APPENDIX D
SAES-422
Multistate Research Activity
Accomplishments Report

Project Number: NC1192
Project Title: An integrated approach to control of bovine respiratory diseases (NC-1027)
Period Covered: January 1, 2017 – December 31, 2017
Date of This Report: November 13, 2018
Annual Meeting Date(s): September 12, 2018

Participants of Annual Meeting:

Chase, Christopher (Christopher.Chase@SDSTATE.EDU) – South Dakota State University; Dewell, Grant (gdewell@iastate.edu) – Iowa State University; Ollivett, Theresa (ollivett@wisc.edu) – University of Wisconsin – Madison; Palomares, Roberto (palomnr@uga.edu) – University of Georgia; Woolums, Amelia (amelia.woolums@msstate.edu) – Mississippi State University; George Smith (smithge7@msu.edu) – Michigan State University, NIMSS representative

Brief summary of minutes of annual meeting:

Annual meeting was held in conjunction with the AABP's Annual Conference in Phoenix, AZ. Station reports were delivered from those members in attendance: Wisconsin, Georgia, Mississippi State, Iowa, and South Dakota State University. Ollivett is current Chairperson and organized the meeting. Palomares is current Secretary.

In short, we discussed the annual reporting requirements, reviewed the objectives of NC 1192, discussed creating a document describing the roles of president and secretary, timeline for planning meeting, accumulating station reports, and creating/submitting the annual report. We also discussed member retention.

RP mentioned having formal protocols for initiating contacts between members and industry for outreach support, contacting local reps, mentioning that we are part of USDA supported NC1192. The group discussed creation of a catch-phrase or nickname in order to brand ourselves for marketing to help relate to non-members.

Discussed the variation between stations and the amount of support that is available to members (travel money, operating dollars, etc). Reminder that experiment station director should be contacted regarding funding available for participation in NC1192. Stations can spend funds using their discretion on multistate projects.

TO will send email to members to check on funding for nc1192 and their specific station. Individuals wishing to leave the project can work through their experiment station director to be formally removed, president can just exclude them from emails, or they can just delete the mass emails. TO to reach out to individuals that haven't submitted station reports. 7 stations sent reports in 2017 (list of participating stations are on NIMSS website).

When faculty retire/move remind them to designate or suggest new station rep or get added to new station program. TO to email with minutes, mention travel support, BRDS 2019 (3 hr business meeting, discuss new project, recruit people to work on), mention looking for nickname/logo to help with recruiting and branding.

GS: Officers include chairperson and secretary. Secretary is chairperson elect, but both are involved in meeting planning and generating the report. Administrator can volunteer assistant for helping get the meeting organized (room arrangements, pricing for block of rooms, etc) (Amy at UCDavis is helping organize BRDS).

RP will be in charge of organizing an abbreviated NC1192 annual meeting (1-2 hours to debrief after symposium) during the BRDS Aug 7-8 2018, at the Renaissance Hotel in Denver, CO. Should be scheduled after BRDS. Station reports won't be discussed during this particular meeting due to time constraints but reports will still need to be submitted for annual report. This brief meeting will really just serve as the Business meeting. Need to determine what time is best. Thursday night – AVC dinner/speaker.

Need to discuss pricing and registering for BRDS by January, discuss with Paula from AVC- rooms have already been discussed. Need to start sending out emails/save the date for BRDS no later than late this Fall.

Timeline for planning annual NC 1192 meeting:

- Chair solicits station reports for presentation at meeting 60 days before meeting with regular reminders; **make sure to denote collaborations in regards to publications. Make this information standout.** Will help in future when writing for the new project. Could use footnotes, for example, to highlight collaborations
- Chair collates the reports prior to meeting
- Plan date/location at annual meeting
- Chair finalizes report and submits within 60 days of annual meeting
- Submitting final report- President can either upload directly or send to George Smith to be uploaded.
- Send annual report to members prior to submission to NIMSS to ask for any additional input/edits; then once finalized, send final report to group.
- Need to get NIMSS authorization by contacting George Smith before telling group of meeting date/location- Authorization can go out anytime, important so participants that get travel need this authorization, **60-90 days before the annual meeting is typical**- email to George should contain two contacts for questions, and the details regarding the meeting location/dates, etc
- Should have meeting organized with the location prior to authorization

- Keep Fred Gingrich (AABP) and Paula (AVC) in the loop each year so they know if our meeting will be affiliated with AABP/AVC for the given year.
- AABP/AVC- coordinates the projector/screen/ room location, shouldn't expect to pay for these services
- Would need to work with hotel to coordinate wifi, beverage and food service (and costs) if desired, can get outside funding for this, invite outside sponsor to lunch (not to the whole meeting, historically).
- Fee to attend covers and food/bev/wifi services if desired.
- Members can invite non-members to attend, benefit of having non-NIMSS list of emails
- **TO DO:** generate list of member and non-member emails

TO: presented UW station report.

GD: presented Iowa report on acclimation studies (establish herd behavior, walk through processing areas, 3 days (2 before processing and 1 day after processing) out of first 5 days of arrival) – 6400 calves, seeing improvements in gain at 180-200 days, .05 lb differences in gain (roughly 20lbs of improved performance), some differences in serum cortisol; also looking at compounds in nasal secretions collected by tissues and breath condensate and also did an AMR study looking at danofloxacin and enrofloxacin; TO offered to provide info on our EBC collection tube from the Mannheimia challenge.

