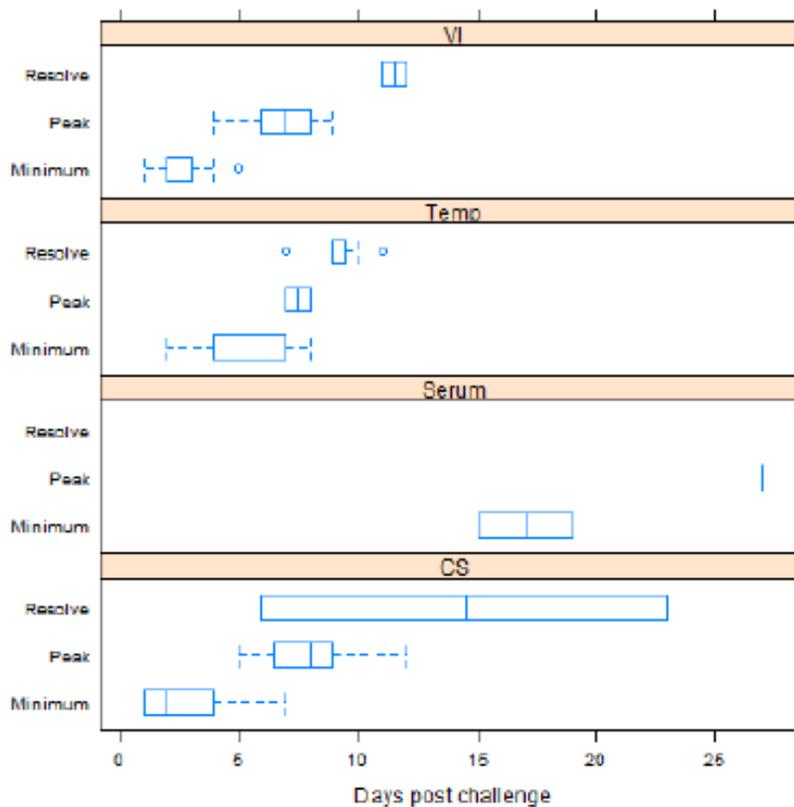


Figure 2.2 Summary of IBR trials.

For virus isolation (VI), minimum is defined as the day when shedding was first detected, peak is when shedding was at the maximum, and resolution when shedding ceased. For rectal temperature (Temp), minimum is defined as the day when rectal temperature first exceeded 40°C, peak when rectal temperature was highest, and resolution defined as when rectal temperature was less than 40°C. For serum neutralization (Serum), minimum is defined as the day when seroconversion was first detected and peak when serum neutralization was highest. For clinical signs (CS), minimum is defined as the day when clinical signs were first detected, peak being when clinical signs were the most severe, and resolution when clinical signs resumed normal limits.

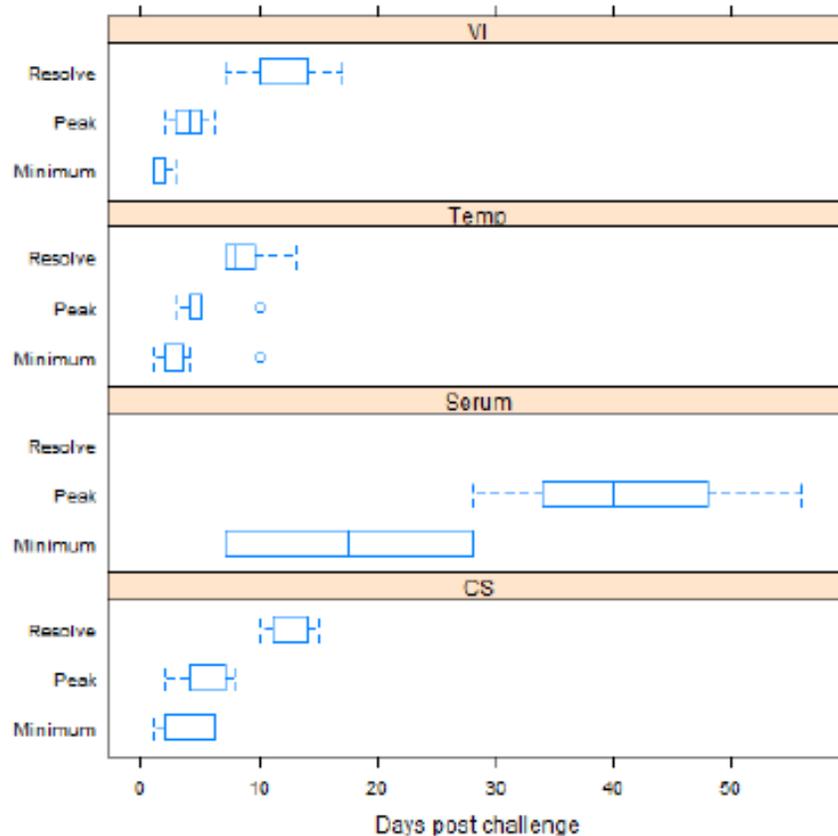


Figure 2.3 Summary of PI-3 trials.

For virus isolation (VI), minimum is defined as the day when shedding was first detected, peak is when shedding was at the maximum, and resolution when shedding ceased. For clinical signs (CS), minimum is defined as the day when clinical signs were first detected, peak being when clinical signs were the most severe, and resolution when clinical signs resumed normal limits.

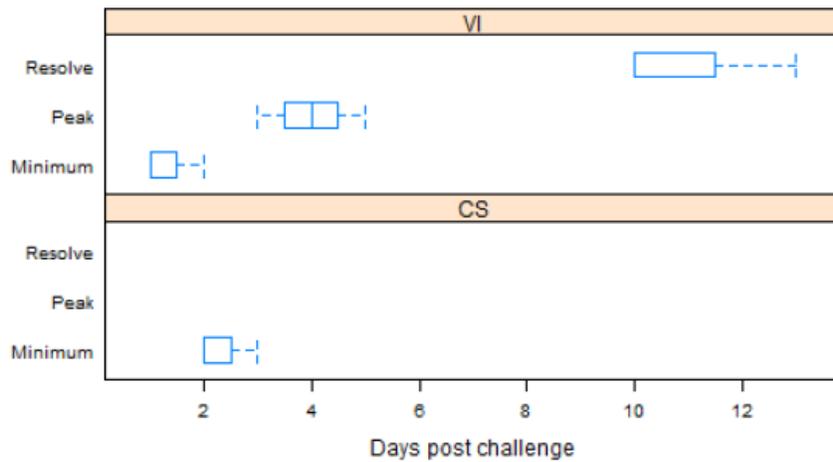


Figure 2.4 Summary of BRSV trials.

For virus isolation (VI), minimum is defined as the day when shedding was first detected, peak is when shedding was at the maximum, and resolution when shedding ceased. For rectal temperature (Temp), minimum is defined as the day when rectal temperature first exceeded 40°C, peak when rectal temperature was highest, and resolution defined as when rectal temperature was less than 40°C. For serum neutralization (Serum), minimum is defined as the day when seroconversion was first detected and peak when serum neutralization was highest. For clinical signs (CS), minimum is defined as the day when clinical signs were first detected, peak being when clinical signs were the most severe, and resolution when clinical signs resumed normal limits.

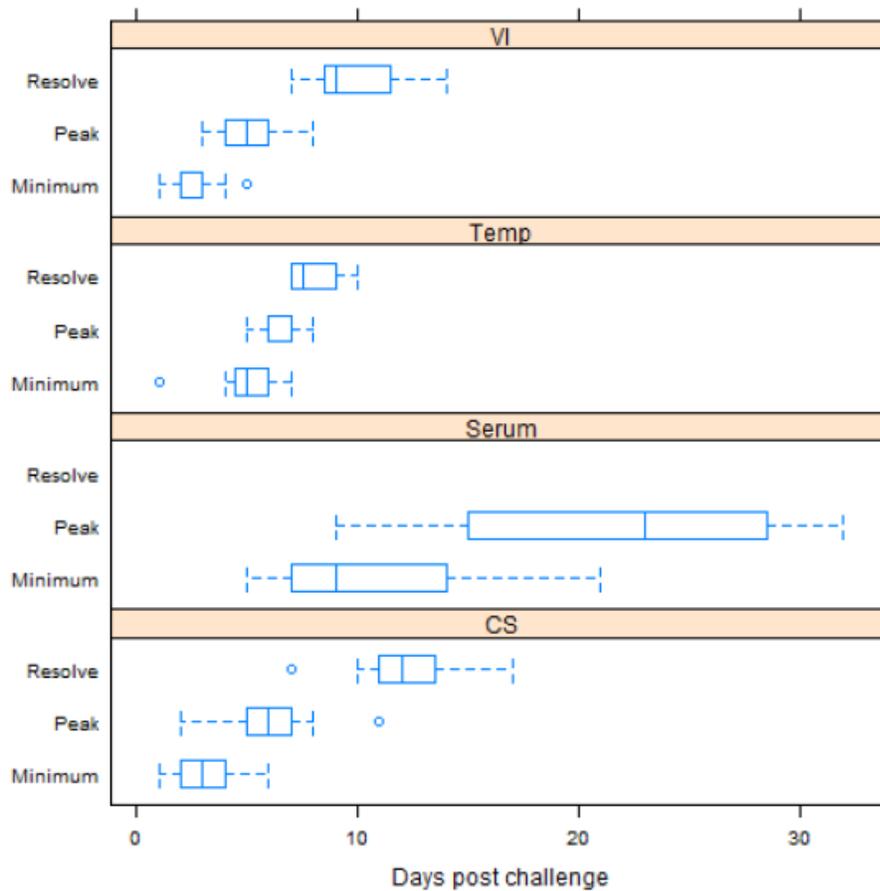


Figure 2.5 Summary of *Mannheimia haemolytica* trials.