RP: presented UGA report

AW: presented MS report, RNAseq on high risk calves, spraying mRNA on mucosal surfaces leads to antibody production and less disease

CC: presented SDSU report; influenza D up to 80% seropositive, tracheitis (neutrophilic), not much clinical relevance, bvdv 1b increasing in prevalence, 2+2 program with UM (vet school at SDSU in combo with MS, 20 students in 2021). BHV4; hemotropic mycoplasma (M. wenyonii) causing acute onset hot joints in fresh heifers, PCR done at MS and NCSU joint fluid

Officers for upcoming year:
Secretary: Ollivett
Chair: Palomares

BRDS Discussion – Terry L in Food Educators Symposium. Discussion limited, will have teleconference to more fully discuss BRDS. Grant and sponsorship was discussed briefly regarding how to handle checks- discuss with Terry L more fully. Per CC, Dawn is willing to handle account/checks etc. Need to get website set up. Lallamand, multimin, zinpro willing to sponsor. BI (craig jones), bayer (Jim Sears) and merck (Brit Meyer) have been consistent sponsors. Zoetis (roger saltmann). Diamond V hasn't been contacted yet. Angie Rousen (potential philbro contact). IDEXX (Jim Rhodes, not contacted yet), Elanco (Gerry Mechor, Mark Hilton); Arm and Hammer (Neil Michaels); LOL (Tom Earlywine); Program is fairly set – TO to email TL to review our discussion.

Group really appreciates George's involvement and contributions to today's Annual NC1192 meeting.

Member activities focus on the project's 5 objectives and are outlined below.

Objective 1:
To elucidate pathways by which host characteristics, pathogen virulence mechanisms, and environmental impacts interact to produce BRD, and to develop strategies to mitigate detrimental factors and enhance protective mechanisms.

Accomplishments – objective 1

At the UW-Madison, the Czuprynski laboratory explored the ability of bacterial BRD pathogens to attach bovine epithelial cells and form a biofilm, in an effort to gain insights into mechanisms by which these organisms colonize the upper respiratory tract in healthy cattle. A previously developed cell culture method was used to assess biofilm formation by *M. haemolytica* and *P. multocida* on bovine respiratory epithelial cells. Key findings included:

- *M. haemolytica* and *P. multocida* mutually antagonize biofilm formation by each other
- Inhibition of *M. haemolytica* biofilm formation required viable *P. multocida* cells, did not occur when *M. haemolytica* and *P. multocida* cells were separated in a Transwell chamber, and was not reproduced by *P. multocida* conditioned medium
- Results suggest that *M. haemolytica and P. multocida* cells must be in close proximity for inhibition to occur

The Czuprynski laboratory also investigated the interactions among endothelial cells and neutrophils (PMNs) in response to *Histophilus somni*, a Gram negative pathogen that causes respiratory, reproductive and central nervous system disease in cattle. The hallmark of *H. somni* infection is diffuse vasculitis and intravascular thrombosis that can lead to an acute central nervous system disease known as thrombotic meningoencephalitis (TME). Because neutrophils are major players in the pathophysiology of septic meningitis, their role in *H. somni*-induced fibrin clot formation was explored *in vitro*. Bovine brain endothelial cells (TBBE cells) were exposed to *H. somni* and conditioned media (CM) collected. Key findings included:

- Greater tissue factor activity in cell lysates and CM from *H. somni*-stimulated TBBE cells than unstimulated control TBBE cells
- PMNs exposed to CM, or extracellular vesicles released by *H. somni*-stimulated TBBE cells, expressed von Willenbrand factor, exhibited increased fibrin clot formation, and displayed greater tissue factor activity than PMNs exposed to CM or extracellular vesicles from unstimulated control TBBE cells

- Results suggest that during *H. somni* infection bovine PMNs might acquire extracellular vesicles from endothelial cells that could contribute to the thrombus formation in bovine brain microvasculature that characterizes thrombotic meningoencephalitis

The Czuprynski laboratory also investigated the interactions between bovine macrophages and *H. somni in vitro* to determine whether macrophages release extracellular vesicles that activate the clotting cascade in a manner that could lead to thrombus formation, a hallmark of *H. somni* infection. Bovine monocyte-derived macrophages were incubated with *H. somni* and conditioned media collected. Membrane-shed extracellular vesicles were isolated from the conditioned media, washed twice with Ca^{2+} and Mg^{2+} free HBSS, and pro-coagulant activity assessed by a one-step plasma clotting assay. Key findings included:

- Greater pro-coagulant activity was observed for extracellular vesicles from *H. somni* stimulated macrophages than from unstimulated control macrophages
- Pro-coagulant activity was inhibited by addition of an anti-tissue factor antibody
- Co-localization of fluorescein-labeled *H. somni* cells and annexin V staining of extracellular vesicles was observed using confocal microscopy
- Results demonstrate that exposure to *H. somni* cells causes bovine monocyte-derived macrophages to release extracellular vesicles that contain tissue factor, the first such report for bovine macrophages. It is possible that if similar events occur in vivo they could amplify thrombus formation in bovine histophilosis

In collaboration with Drs. Suen and Ollivett, the Czuprynski lab is working to define the upper respiratory tract microbiome in cattle, and to define the microbiome of bedding sand used for dairy cattle.

From the SDSU, an in vitro study was performed to determine the direct effects of BVDV and BHV-1 infections of monocyte-derived dendritic cells (MDDC) on cytokine mRNA expression. For both viruses, a high virulent strain (1373 for BVDV and Cooper for BHV-1) and a lower virulent strain (20508 for BVDV and Los Angeles for BHV-1) was used and ten cytokines were measured: interferon-alpha (IFN-alpha), interferon-beta (IFN-beta), interferon-gamma (IFN-gamma), interleukin-1a (IL-1a), interleukin-1b (IL-1b), interleukin-6 (IL-6), interleukin-8 (IL-8), interleukin-10 (IL-10), interleukin-12 (IL-12), and tumor necrosis factor (TNF). Key findings included:

- BVDV infection down regulated all cytokine mRNA compared to the control
    - IL-1b had the greatest down regulation with more than a 40-fold decrease
    - The three IFN cytokines had less than a 2-fold decrease
    - The other IL cytokines had less than 10-fold decreases
- BHV-1 infection altered regulation of cytokines in multiple ways
    - Five cytokines were down regulated
    - The IFN family were up regulated
        - IFN-alpha had greater than a 10-fold increase
        - IFN-beta had greater than a 3-fold increase
        - IFN-gamma had less than a 1-fold increase
    - The other 5 IL cytokines were down regulated with less than a 4-fold decrease
- For both BVDV and BHV-1
    - IFN-alpha and IL-8 showed statistical significance between the two strains

        - BVDV 1373 down regulated both IFN-Alpha and IL-8 more than 28508
        - BHV-1 Cooper strain up regulated IFN-Alpha and down regulated IL-8 as compared to the Los Angeles strain
- Breed differences were noted:
    - For BVDV, Brown Swiss calves had higher MDDC yields than Holstein Friesian calves after MDDC preparation
    - Cytokine mRNA expression had a greater up regulation and less down regulation in Holstein Friesian calves
    - BHV-1 infection of MDDC resulted in decreased MHCI, MHCII, and CD86 surface marker expression

In a second line of research at SDSU, using neutrophils isolated from dairy heifers, viability, apoptosis, expression of the surface markers CD14, CD18 and L-selectin and NET formation were measured after neutrophil infection with several strains of BVDV. Key findings included:

- Only highly virulent strains, such as BVDV 1373 affected neutrophil viability and apoptosis, while moderate virulent ncp strains, such as BVDV 28508, do not significantly affect neutrophil viability
- BVDV 1373 and 28508 do not affect CD18 and neutrophil function and migration, while BVDV 1373 increases CD14 expression, thus increasing the bacterial recognition and phagocytosis of bacteria
- In regard to NET formation, all BVDV strains, regardless of the biotypes and genotype down-regulate NET capacity of bovine neutrophils.

At Mississippi State, whole blood transcriptome and differential gene expression is being evaluated in high risk stocker cattle. More specifically, researchers are beginning to develop a reference whole blood transcriptome from 12 beef stocker cattle sampled on the day of arrival. Six of these cattle were treated for BRD over the following 84 days while the other 6 animals were never treated for BRD. Comparing these healthy and BRD-affected cattle, researchers will look for differentially expressed genes to search for one or more biomarkers that may be useful to develop targeted management practices that may improve health of high risk stocker cattle while decreasing use of antimicrobials.

Impacts – objective 1

- Furthered our understanding of how common bacterial and viral respiratory pathogens interact with each other and with the host cells to enhance propagation of disease. Manipulating these mechanisms may enhance resilience of susceptible cattle.

Objective 2:

To develop and validate methodologies for accurate BRD diagnosis, objective risk assessment, and surveillance to detect new trends in BRD occurrence.

Accomplishments – objective 2

The Wisconsin Veterinary Diagnostic Laboratory (WVDL) evaluated 1,432 samples for respiratory viruses and bacteria using **molecular diagnostics** for bovine respiratory syncytial virus, respiratory corona virus, viral diarrhea virus, herpes virus, *Mycoplasma bovis*, *Mannheimia haemolytica, Pasteurella multocida* and *Histophilus somni*. The majority of samples are collected from dairy cattle within Wisconsin.

- The greatest percentage of rtPCR positive samples were:
    - *P. multocida* (30%)
    - *M. bovis* (24%)
    - *B*ovine respiratory corona virus (24%)
    - *M. haemolytica* (22%)
    - *H. somni* (17.8%)
- *M. bovis* positive samples decreased 5.8% since the previous year
- Bovine respiratory corona virus increased 4.1% since the previous year

The WVDL also performed culture for bacteria organisms although WVDL data has demonstrated that rtPCR is more sensitive than culture. Most clients submit a swab in viral transport media for *M. bovis* PCR; therefore, we did not include the culture rates for *M. bovis*. Bacteriology evaluated 1727 samples, the majority of which were collected from dairy cattle within Wisconsin.