For rectal temperature (Temp), minimum is defined as the day when rectal temperature first exceeded 40°C, peak when rectal temperature was highest, and resolution defined as when rectal temperature was less than 40°C. For clinical signs (CS), minimum is defined as the day when clinical signs were first detected, peak being when clinical signs were the most severe, and resolution when clinical signs resumed normal limits.

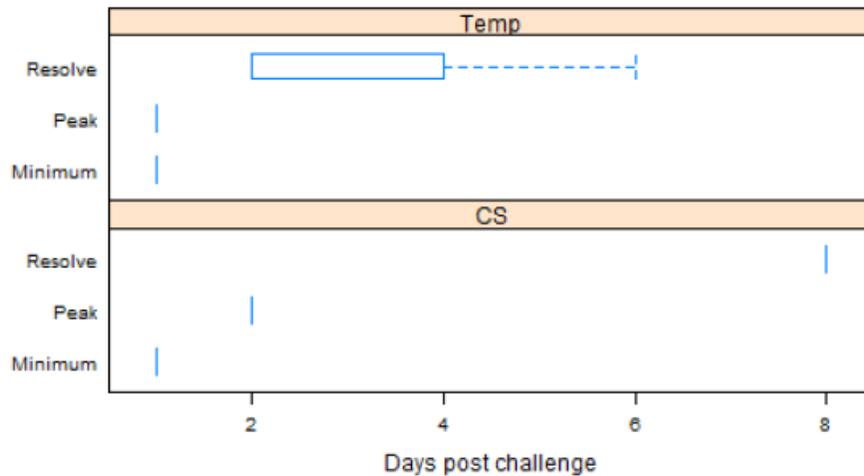


Figure 2.6 Summary of *Mycoplasma bovis* trials.

For rectal temperature (Temp), minimum is defined as the day when rectal temperature first exceeded 40°C, peak when rectal temperature was highest, and resolution defined as when rectal temperature was less than 40°C. For serum neutralization (Serum), minimum is defined as the day when seroconversion was first detected and peak when serum neutralization was highest. For clinical signs (CS), minimum is defined as the day when clinical signs were first detected, peak being when clinical signs were the most severe, and resolution when clinical signs resumed normal limits.

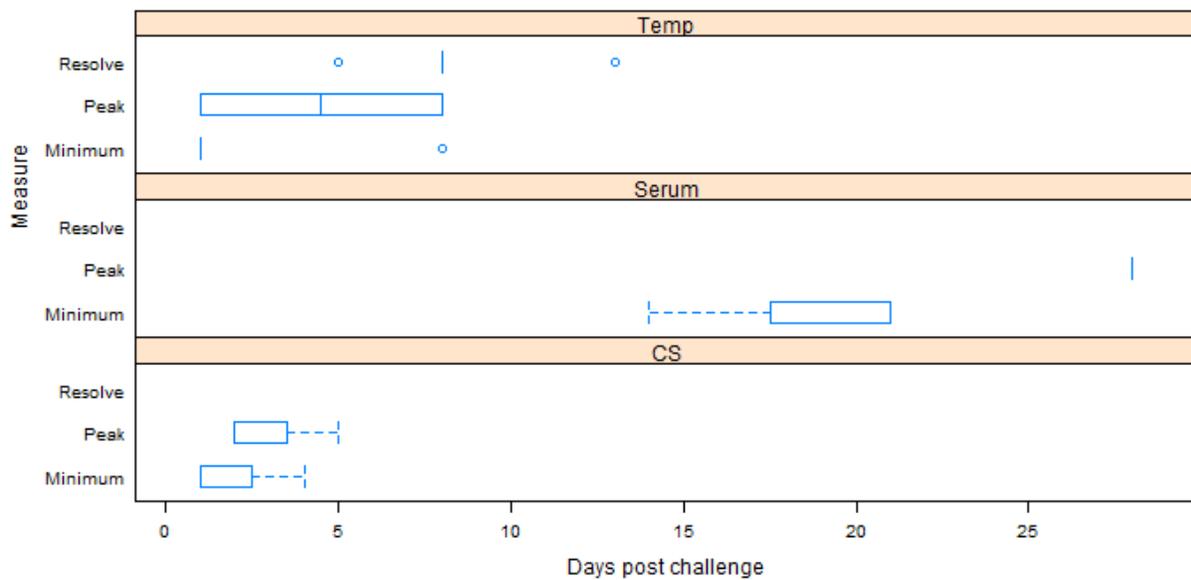
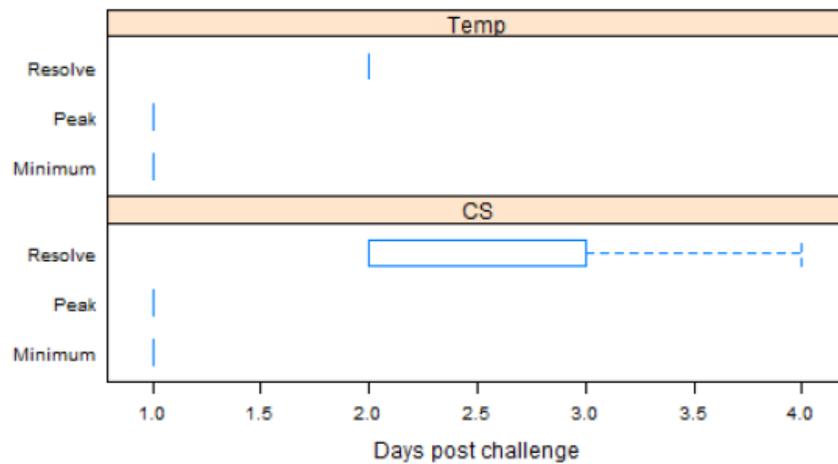


Figure 2.7 Summary of *Pasteurella multocida* trials.

For rectal temperature (Temp), minimum is defined as the day when rectal temperature first exceeded 40°C, peak when rectal temperature was highest, and resolution defined as when rectal temperature was less than 40°C. For clinical signs (CS), minimum is defined as the day when clinical signs were first detected, peak being when clinical signs were the most severe, and resolution when clinical signs resumed normal limits.



Chapter 3 - Effect of Ambient Temperature on Viral Replication and Serologic Titers Following Administration of a Commercial Intranasal Modified-Live Bovine Rhinotracheitis-Parainfluenza-3 Viral Vaccine in Beef Cattle Housed in High and Moderate Ambient Temperature Environments

Bovine respiratory disease is a prominent disease of the feedlot industry with 96.9% of feedlots having cattle affected with BRD.(USDA 2011) Bovine herpes virus-1 and PI-3 are both considered pathogens in the BRD complex. A variety of clinical manifestations are associated with IBR virus including: rhinotracheitis, vulvovaginitis, balanoposthitis, abortion, and encephalitis.(Radostits 2001; Smith 2009) Additionally, IBR plays an important role in the BRD complex through immune suppression, increasing susceptibility to secondary bacterial infections. The biggest role of PI-3 is predisposing the bovine respiratory tract to subsequent infections of other viruses and bacteria.(Smith 2009) One study of incoming feedlot cattle showed antibody titers of 12% for PI-3 and 23% for IBR.(Martin, Bateman et al. 1989) Due to the prominent role of IBR and PI-3 in the BRD complex, significant focus is placed on prevention.

One mainstay of BRD prevention is vaccination. In 2011, the National Animal Health Monitoring Systems survey identified that 93.7% of feedlots were vaccinated for IBR (53.7% via intranasal route) and 85.1% were vaccinated for PI-3.(USDA 2011) Viral vaccines can be either inactivated or MLV with MLV vaccines considered to have superior efficacy due to their ability to induce humoral immunity, cell-mediated immunity, and interferon. (Tizzard 2000) Antibody titers measured in calves two weeks after booster vaccination showed higher IBR antibody titers

in calves vaccinated with MLV compared to an inactivated product.(Tollis, Di Trani et al. 1996) Infectious bovine rhinotracheitis/PI-3 MLV vaccines are administered intranasally to cattle, with the goal of providing immunity against initial viral establishment in the nasal passages and subsequent disease development.(Todd 1976) Intranasal administration results in systemic immunity, as well as faster onset of local immunity than systemically administered IBR/PI-3 MLV vaccine.(Todd 1976) Disadvantages of MLV vaccines include the need for special storage and handling. Temperature sensitive vaccines have been shown to have diminished viral replication at temperatures $> 39^{\circ}\text{C}$ in vivo models; thus, IN is the preferred route as nasopharyngeal temperatures only reach $32\text{-}34^{\circ}\text{C}$ which allows the virus to replicate and produce an immune response.(Mills, Chanock et al. 1971) Improper vaccine handling and extreme environmental temperatures may lead to vaccine failure in calves. To the authors' knowledge, there has been no research evaluating the immunological response of IN MLV vaccine at extreme environmental conditions.

Intranasal vaccines are often administered to cattle at times when environmental conditions of ambient temperature and humidity may be adversely high. One study conducted in high ambient temperatures (daily temperatures $\geq 32^{\circ}\text{C}$ and humidity $\geq 40\%$) showed nasal mucosal temperatures exceed 39°C in up to 25% of healthy calves and most (90%) of calves with induced respiratory disease.(Theurer, Anderson et al. 2013) The impact of ambient temperature on viral replication and serologic response following calf intranasal vaccination is unknown. The purpose of this trial was to compare calf serologic response (antibody titers) and viral replication (virus isolation) in beef calves administered a MLV intranasal vaccine during summer conditions (high ambient temperatures in outside pens) and moderate conditions (indoor climate-controlled temperatures). Rectal and nasal mucosal temperatures were measured as potential

factors that may impact vaccine response. Our hypothesis was that calves housed in a high ambient temperature environment would have decreased serologic response and decreased virus replication when compared to calves housed in a climate-controlled environment. Conclusions from this trial may impact timing of vaccine administration and education of animal health managers.

Methods

Trial Design

This randomized clinical trial consisted of 28 weaned black beef heifer calves. All study procedures were conducted in accordance with a protocol approved by the Kansas State University Institutional Animal Care and Use Committee (protocol # 3163). The study was conducted during July and August at the Kansas State University Large Animal Research Center in Manhattan, Kansas with maximum daily temperatures was forecasted to be $> 32.2^{\circ}\text{C}$. Upon arrival, the calves were randomly allocated to 1 of 4 treatment groups:

- *High Ambient Temperature, HAT*: Received vaccine; housed in high ambient temperatures (as defined) in outdoor pen (12.2 x 24.4 m) during peak summer heat. (n=10).
- *High Ambient Control, HAC*: Did not receive vaccine; housed in identical conditions as HAT in separate pen (12.2 x 24.4 m) (n=4).

- *Moderate Ambient Temperature, MAT*: Received vaccine; housed in indoor, climate-controlled pen (6.1 x 9.1 m) with moderate ambient conditions (temperature approximately 21°C; n=10).
- *Moderate Ambient Control, MAC*: Did not receive vaccine, housed in identical conditions as MAT with 2 calves in each pen (3.0 x 3.0 m) (n=4).

The trial design resulted in a 2x2 allocation of housing (high ambient temperature or low (controlled) ambient temperature) and vaccine status (receiving intranasal modified-live bovine rhinotracheitis-parainfluenza-3 viral vaccine or saline treated controls). The housing environment and difference in number of calves allocated into treatment and control groups prevented blinding of this study.

Calf arrival

The potential study population included 28 calves intended for use in the study and 2 calves for use as substitutes for any calf that developed clinical illness during the 21 day observation period after arrival and prior to vaccine administration. One alternate calf was housed with the outdoor control group pen (HAC) and one alternate calf was housed with the indoor control group pen (LAC). The calf in each group would thus be acclimatized if needed.

After initial processing, all calves were placed in their appropriate group and housing environment. The HAT and HAC groups were housed in separate outdoor pens on opposite sides of a 12 foot wide alley, providing separation and preventing direct contact between groups. The HAT and HAC groups were provided an open faced shed for shelter. Groups were handled and processed separately at the same outdoor chute to prevent nose to nose contact between

groups. The HAC group was always processed first to limit potential vaccine virus exposure. The MAT group was housed in one pen in the indoor climate controlled facility. The MAC group was housed in 2 pens (n=2 per pen) separate from the MAT group without the possibility of direct contact. An indoor chute and alley was used for the MAT and MAC groups to maintain proper temperature acclimation. Each group was processed separately to prevent contact among groups.

Upon arrival at KSU, all calves were weighed, and treated with ceftiofur crystalline free acid^b at 6.6 mg/kg. Individual animal identification ear tags were also applied at this time. Eight days after arrival, biothermal radiofrequency chips^c were implanted submucosally in the right nares approximately 100 mm caudal to the alar cartilage and 2 mm deep in the nasal mucosae using a previously developed technique.(Theurer, Anderson et al. 2013; Theurer, White et al. 2013) A digital electronic microchip scanner was used to measure the biothermal chip temperature.^c Intranasal biothermal chips were implanted in all HAT calves (n=10) and randomly selected MAT calves (n=7). A randomization program was used to determine the seven calves receiving nasal thermometers in the MAT group.^a

Throughout the trial, calves were fed a complete starter grain ration^d and free choice grass hay, supplemental salt block, and water. The ration contained 13.5% crude protein and 2.79% crude fat. For the first week after arrival, calves were fed 1.4 kg of this starter ration per head per day. The amount was increased to 1.6 kg per head per day for the last two weeks of the study.

Clinical observations

Calves were observed twice daily for 21 days prior to vaccine administration by the same veterinarian trained in the detection of clinical illness and bovine respiratory disease. Each calf

was given a clinical illness score (CIS) based on the health characteristics at each observation. The CIS ranged from 1 to 4 with the following criteria used for each level: 1 = normal behavior, 2 = slight illness, mild lethargy and/or a cough, 3 = moderate illness, severe lethargy, labored breathing, and/or cough, and 4 = severe illness, moribund or have little response to human approach. During the pre-vaccine administration period, calves receiving a $CIS \geq 2$ had a physical examination performed including measurement of rectal temperature. Calves with $CIS \geq 2$ and rectal temperature $\geq 40^{\circ}C$ in the pre-vaccine administration period were given appropriate treatments and removed from potential consideration for inclusion in the vaccine administration portion of the trial. After vaccine administration, the same trained veterinarian observed each calf twice daily and assigned a CIS at each observation until completion of the trial. After vaccine administration, calves receiving a $CIS \geq 2$ and a rectal temperature $\geq 40^{\circ}C$ had physical examinations performed and were given appropriate treatments. Calves treated after vaccination remained in the study with their respective treatment group and continued to have data collected.

Vaccine administration

Study initiation was determined based on predicted outdoor temperatures consistently exceeding $32.2^{\circ}C$ for the first week of the trial. Vaccine administration occurred 21 days after arrival (study day 0; Table 3.1). On study day 0, all baseline measurements and samples were collected immediately prior to vaccine administration. After baseline data collection, calves in the HAT and MAT groups were administered a commercial intranasal modified-live bovine rhinotracheitis-parainfluenza-3 viral vaccine.^e All calves were restrained in a head gate and 1 ml of vaccine was administered in each nostril according to label instructions. After vaccine

administration to the HAT and MAT groups, personnel changed attire and the chutes were disinfected in order to prevent exposure of the control calves to the modified live vaccine. Calves in the HAC and MAC groups were administered sterile saline in the same manner as the vaccine-administered group. The same trained veterinarian administered all treatments and was not blinded to treatment group.

Temperature Monitoring

On study day 0, baseline nasal mucosal temperature and rectal temperature were obtained on all calves prior to vaccine or saline administration. To determine if nasal mucosal temperatures exceeding 39°C could inactivate viral replication during the expected peak replication period, nasal mucosal and rectal temperatures were measured every two hours between the hours of 8am and 8pm during the first 48 hours after vaccination. Following the first 48 hours, nasal mucosal and rectal temperatures were obtained twice a day for the first week of the trial. After study day 7, nasal mucosal and rectal temperatures were obtained at weekly processing.