- The greatest percentage of positive cultures were:
    - *Pasteurella multocida* (24%)
    - *Mannheimia haemolytica* (15%).

Additionally, the WVDL performs susceptibility testing using minimum inhibitor concentrations on all samples. Therefore, this data is not exclusive to only respiratory samples.

- Susceptibility of *B. trehalosi* to neomycin decreased 13% as compared to 2016
- Susceptibility of *P. multocida* to gamithromycin decreased 8% as compared to 2016
- Susceptibility of *M. haemolytica* to ampicillin, penicillin, tilmicosin, tulathromycin, and chlortetracycline decreased 3%, 13%, 12%, 11%, and 8%, respectively compared to 2016
- Susceptibility of *H. somni* to tilmicosin and tulathromycin decreased 105 and 4%, respectively compared to 2016.
- Susceptibility of *E. coli* to sulphadimethoxine decreased 3% compared to 2016
- Susceptibility of *Salmonella* species to chlorotetracycline and oxytetracycline decreased 2% and 1%, respectively, compared to 2016.

At SDSU, the infectious agents associated with bovine respiratory disease complex were monitored by bacterial culture, virus isolation, and fluorescent antibody techniques. Bacterial agents isolated from bovine pneumonic lungs, tracheal swabs, and nasal swabs were as follows for July 1, 2017 – June 30, 2018:

- M. haemolytica (n = 265)
- P. multocida (n = 246)
- H. somnus (n = 229)

- B. trehalosi (n = 29)

Viral agents from **bovine pneumonic lungs** are as follows for July 1, 2017 – June 30, 2018. This data was not available yet at the time of the writing the report isolations:

- BVDV (n = 15; 7 NCP and 8 CP)
- BHV-1 (n = 6)
- BHV-4 (n = 15)
- PI3 (n = 4)

Individual ELISA ear notch BVDV tests were run on 5889 samples and 63 were positive (1%).

PCR tests were done for BVDV, BHV-1, BCV, BRSV and Mycoplasma.

- Pooled ear notch was done on 2150 submissions with 152 positive samples, 7% case positive.
- BVDV PCR was also done on nasal swabs, tissue, whole blood/serum /milk samples (478 submissions 32 positives, 7% positive).
- BHV-1 PCR was also done on nasal swabs, tissue, whole blood/serum (257 submissions 25 positive, 10%).
- BCV PCR was also done on nasal swabs and tissue (530 submissions 78 positive, 15%).
- BRSV PCR was also done on nasal swabs and tissue (585 submissions 122 positive 21%).
- Mycoplasma bovis PCR positive samples had 304 with 198 positive, 65%).

Characterization of BHV-1 Field isolates resulted in 8 reproductive isolates between July 1, 2017 – June 30, 2018 indicating that they are all vaccine strains. Three respiratory isolates were also sequenced, of which 2 were vaccine isolates.

BVDV typing for 2018 resulted in 1a (n = 10/38), 1b (26/ 38), and 2a (2/38) positive tests compared which was increased for each type compared to 2017 (1a, n = 0; 1b, n = 10; 2a, n = 0).

At Mississippi State, researchers assessed the diversity of *Mannheimia haemolytica* genotypes and phenotypes on bacterial culture plates from bovine nasopharyngeal swabs in order to determine how many colonies should be selected from the plate in order to have a high confidence that all relevant genotypes and phenotypes have been selected. Researchers have also begun a project to determine the number of different antimicrobial resistance phenotypes that can be identified on a blood agar plate.

- At least 1 colony consistent with *M. haemolytica* was grown from 13/20 animals sampled; 437 isolates were banked.
    - These isolates will be tested to confirm identity and to characterize the antibiogram for all antimicrobials with CLSI defined breakpoints.
    - The number of unique antibiograms on each plate will be used in the program developed by Döpfer et al. to estimate the number of colonies that need to be

selected from NPS culture plates provide 95% certainty that we have identified all the antibiograms (i.e. phenotypes) on the NPS. The program of Döpfer et al. uses Bayesian statistical inference in a WinBUGS code to calculate the number of isolates that need to be selected. The program requires an estimate of the number of different phenotypes likely to be identified ("prior probabilities"); the data collected in the work proposed here will provide the prior probabilities required by the program. Although funding awarded for this project was not adequate to support genotyping the isolates, we are actively looking for support to also genotype all the isolates by PFGE. The number of unique genotypes may not be the same as the number of unique phenotypes; we hope to assess this in the near future.

Researchers in UCCE, VMTRC at UCD SVM developed a clinical scoring system to detect BRD in preweaned dairy calves. The system uses 6 clinical signs, each categorized as normal or abnormal: nasal discharge, eye discharge, cough, fever, breathing difficulty/rate, and ear & head position. The simple dichotomy of clinical signs is pivotal to its reliability and robust results between different users of different experience levels. The sum of points from all 6 signs is used to classify the calf as healthy or BRD positive (≥ 5 points). More than 500 calves from three dairies and two calf ranches were evaluated for BRDC and scored. The new system correctly identified 72.2% of calves with BRD and 89.9% of healthy calves. The scoring system is also available as a free mobile application that can be used to calculate and track BRD prevalence.