Environmental Monitoring

Outdoor temperature and humidity was measured hourly using a wireless weather monitoring technology^f throughout the trial. For the first 48 hours, indoor temperature and humidity were measured hourly using an environmental data monitor.^g After the first 48 hours, the indoor temperature and humidity maximums were measured using a commercial monitor.^h

PCR

Nasal swabs obtained for virus isolations were also submitted to the Kansas State University diagnostic lab for PCR analysis of IBR and PI-3 on trial day 0 to determine any potential infection prior to initiation of trial. The individual performing PCR analysis was blinded to the treatment group of the sample.

Virus isolation

Nasal swabs were obtained from both nostrils of each calf immediately prior to vaccination and every 24 hours after vaccination on days 1-7. Control calves (HAC, LAC) were processed first to minimize potential contamination with vaccine strains of IBR and PI-3. The nasal swab samples were obtained approximately 10cm caudal to the alar cartilage using individual sterile cotton tipped swabs and placing them both in the same viral culture media. Immediately after collection, the samples were placed in a -80°C freezer until sample submission. At completion of study day 7, the nasal swab-samples from all collection time points were submitted to the Kansas State Veterinary Diagnostic Lab for virus isolation of IBR and PI-3. All samples positive on virus isolation had a TCID-50 measured. The TCID-50 was determined as the endpoint dilution required to produce a cytopathic effect in 50% of inoculated tissue culture cells.(LaBarre and Lowy 2001) The lab was blinded to the treatment group assignment and time frame of sample collection.

Antibody titers

Venous blood samples were collected on all calves immediately prior to vaccine administration (study day 0), and on days 7, 14, 21, and 28. After collection, the samples were

centrifuged and the serum was placed in a -80°C freezer until analysis. At completion of the study day 28, all samples from all collection time points were submitted to the Kansas State Veterinary Diagnostic Lab for measurement of serum neutralization for IBR and PI-3. The lab was blinded to the treatment group assignment and time frame of sample collection.

Seroconversion for IBR was defined as an antibody titer of 1:4 or greater.(Richeson, Beck et al. 2008)

Statistical Analysis

Trial data were imported into a commercial statistical software packageⁱ for descriptive and statistical analyses. Generalized linear models were used to evaluate potential associations between outcomes of interest (serology, TCID-50, nasal temperatures, and rectal temperatures) with treatment group (HAT, HAC, MAT, MAC), time of sample collection relative to vaccine administration, and the potential interaction between treatment group and sample timing. Non-normally distributed variables (serology) were log-transformed for the analyses, and means back-transformed for reporting along with 95% confidence intervals. All analyses included a random effect to account for repeated measurements on individual calves.

Results

On arrival, calves weighed an average \pm SD of 206.8 ± 25.4 kg. Prior to vaccine administration (study day 0), two calves (HAT, n=1; LAC, n=1) were given a CIS =2. These two calves had rectal temperatures $>40^{\circ}\text{C}$, were diagnosed with BRD, treated appropriately, and removed from the study. Both calves were replaced with alternates already housed in the same environmental conditions.

After vaccination on trial day 14, one LAC calf was treated for frothy bloat without any complications and remained in the study with all data included in analysis. Four calves (MAT n = 1 study day 1; MAT n=2 calves day 9; HAT n=1 calf day 12) were given a CIS >1 after vaccination. All four calves had a rectal temperature greater than >40°C, diagnosed with bronchopneumonia, and treated with florfenicol^j at 40 mg/kg subcutaneously, and remained in their respective housing groups. No temperature data from the calf treated on study day 1 were included in the analyses; however temperature data was included for calves diagnosed with BRD on days 9 and 12. The HAT calf identified as ill on day-12 died on study day 15, and necropsy findings included bronchopneumonia and hepatic lipidosis. All data from the HAT calf that died were included in the analysis of the results.

On study day 0, outdoor temperature maximum reached 38.7°C, and outdoor temperature maximum reached 42.1°C on study day 1. The indoor climate controlled environment maintained an average \pm SD temperature of $19.9 \pm 0.28^\circ\text{C}$ for the first 48 hours of the trial (Figure 3.1). Average \pm SD relative humidity was $70.9 \pm 5.1\%$ for study days 1 and 2 for indoor treatment groups (MAT, MAC). Maximum humidity of study day 0 and 1 was 49% and 66% respectively for the outdoor groups (HAT, HAC). Average \pm SD relative humidity was $40.0 \pm 12.7\%$ for study days 1 and 2 for outdoor treatment groups (HAT, HAC).

A significant interaction ($P < 0.01$) was identified in the relationship between nasal mucosal temperatures and treatment group (HAT and MAT), although all calves displayed an increase throughout the day during the intensive measurement period (Figure 3.2). Maximum nasal temperature was 36.6°C in the HAT group and 35.1°C in the MAT group in the first 48 hours of the trial. Study hours 6, 30, 32, 34, 38 were the only times there were statistically

significant differences within study hours between nasal temperatures of HAT and MAT based on treatment group and study hour.

There was a significant interaction ($P < 0.01$) between timing of measurement and treatment group for rectal temperature, but there were no statistically significant differences between treatment groups at any timing of measurement for HAT and MAT (Figure 3.3). Rectal temperature was significantly less for MAC compared to MAT, HAC, and HAT at study hours: 5, 6, 7, 8, and 14.

Bovine herpes virus-1 and PI-3 PCR was performed on samples from nasal swabs collected from all calves on study day 0. All calves were PCR negative for IBR. Two calves (HAT, n=1; MAC, n=1) were positive for PI-3 by PCR and virus isolation on trial day 0. Due to the pre-existing infection of PI-3 based on PCR and virus isolation, serology and subsequent virus isolation data for PI-3 were not analyzed.

Virus isolation was negative for IBR for all calves on study day 0. All control calves (HAC, MAC) remained negative on virus isolation for IBR during the first week of the trial. Virus isolation was positive for all vaccinated calves (HAT, MAT) on trial days 1 to 6 (Figure 3.4). On trial day 7, virus isolation was positive for all vaccinated calves except 1 calf in the HAT group. There was a significant interaction ($P < 0.01$) between TCID-50 and vaccinated calves (HAT, MAT) by study day; however, there were no statistically significant differences within study day between HAT and MAT groups on trial days 0 to 7.

On study day 0, all calves were serologically negative for IBR, and all control calves (MAC, HAC) remained serologically negative for IBR for the duration of the trial (Figure 3.5). Although there was a significant interaction ($P < 0.01$) between trial day and treatment group for IBR serologic status, mean antibody titers for HAT and MAT did not differ at any time point

during the trial. All calves in the MAT and HAT groups had seroconverted with a titer of $\geq 1:4$ by study day 28.

Discussion

The objective of this trial was to determine if exposure to extreme environmental conditions would inhibit serologic and viral replication response of cattle to IN vaccination with a MLV IBR/PI3 product. We were able to achieve exposure of calves to natural ambient temperatures exceeding 32.2°C. The climate-controlled environmental building allowed for comparison to moderate ambient temperature maintained near 19.4°C. Maximum nasal temperatures did not exceed 39°C for any calf in any treatment group in this study, which differs from previous work where calves housed in high ambient temperatures occasionally had nasal temperatures in excess of 39°C.(Theurer, Anderson et al. 2013; Theurer, Anderson et al. Accepted, In press) The difference in maximum temperatures may be related to other environmental factors such as relative humidity. In a previous study, humidity ranged from 64 to 89%, while in the current study, relative humidity only reached 49 and 66% for study days 0 and 1 respectively.(Theurer, Anderson et al. 2013) The lower relative humidity in this trial may explain the lower nasal temperatures observed.

Interestingly, indoor vaccinated calves (MAT) followed a similar rectal temperature trend compared to the outdoor calves (HAT, HAC) and were higher relative to MAC group at several time points during the first 48 hours. The reason for greater rectal temperatures in the MAT relative to MAC is unknown, but could be associated with subclinical BRD in this group or an immunological response produced from vaccine administration. Outdoor calves were housed in extreme environmental conditions and reached maximum physiological capabilities, thus

limiting the potential temperature response to vaccination.(Theurer, Anderson et al. Accepted, In press)

Due to the pre-existence of PI3 virus in calves in all treatments, data regarding serologic or viral replication for PI3 were not evaluated. Calves in the HAT and MAT groups both mounted similar IBR antibody responses following vaccine administration. All calves in both vaccinated groups (HAT, n=9 and MAT, n=10) seroconverted by trial day 28. This demonstrates that high ambient temperature had no effect on the calves' response to vaccine administration when compared with controlled ambient temperature environment. Other studies have shown an antibody titer peak at 21 day post vaccination, which coincides with the results of our study.(Patel 2005)

Calves in the HAT and MAT groups both demonstrated ability to produce viable IBR virus after administration of vaccine. All vaccinated calves had viable virus recovered from nasal swabs on the first 6 trial days. While one calf failed to have virus recovered on trial day 7, 90% of the high ambient temperature calves had virus recovered, suggesting high ambient temperature had minimal effect on the viability of the virus after vaccination when compared to controlled ambient temperature calves. Similar studies have demonstrated the ability to recover viable virus 8 days post vaccination with peak recovery occurring on day 4 post-vaccination.(Patel 2005) Our study showed the amount of virus recovered (as measured by TCID-50) did not statistically decrease throughout the study period for either group. Determination of a decrease in viral shedding would require research evaluating more days beyond vaccination.