The UCD researchers then visited 100 dairies to survey management practices and estimate BRD prevalence in over 4,000 calves using the newly developed scoring system. These management practices were analyzed to determine their association with BRD prevalence on different dairies. With this information in hand, the team followed over 10,000 calves on six different dairies from birth to weaning, keeping careful records on cases of BRD and associated management practices. Available in both paper and mobile application forms, the scoring system can be used to evaluate a herd's BRD status and the effectiveness of recommended management practices. The mobile application allows for real time prevalence calculations and stores data in an electronic format monitor herd progress over time. With this data, the team has identified the primary risk factors for BRD in young dairy calves and has created a tool for dairy farmers.

Impacts – objective 2

- *Pasteurella multocida* and *Mannheimia haemolytica* continue to be the predominant pathogens isolated from respiratory samples submitted to 2 different diagnostic laboratories.
- Data has been collected to improve our understanding of how individual bacterial colonies, and the methods by which we sample from blood agar plates, impact antibiogram results.
- A risk assessment tool has been created to help dairy producers manage BRD

Objective 3:

To develop and validate management practices and responsibly applied therapeutic and preventative interventions, such as vaccines, antimicrobials, and immunomodulators, to minimize the impact of BRD on cattle, producers, and society

Accomplishments – objective 3

At Mississippi State, over the past 3 years, researchers investigated the impact of on-arrival vaccination of high risk stocker cattle with modified-live 5-way viral and multivalent clostridial vaccines, and/or deworming, on BRD morbidity, mortality, seroconversion to BHV-1 and BVDV1, and weight gain over an 84-day backgrounding period. Trials were run in 2015, 2017, and 2018. Preliminary analysis of the results of all 3 trials has been completed.

- On-arrival vaccination tended to increase the odds of BRD treatment over the next 84 days
- On-arrival deworming tended to increase weight gain over the next 84 days
- Cattle vaccinated on arrival seroconverted to BHV-1 and BVDV1 in spite of their tendency for being at higher odds for BRD treatment.

Researchers at MS also evaluated whether or not the stress of transportation has an impact on immunity. In the summer of 2017, researchers conducted a study to assess the impact of a 9-hour episode of transportation on the immune response to intranasal vaccination with a modified live BHV-1/BRSV/PI3V vaccine and SC MLV BVDV vaccine. Three groups of 25 cattle received either 1) transportation for 6 hours, 2 hours rest, then transportation for 3 hours, followed by vaccination 12 hours later; 2) transportation but no vaccination; or 3) no transportation but vaccination. Serum neutralizing antibody titers to BHV-1 and BRSV, concentrations of IgA directed against BHV-1 and BRSV in nasal secretions, and concentrations of interferon alpha, beta, and gamma in nasal sections from samples collected at multiple time points over 21 days after vaccination have been measured.

- Analysis currently underway

To facilitate efficient treatment and minimize stress related to gathering cattle following daily assessment of cattle for BRD, many producers use remote delivery devices (RDD, dart guns) to administer antimicrobials. However, little research has tested the plasma concentrations of drug following administration of common antimicrobials by RDD. MS researchers assessed the plasma concentrations of tulathromycin in cattle following administration by CO2-powered dart gun, air pump dart gun, or needle injection, in a 3-way crossover study.

- Three darts administered by the air pump RDD did not discharge.
- Excluding the 3 cases where darts did not discharge, we found that maximum plasma concentration or the area under the concentration time curve were similar and not significantly different between methods of administration

- Results suggest that PK of tulathromycin following RDD are similar to subcutaneous injection; however failure of RDD darts to discharge delivered by air pump dart gun can cause a proportion of cattle to fail to receive drug as expected.

Researchers at UGA completed a study to compare the immune response to subcutaneous versus intranasal booster vaccination in young beef calves that were prime vaccinated with IN vaccine early in life.

- Booster vaccination 60 days after priming (at 3-4 weeks of age) did not induce a significant increase in SNA titers against BHV1, BRSV and PI3V in beef calves that received a primary IN MLV vaccine in the face of MA.
- A sustained and gradual increase in BHV1-specific IgA titers in nasal secretions was observed in both (IN and SC) groups after both priming and booster vaccinations.
- Calves receiving IN vaccination tended to have higher ($P=0.09$) BHV-1 IgA titers on day 14 post booster vaccination.

In collaboration with Dr. Amelia Woolums from Mississippi State University, researchers also compared the BRSV-specific IgA concentration in nasal secretions.

- There was a comparable induction of BRSV-specific IgA in both groups during the first 2 weeks after booster vaccination
- There was a significantly higher concentration of IgA on days 21 ($P=0.03$) and 42 ($P<0.01$) in calves receiving SC vaccination compared to calves in the IN group
- These results of IgA concentration in nasal secretions might suggest differences between BHV-1 and BRSV pathogenesis and the immune responses induced by IN versus SC vaccination

Gene expression analysis of proinflammatory (TNF-a, IL1B) Th1 (IFN-a, IL2) and Th2 (TGFb, IL10) cytokines on days 0, 14, 21, 28 and 42 was also performed.