Limitations of this study include study observers not being blind to treatment group as treatment groups were geographically spaced and numbers of calves assigned to each treatment

group were uneven. The lack of observer blinding resulted in minimal bias as the diagnostic laboratory was blinded and primary study outcomes were antibody titer production and viable virus recovery. Due to the limited availability of the biothermal chips at time of the trial, we were unable to implant the chips in all of the calves; therefore, we were not able to compare the nasal mucosal temperature between vaccinated and unvaccinated control calves.

This research illustrates that administration of a modified-live IBR-PI3 intranasal vaccine stimulated an IBR immune response in calves housed in both a temperature-controlled and a high ambient temperature environment. All calves in both vaccinate groups seroconverted to IBR by study completion and viable IBR virus was recovered from all calves (HAT, MAT) through six days post-vaccination. These findings indicate that despite high ambient temperatures, administration of this modified-live intranasal vaccine stimulated a similar response to calves housed in a controlled temperature environment.

- a. Microsoft Office Excel 2007; Microsoft, Redmond, WA
- b. Excede, Pfizer Animal Health, New York, NY
- c. Biothermal RFID Chip, Destron Technologies, Round Rock, TX
- d. Key Feeds, Clay Center, KS
- e. Nasalgen IP Merck Animal Health Summit, NJ
- f. La Crosse Technology Wireless Weather Station
- g. HOBO U12 Temperature/Relative Humidity/Light/External Data Logger
- h. Daily, Springfield 91551 Digital Humidity and Temperature Monitor
- i. JMP, Cary, NC
- j. Nuflor, Merck Animal Health, Summit, NJ

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Figures and Tables

Figure 3.1 Indoor (solid grey line) and outdoor (solid black line) environmental temperatures for first 48 hours after vaccine administration (8 am study day 0).

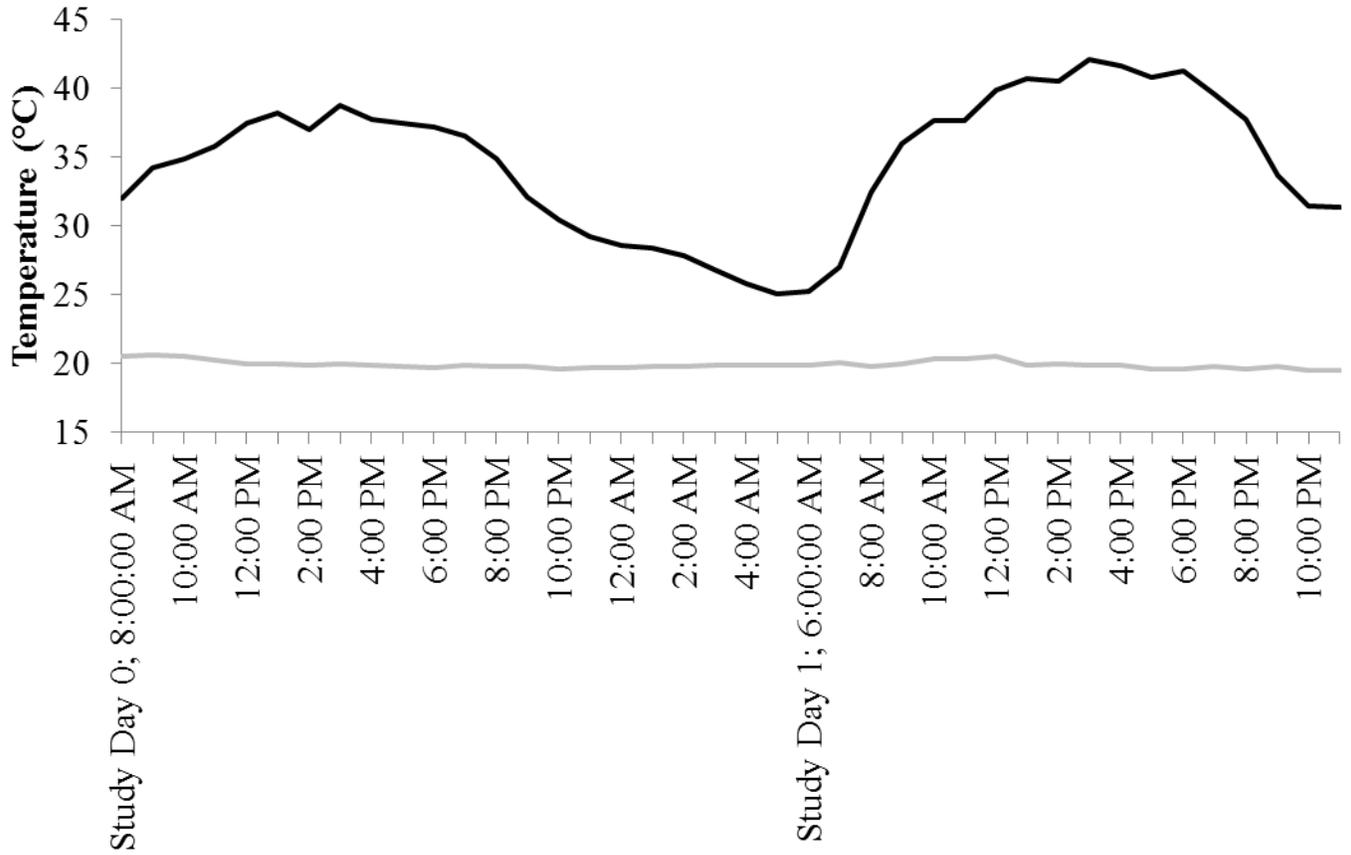


Figure 3.2 Nasal mucosal temperatures for calves in the high ambient temperature vaccinate group (HAT, n=10; solid black line) and moderate ambient temperature vaccinate group (MAT, n=9; solid gray line) for the first 48 hours after vaccine administration (study hour 0, 8 am on study day 0). Temperatures not measured between study hours 14 and 24. One calf in the MAT group received an abnormal CIS on study day 1 and temperatures from this calf were removed from the analysis. Significant differences ($P < 0.01$) between treatment group within study hour denoted by *.

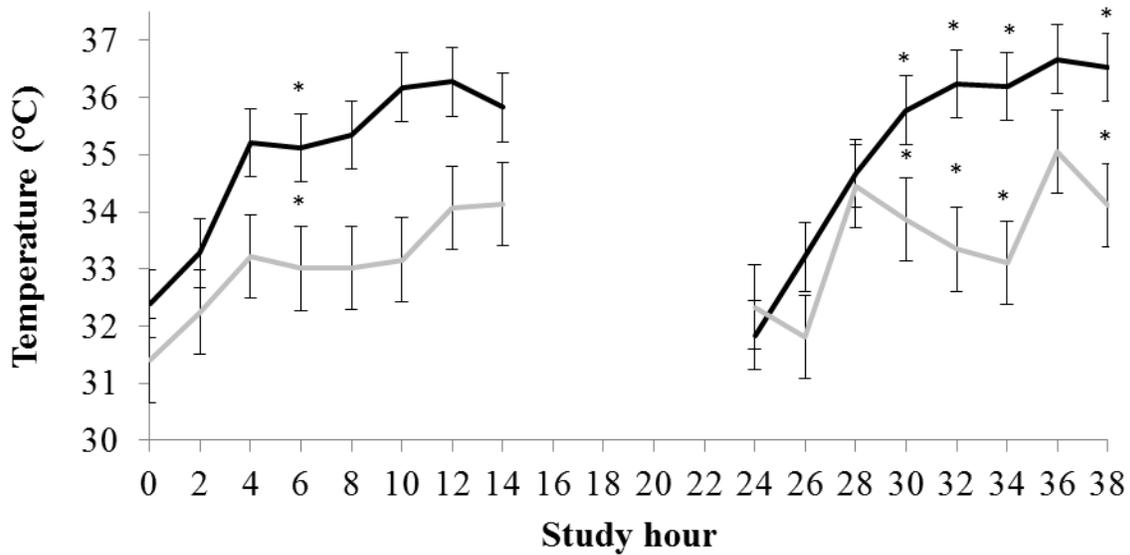


Figure 3.3 Rectal temperatures for calves in the high ambient temperature vaccinate group (HAT, n=10; solid black line), moderate ambient temperature vaccinate group (MAT, n=9; solid grey line), high ambient temperature control group (HAC, n=4; dashed black line), and moderate ambient temperature control group (MAC, n=4; dashed grey line) for the first 48 hours after vaccine administration (study hour 0, 8 am on study day 0).

Temperatures not measured between study hours 14 and 24. One calf in the MAT group received an abnormal CIS on study day 1 and temperatures from this calf were removed from the analysis.

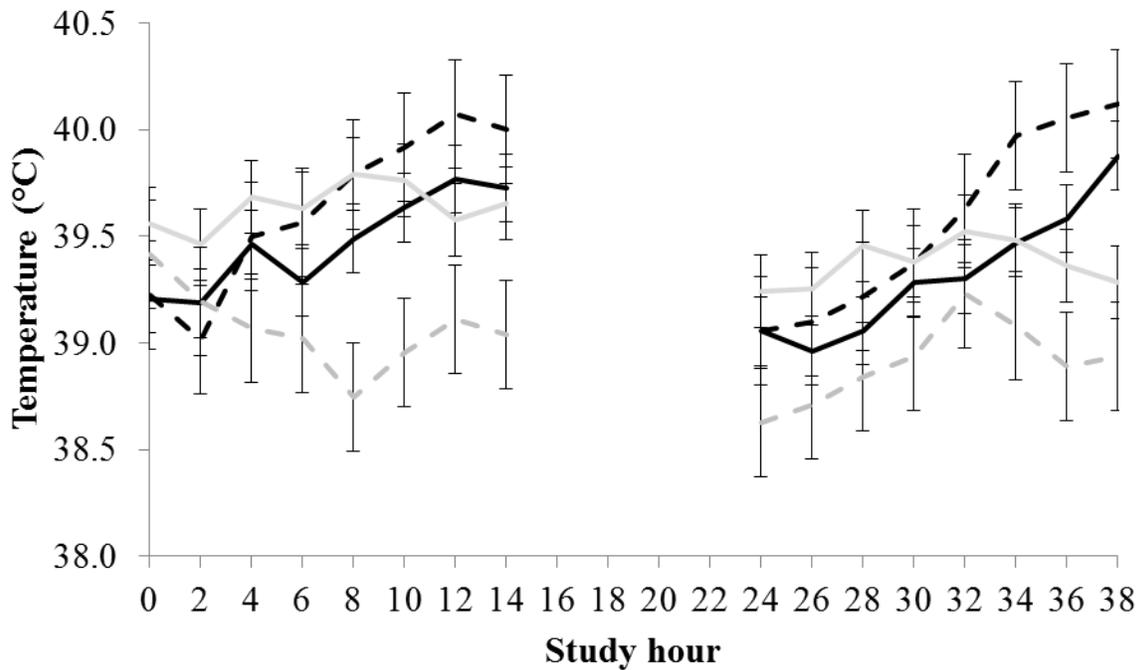


Figure 3.4 Model estimated TCID-50 results (and 95% confidence intervals) for IBR for calves in the high ambient temperature vaccinate group (HAT, n=10, black line) and moderate ambient temperature vaccinate group (MAT, n=9, grey line) for the first 7 trial days.

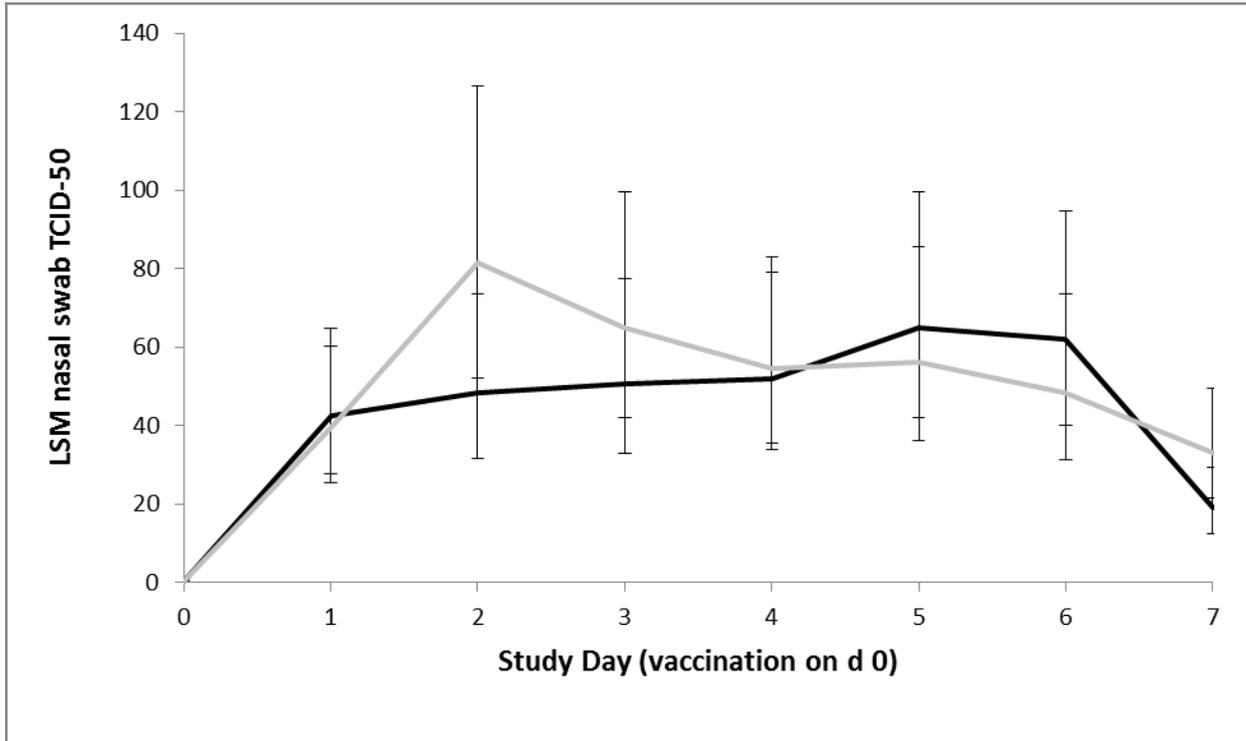


Figure 3.5 Model estimated antibody titers (and 95% confidence intervals) for IBR for calves in the high ambient temperature vaccinate group (HAT, n=10, black line) and moderate ambient temperature vaccinate group (MAT, n=9, grey line) for study days 0, 7, 14, 21, 28. HAC and MAC were not included because titers for IBR were 0 for entire trial.