- Results revealed comparable mRNA transcription in both groups
- In conclusion, both routes of booster vaccination stimulated similar systemic and mucosal antibody response in beef calves intranasally prime vaccinated during the first month of life
- Some virus-associated differences in the IgA response were observed between vaccination groups

UGA researchers performed a similar study in dairy calves to compare the immune response to SC and IN booster vaccination in dairy calves that were prime vaccinated with IN vaccine at one month of age.

- Similar to beef calves, booster vaccination did not induce a significant antibody response against BHV1, BRSV and PI3V in dairy calves in the face of maternal antibodies
- BRSV antibody titers were significantly higher ($P=0.01$) on day 42 after booster in the IN calves compared to SC calves

- Similar to the results of our previous study in beef calves, dairy calves receiving IN booster vaccination had higher (P=0.04) BHV-1 IgA titers on day 21 post booster vaccination, and this values also tended to be greater on day 35 compared to the SC group calves

The effect of injectable trace minerals (ITM) and the impact of route of concurrently administered MLV booster vaccination (IN vs. SC) on the systemic and mucosal immune response was investigated before and after experimental infection with BVDV-2 and BHV1 infection in dairy calves primed with IN vaccine. All calves were inoculated IN with BVDV2 strain 890 ($5x10^5$ $CCID_{50}$) on day 49 after booster and with BHV1 ($8x10^6$ $CCID_{50}$) one week later (day 56 after booster). Endoscopic evaluation of the upper respiratory tract (nasal cavity, pharynx, larynx, trachea, and bronchi) was performed at multiple time points before and after challenge.

- Booster vaccination 10 weeks after intranasal priming did not induce a significant increase in SNA titers against BHV1, BRSV and PI3V

- For calves that received IN booster vaccination
    - ITM administration resulted in similar antibody titers than those for the saline-injected calves.
    - A sustained and gradual increase in BHV1-specific IgA titers in nasal secretions was observed in both groups after IN booster vaccinations.
    - Calves receiving ITM tended to have higher (P=0.1) BHV-1 IgA titers on day 35 post booster vaccination than the saline-treated

- For calves that received SC booster vaccination
    - treatment with ITM tended to induce higher (P=0.1) SNA titers against BHV1 on day 21 post booster compared to saline treated-calves
    - No significant differences were observed for BHV-1 IgA titers in nasal secretions or BRSV and PI3V SNA titers
    - Additionally, we will be performing a cytokine gene expression analysis as a reference for the cell mediated immunity after booster vaccination

- Unvaccinated calves had significantly higher clinical scores post challenge than vaccinated calves on days 6, 10 and 12.
- Clinically relevant differences in the health score were not observed between calves that were vaccinated by SC or IN route and were treated with ITM or not.
- Overall, unvaccinated calves had the highest endoscopic respiratory scores after BVDV+BHV1 intranasal inoculation, which was significantly greater than the values for ITM ($P< 0.01$) and Saline ($P <0.05$) groups.
- Severe hyperemia with abundant purulent secretions were observed in the upper respiratory tract of unvaccinated calves.
    - Treatment with ITM at the time of booster vaccination had a significant effect reducing the severity of lesions caused by BVDV-BHV1 in the upper respiratory tract, when compared to vaccinated saline-injected calves ($P< 0.01$).

- Leukopenia occurred between d3 and d10 in the unvaccinated calves, but not in vaccinated calves (treated with ITM or not)

- A significant reduction in $CD4^+$ T cells was observed in unvaccinated calves between d3 and d12 ($P<0.05$). Further, $CD8^+$ T cells were significantly reduced in the unvaccinated calves between d3 and d10.

- <u>In calves receiving IN booster vaccination</u>
    - CD4 T cell population was not different between calves receiving ITM or saline. Further, ITM calves had consistently higher $CD4^+$ T cell values on days 3 (P=0.04), 6 (P<0.001) and 14 (P=0.06) compared to unvaccinated calves.
    - A less pronounced decay in $CD8^+$ T cells was observed in IN vaccinated calves after d0.
        - On d6, IN vaccinated calves had a rebound on CD8 T cell population, with a significantly higher cell number observed in ITM calves than saline treated calves (P=0.04).
        - Accordingly, ITM-treated calves had consistently greater values of CD8 T cells than unvaccinated calves on d3 (P=0.05), d6 (P<0.001) and d7 (P=0.04).

- <u>In calves receiving SC booster vaccination</u>
    - $CD4^+$ T cell number was higher on day 3 after BVDV challenge in calves treated with ITM compared to the saline-treated group.
    - The number of $CD8^+$ T cells was significantly lower (P=0.01) on day 7 after BVDV inoculation for ITM group compared with the saline group.

- $WC1^+$ T cells significantly dropped in all groups on d3-d6, with a notable recovery in vaccinated calves after d6.
- Circulating $CD25^+$ T cells decreased after BVDV infection with comparable pattern among groups.
- Administration of ITM concurrent with IN vaccination consistently mitigated the reduction in circulating $CD4^+$ and $CD8^+$ T cells observed in dairy calves after BVDV + BHV1 infection.