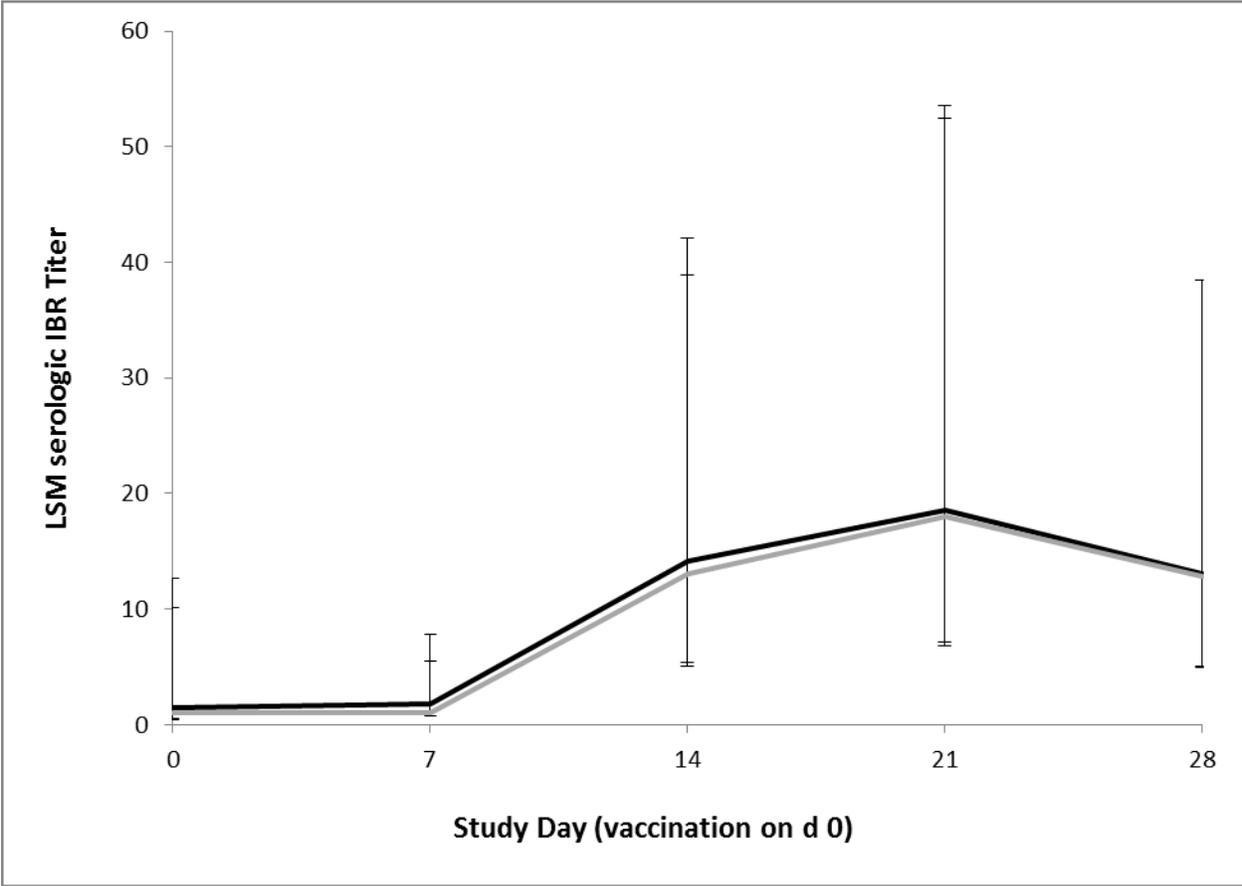


Table 3.1 Trial timeline of events

Study day^a	Procedures
-21	Arrival, identification tag, weights, metaphylaxis, clinical signs monitoring
-13	Nasal biothermal chip implantation
0	Baseline sample (nasopharyngeal swab, serum) collection for antibody titers and virus isolation, Administration of vaccine
0,1	Nasal and rectal temperature monitoring every 2 hrs (between 8 am and 8 pm)
1-7	Daily sample (nasopharyngeal swab) collections for virus isolation; twice daily rectal and nasal temperature monitoring
7, 14, 21, 28	Collect samples for antibody titers, individual weights, rectal and nasal mucosal temperatures

^aDay 0=Induction of trial

Chapter 4 - Thesis Conclusion

Bovine respiratory disease research remains extensive in evaluation of pathogenesis, treatments, and prevention. Knowledge of disease characteristics remains vital for implementing proper treatment and management of BRD, and prevention continues to be essential to reducing the morbidity and mortality from BRD.

Disease challenge models remain instrumental for evaluation of BRD pathogenesis. The structured literature table from the systemic review showed the substantial number of articles published about BRD alone. Clinically important points obtained from the systemic review include: shedding on average will resolve approximately 3 day prior to resolution of clinical signs, rectal temperature will return to normal limits 4 to 5 days prior to resolution of clinical signs, and seroconversion occurs between 10 to 21 days for the common BRD pathogens.

The second study showed the serologic and viral responses of calves administered an intranasal modified-live vaccine in high ambient temperatures compared to a controlled moderate ambient temperature. This most important finding was that serologic responses and viral vaccine replication were comparable between the treatment groups when outdoor ambient temperature exceeded 32.2°C. Clinically, these results indicate that vaccinating during times of extreme heat results in normal vaccine response with this intranasal vaccine.

Certainly, BRD continues to be a frustrating disease for researchers, producers, and veterinarians. Further research is needed to continue to understand the disease, prevention, and treatment. Review of current challenge models in the literature offers a substantial source of information for understanding the disease course and how it relates to other physiological

processes regarding clinical signs, shedding, and seroconversion. Further research and/or review of dual pathogen exposure may provide further resources for extrapolation of literature to clinical scenarios. Use of the intranasal vaccine in this study showed proper vaccine response in high ambient temperatures thus hopefully providing adequate protection for cattle.

Appendix A - Systemic Review Reference Tables

Tables

Table A.1 References reviewed for BVD challenge studies

Reference	Study description
Salt 2007 <i>The Veterinary Journal</i>	Random allocation of 2-5 month old male/female dairy and beef cross calves. Calves were confirmed seronegative to BVD. Calves were challenged intranasally with strain 11249. Calves were monitored for 14 days after challenge
Peters 2004 <i>Preventive Veterinary Medicine</i>	Randomized control trial of 5-6 month calves of beef and dairy calves of both sexes. Calves were blocked by BVD antibody titer prior to challenge and allocated to equal treatment groups. Calves were challenged intranasally with strain 11249.
Galav 2007 <i>Research in Veterinary Science</i>	Random allocation of 4 male Holstein Friesian crossbred calves 7-9 months of age. Calves were confirmed seronegative. Inoculated intranasally with strain IND S-1449 and monitored for 27 days.
Xue 2010 <i>Vaccine</i>	Unknown allocation of calves negative for BVD, BHV-1, and PI-3. Sixteen calves were challenged via aerosolization for BVD type 1 and 15 calves for BVD type 2. Calves were monitored for 14 days post-challenge.
Xue 2011 <i>Vaccine</i>	Unknown allocation of calves seronegative and persistently infected negative for BVD. Four different trials challenging calves with BVDV-1b intranasally with a 14 day monitoring post-challenge. Each trial included 12, 14, 14, and 10 calves respectively.

Kelling 2007 <i>American Journal of Veterinary Research</i>	Unknown allocation of 5 crossbred beef calves 5-7 months old. All calves were seronegative and virus isolation negative for BVD. All calves were challenged via intranasal aerosolization with BVD type 2 strain 890 and were monitored for 9 days post-challenge.
Ganheim 2003 <i>Journal of Veterinary Medicine</i>	Random allocation of 6 calves purchased from a BVD free herd. Calves were challenged intranasally with BVD type 1 and monitored for 23 days post-challenge.
Broderson 1998 <i>American Journal of Veterinary Research</i>	Unknown allocation of 5 mixed breed beef calves seronegative for BVD and BRSV. Calves were aerosolized with BVD and monitored for 9 days post-challenge.

Table A.2 References reviewed for IBR challenge studies

Reference	Study description
Salt 2007 <i>The Veterinary Journal</i>	Unknown allocation of male and female both dairy and beef crosses 5-9 months of age. Calves were seronegative to BHV-1. Challenge starting was ED3 and calves were monitored for 21 days post-challenge.
Peters 2004 <i>Preventive Veterinary Medicine</i>	Randomized clinical control trial utilizing 8 (beef and dairy) calves 5-6 months of age both sexes. Calves were confirmed seronegative for BHV-1. Strain ED3 was used for intranasal inoculation.
Xue 2010 <i>Vaccine</i>	Unknown allocation of calves negative for BVD, BHV-1, and PI-3. Fourteen calves were aerosolized challenged with BHV-1. Calves were monitored 14 days post challenge.
Meyer 2001 <i>Archives of Virology</i>	Calves 2.5-4 months of age dairy calves originating from BHV-1 and BHV-5 negative farms and serologically negative for BHV-1, BHV-5, and BVD. Allocation was unknown. Eight calves

	were intranasally challenged with BHV-1 and monitored for 17 days.
Castrucci 1998 <i>Comparative Immunology Microbiology and Infectious Diseases</i>	Unknown allocation of Friesian calves 2-3 months of age. Animals were allowed 1 week of acclimation prior to initiation of study. Three calves were intranasally challenged with BHV-1 and monitored for 30 days post-challenge.
Castrucci 1995 <i>Comparative Immunology Microbiology and Infectious Diseases</i>	Unknown allocation of 3 month old Friesian calves seronegative for BHV-1 neutralizing antibody. Four calves were intranasally challenged with BHV-1 and monitored for 30 days post-challenge.
Gilliam 1993 <i>Archives of Virology</i>	Unknown allocation of 4 week old Jersey calves. Calves were confirmed negative for BHV-1 via serum neutralization and virus isolation. Three calves were aerosolized with BHV-1 wild type strain, 3 calves were aerosolized with BHV-1 B1, and 4 calves with BHV-1 3A. Calves were monitored for 55 days after inoculation.