Impacts – objective 3

- On-arrival vaccination of high risk stocker cattle can be associated with BRD treatment
- By the time high risk cattle arrive, vaccination may be too late to help prevent disease
- Remote delivery devices have the potential to deliver antibiotics in an efficient manner, however, failure of darts to discharge when delivered by air pump dart gun can cause a proportion of cattle to fail to receive drug
- Information regarding the measurement of circulating T cell phenotype during acute BVDV and BHV-1 infections to assess differential CMI responses between vaccinated and naive calves, was obtained and may serve as a tool to evaluate management practices relative to vaccination.

- Endoscopic evaluation of the upper respiratory tract in calves inoculated with BVDV-2 and BHV1 was performed successfully and offered an objective and effective tool to determine the severity of the inflammatory lesions caused by virus infection.
- More evidence about comparable systemic and mucosal immune response induced by IN or SC booster vaccination in dairy and beef calves that received an IN prime vaccination early in life (1 month of age) was obtained
- New information regarding the impact of trace mineral supplementation at the time of vaccination was gain and could help veterinarians and producers improve the ability of cattle to resist respiratory infection.
- Gained new knowledge regarding the interaction between common pathogens of cattle (BVDV and BHV-1) and T lymphocytes, a population of immune cells that have special importance in the bovine immune response.
- Administration of ITM concurrent with IN booster vaccination mitigated the reduction in CD4 and CD8 T lymphocytes caused by BVDV + BHV1 infection and reduced the severity of inflammatory lesions (evidenced by endoscopy) of the upper respiratory tract, without showing clinically relevant effects on the general health status.
- Administration of ITM concurrent with SC booster vaccination also mitigated the reduction in CD4. Subcutaneous booster vaccination concurrent with ITM or not mitigated the decay in CD8 T lymphocytes caused by BVDV + BHV1 infection.

Objective 4:

To determine how attributes of cattle production systems including epidemiologic, societal, and economic forces contribute to BRD, and to develop ways to catalyze change in those systems to reduce the occurrence of BRD and improve cattle health, welfare, and productivity.

Accomplishments – objective 4: NA

Objective 5:

To promote dialogue and exchange among scientists, veterinarians, allied industry professionals and cattle producers to advance BRD research initiatives, to implement outreach, to disseminate research results, and to facilitate the translation of research findings to practical field applications.

Accomplishments – objective 5

At SDSU, members have been active in preparing for a 2019 BRD symposium to be held in August 2019.

At MS, annual continuing education meeting for veterinarians engaged in stocker cattle practice: In December 2017 researchers at MS have organized an annual 2-day conference for veterinarians engaged in stocker cattle practice. Planning for 2019 BRD Symposium: Researchers at MS are participating in organizing the 3rd BRD Symposium which will be presented by NC1192 in August 2019. The Symposium will be held in Denver CO in conjunction with the Summer Academy of Veterinary Consultants (AVC) meeting.

Impacts – objective 5

- The BRD Symposium will be held in Denver, CO August 2019

Journal Publications:

Bittar JHJ, Hoyos-Jaramillo A, Hurley DJ, Woolums AR, Havenga LJ, Lourenço JM, Barnett G, Gomes V, Saliki JT, Harmon DD, **Palomares RA**. Effects of injectable trace minerals administered concurrently with a modified live virus vaccine on long-term protection against bovine viral diarrhea virus acute infection in dairy calves. Res Vet Sci. 2018 119:250-258.

Woolums AR, Karisch BB, Parish JA, Park J, Seo KS, Badial P, Olsen SC. Effect of a DNA-based immunostimulant on growth, performance, and expression of inflammatory and immune mediators in beef calves abruptly weaned and introduced to a complete ration. In review.

Woolums AR, Karisch BB, Frye JG, Epperson W, Smith DR, Blanton J, Austin F, Kaplan R, Hiott L, Woodley T, Gupta S, Jackson CR, McClelland M. Multidrug resistant *Mannheimia haemolytica* isolated from high-risk beef stocker cattle after antimicrobial metaphylaxis and treatment for bovine respiratory disease. Vet Micro 2018, 221:143-152.

Griffin CM, Scott JA, Karisch BB, Woolums AR, Blanton Jr. JR, Kaplan RM, Eppereson WE, Smith DR. A randomized controlled trial to test the effect of on-arrival vaccination and deworming on stocker cattle health and growth performance. Bov Pract 2018, 52:26-33.

Woolums AR**,** Berghaus RD, Smith DR, Daly RF, Stokka GL, White BJ, Avra T, Daniel AT, Jenerette M. A case-control study to determine herd-level risk factors for nursing calf bovine respiratory disease (BRD) on cow-calf operations. J Am Vet Med Assoc 2018, 252:989-994.

5. Scientific and outreach oral presentations:

Pharmacokinetics of tulathromycin following administration with remote delivery devices
A.R. Woolums, J. D. Rivera, S. Giguère, J. T. Johnson, A. G. Lutz, P.N. Tipton, W. Crosby, I. Hice, M. Thoresen. Academy of Veterinary Consultants Summer Meeting, Denver CO. Aug 9-11, 2018.