Table A.3 References reviewed for PI-3 challenge studies

Reference	Study description
Salt 2007 <i>The Veterinary Journal</i>	Random allocation of animals to treatment group. Calves (beef and dairy) were male and female between 3-4 months of age. Challenge was intranasal and intratracheal with strain J121. Monitored 14 days post-challenge.
Peters 2004 <i>Preventive Veterinary Medicine</i>	Randomized clinical trial utilizing 7-9 week old calves (beef and dairy) 7-9 weeks old both male and female. The 15 calves were confirmed seronegative. Strain J121 was used for intranasal and intratracheal inoculation.
Xue 2010 <i>Vaccine</i>	Random allocation of calves seronegative for BVD, BHV-1, and PI-3. Sixteen calves were challenged with PI-3 aerosolized and

monitored for 14 days post-challenge.

Table A.4 References reviewed for BRSV challenge studies

Reference	Study description
Salt 2007 <i>The Veterinary Journal</i>	Calves 2-4 months of age were randomly allocated. Both male and female dairy and beef crosses were utilized. Calves were inoculated with strain SNK both intranasally and intratracheally. Post-challenge monitoring was performed for 14 days.
Peters 2004 <i>Preventive Veterinary Medicine</i>	Randomized clinical trial with calves 6 months of age beef and dairy origin (male and female). The 10 calves were seronegative at initiation of trial. Calves were inoculated with strain 165 intranasally.
Xue 2010 <i>Vaccine</i>	Unknown allocation of calves seronegative for BVD, BHV-1, and PI-3. Seven calves were challenged with BRSV via aerosolization and monitored for 8 days after challenge.
Otto 1996 <i>Comparative Immunology Microbiology and Infectious Diseases</i>	Nine Friesian crossbred calves male and females were inoculated with BRSV via aerosolization using a face mask. Calves were monitored for 7 days and euthanized.
Elvander 1998 <i>Acta Veterinaria Scandinavica</i>	Unknown allocation of male calves of Swedish Red and White breed and Friesian 14-17 weeks of age. Calves were from a BVD, IBR, and bovine leukemia virus free herd and tested seronegative for BRSV antibodies. Calves were inoculate intranasally and intratracheally with strain SVA 187/92 and monitored for 21 days after challenge.
Gershwin 2000 <i>American Journal of Veterinary Research</i>	Twelve 6-8 week old Holstein bull calves with lymphatic cannulation were inoculated with BRSV strain CA-1 via aerosolization and face mask. Calves were monitored for 10 days post-challenge.
LeBlanc 1991 <i>American</i>	Six Holstein bull calves 4 days of age were seronegative for

<i>Journal of Veterinary Research</i>	BRSV, BVD, IBR, and PI-3. Calves were intranasally and intratracheally inoculated with BRSV for 4 consecutive days and monitored for 30 days post-challenge.
Ellis 2001 <i>Journal of the American Veterinary Medical Association</i>	Randomized treatment allocation of 9 week old dairy calves. Calves were seronegative to BRSV. Challenge was administered via aerosolization using a nebulizer and face mask. Calve were monitored 8 days post-challenge.
Vangeel 2007 <i>The Veterinary Journal</i>	Random allocation of 3 day old dairy calves blocked by BRSV antibody titers. Ten calves with pre-existing BRSV antibodies and 20 calves BRSV antibody negative were challenged via intranasal aerosolization using a nebulizer. Calves were monitored for 14 days post-challenge.
Woolums 1999 <i>American Journal of Veterinary Research</i>	Twelve week old Holstein male calves 14-16 weeks old were seronegative to BRSV or had very low titers and were blocked to their treatment groups based on the titer with equal distribution in each group. Twelve calves were challenged via aerosolizstion using a nebulizer and face mask. Calves were monitored until euthanasia on day 10.
Van der Poel 1996 <i>The Veterinary Quarterly</i>	Unknown treatment allocation. Six specific-pathogen-free calves were inoculated intranasally with BRSV. Calves were monitored for 16 days post-challenge.
West 2000 <i>Vaccine</i>	Random allocation of dairy calves 2-4 months of age. Nine calves were challenged via aerosolization using a face mask. Calves were monitored until euthanasia on day 8 post-challenge.
Ellis 2007 <i>Journal of the American Veterinary Medical Association</i>	Unknown treatment allocation of dairy calves. Nasal swabs were obtained prior to challenge for BRSV. Three trials were performed based on type of vaccine administration. Eight calves were in each trial group 2-9 weeks of age challenge via aerosolization in an enclosed room. Calves were monitored for 8

	days (euthanized on day 8).
Mawhinney 2005 <i>The Veterinary Record</i>	Three-week old Holstein crossbred calves were randomly allocated and blocked based on BRSV titers. Calves were challenged intratracheally and intranasally and monitored for 21 days post-challenge.
Ciszewski 1991 <i>Veterinary Microbiology</i>	Unknown treatment allocation of Holstein calves 2-6 days old. Six calves were intranasally and intratracheally inoculated with BRSV for 4 consecutive days and euthanized during maximum clinical response. Six calves were intranasally and intratracheally inoculated with BRSV for 4 consecutive days and maintained for 21 days after inoculation and then necropsied.

Table A.5 References reviewed for *Mannheimia haemolytica* challenge studies

Reference	Study description
Amrine 2013 <i>Journal of Animal Science</i>	Calves were randomly allocated to treatment groups. Three crossbred heifers were endobronchially challenged with <i>Mannheimia haemolytica</i> and monitored for 3 days prior to euthanasia.
Fajt 2004 <i>American Journal of Veterinary Research</i>	Eight 6-month old Angus-cross heifers were randomly allocated. Heifers did not receive vaccines against <i>Pasteurella</i> or <i>Mannheimia</i> organisms. Calves were intrabronchially challenged with <i>M. haemolytica</i> and monitored for 4 days after challenge.
Olchowy 2000 <i>American Journal of Veterinary Research</i>	Calves were randomly allocated to treatment groups. Twelve male Holstein calves were intratracheally inoculated with <i>M. haemolytica</i> and monitored for 15 days post-c challenge.
Hawson 2011 <i>American Journal of Veterinary Research</i>	Unknown allocation of crossbred beef heifers. Nineteen heifers were endobronchially inoculated with <i>M. haemolytica</i> and monitored for 84 days post-challenge.
Theurer 2013 <i>Journal of</i>	Black beef heifers weighing an average of 240 kg were blocked

<i>Animal Science</i>	by body weight and group participation from a previous transportation trial and then randomly allocated. Calves were observed to be free of clinical disease prior to challenge. Calves were endoscopically inoculated with <i>M. haemolytica</i> using bronchoselective endoscopy and monitored for 9 days post-challenge.
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Table A.6 References reviewed for *Mycoplasma bovis* challenge studies

Reference	Study description
White 2012 <i>American Journal of Veterinary Research</i>	Random allocation of male Holstein calves 5-9 weeks of age. Calves were confirmed negative for <i>M. bovis</i> via culture of serum and nasal swabs. Twenty calves were intranasally inoculated with <i>M. bovis</i> and monitored for 22 days.
Howard 1983 <i>Veterinary Microbiology</i>	Unknown allocation of gnotobiotic Friesian-cross, Aberdeen-Angus, or conventionally reared Ayrshire calves. Eight conventionally reared calves were intratracheally challenged with <i>M. bovis</i> and 8 gnotobiotic calves were intratracheally challenged with <i>M. bovis</i> . Calves were monitored for 28 days after challenge.
Dudek 2013 <i>Bulletin of the Veterinary Institute of Pulawy</i>	Unknown allocation of 5 week old Black and White breed calves. Calves were considered free of <i>M. bovis</i> based of screening culture and serology. Three trials of 18 calves were inoculated with <i>M. bovis</i> strains originating from a Polish cow with mastitis, an English calf with pneumonia, and an English calf's stomach contents. Calves were intratracheally inoculated and monitored for 28 days post-challenge.
Godinho 2005 <i>Veterinary Therapeutics</i>	Unknown allocation of male and female dairy or dairy-cross calves 3-9 weeks of age. Calves were confirmed negative via PCR testing of nasopharyngeal swabs. Eighteen calves were

intratracheally challenged with *M. bovis* strain 956 and 18 calves were intratracheally challenged with *M. bovis* strain 16150. Calves were monitored for 14 days post-challenge.

Table A.7 References reviewed for *Pasteurella multocida* challenge studies

Reference	Study description
Dowling 2002 <i>Research in Veterinary Science</i>	Randomly allocated Holstein cross-Friesian male calves 2 weeks of age. Calves were nasal swab and antibody negative for <i>P. multocida</i> . Calves were challenged in a 2X2 factorial design of 2 different concentrations of bacterial inoculum and 2 different volumes of inoculum. The 4 groups had 4 calves in each group challenged intratracheally and monitored for 4 days post-challenge.