Pharmacokinetics of tulathromycin following administration with remote delivery devices

A.R. Woolums, J. D. Rivera, S. Giguère, J. T. Johnson, A. G. Lutz, P.N. Tipton, W. Crosby, I. Hice, M. Thoresen. American Association of Bovine Practitioners Annual Convention, Phoenix AZ, Sept. 12 – 15, 2018.

Doyle, D; Credille, B; Lehenbauer, TW; Berghaus, R; Aly, SS; Champagne, J; Blanchard, P; Crossley, B; Berghaus, L; Cochran, S. Agreement Among 4 Sampling Methods to Identify Respiratory Pathogens in Dairy Calves with Acute Bovine Respiratory Disease. Journal of Veterinary Internal Medicine 31,3, 954-959. 2017.

Owen, Joseph R; Noyes, Noelle; Young, Amy E; Prince, Daniel J; Blanchard, Patricia C; Lehenbauer, Terry W; Aly, Sharif S; Davis, Jessica H; O'Rourke, Sean M; Abdo, Zaid. Whole-Genome Sequencing and Concordance Between Antimicrobial Susceptibility Genotypes and Phenotypes of Bacterial Isolates Associated with Bovine Respiratory Disease G3: Genes, Genomes. Genetics 7,9, 3059-3071. 2017.

Rajput MKS, Abdelsalam K, Darweesh MF, Braun LJ, Kerkvliet J, Hoppe AD., Chase CC. 2017. Both cytopathic and non-cytopathic bovine viral diarrhea virus (BVDV) induced autophagy at a similar rate. Vet Immunol Immunopathol 193-194:1-9. doi:10.1016/j.vetimm.2017.09.006. https://doi.org/10.1016/j.vetimm.2017.09.006

Kramer L, Mayes M, Fritz-Waters E, Williams J, Downey E, Tait, Jr. R, Woolums A, Chase C, Reecy J. 2017. Evaluation of responses to vaccination of Angus cattle for four viruses that contribute to bovine respiratory disease complex. J Anim Sci,95:4820-4834. doi: 10.2527/jas2017.1793.

Chase CCL. 2018. Enteric Immunity: Happy Gut, Healthy Animal. Vet Clin Food Anim, 34:1-18. Doi:10.1016/j.cvfa.2017.10.006
Darweesh MF, Rajput MKS, Braun LJ, Rohlia JS, Chase CCL. BVDV Npro protein mediates the BVDV induced immunosuppression through interaction with cellular S100A9 protein. Microbial Pathogenesis 2018;121:341–349.

Morarie-Kane SE, Smirnova NP, Hansen TR, Mediger J, Braun L, Chase C. Fetal Hepatic Response to Bovine Viral Diarrhea Virus Infection in Utero. Pathogens 2018;7.

Chase, CCL. 2017. Vaccinology. In Blackwell's Five-Minute Veterinary Consult: Ruminant 2nd edition, ed. Chase, Lutz, McKenzie & Tibary. Wiley-Blackwell, Ames, IA, on-line, www.fiveminutevet.com/ruminant.

Chase, CCL, Lutz K, McKenzie E, & Tibary A. 2017. Blackwell's Five-Minute Veterinary Consult: Ruminant 2nd edition. Wiley-Blackwell, Ames, IA.

In review:

Epidemiology of Bovine Respiratory Disease in Preweaned Calves on California Dairies; S.A. Dubrovsky, A.L. Van Eenennaam, B.M. Karle, Paul V. Rossitto, T. W. Lehenbauer, S. S. Aly

Management factors associated with bovine respiratory disease (BRD) in pre-weaned dairy calves on California dairies: the BRD 100 study; Gabriele U. Maier, William J. Love, Betsy M. Karle, Sasha A.

Dubrovsky, Deniece R. Williams, John D. Champagne, Randall J. Anderson, Joan D. Rowe, Terry W. Lehenbauer, Alison L. Van Eenennaam, Sharif S. Aly

Economic Cost of Bovine Respiratory Disease and Cost-Benefit of Implementation of Preventative Measures in Dairy Calves; S.A. Dubrovsky, A.L. Van Eenennaam, S. S. Aly, B.M. Karle, Paul V. Rossitto, T. W. Lehenbauer, J.G.Fadel

Association between plasma Haptoglobin concentration and other biomarkers and bovine respiratory disease status in pre-weaned dairy calves; Moisá, S. J., Aly, S. S., Lehenbauer, T.W., Love, W. J., Rossitto, P. V., Van Eenennaam, A.L., Trombetta, S. C., Luo, Y., and L.E. Hulbert

Risk Assessment of Bovine Respiratory Disease in Preweaned Calves; Gabriele U. Maier, William J. Love, Betsy M. Karle, Sasha A. Dubrovsky, Deniece R. Williams, John D. Champagne, Randall J. Anderson, Joan D. Rowe, Terry W. Lehenbauer, Alison L. Van Eenennaam, Sharif S. Aly

Regional Management Practices and Prevalence of Bovine Respiratory Disease in California's Preweaned Dairy Calves, Betsy M. Karle, Gabriele U. Maier, William J. Love, Sasha A. Dubrovsky, Deniece R. Williams, Randall J. Anderson, Alison L. Van Eenennaam, Terry W. Lehenbauer, Sharif S. Aly

Effect of temperature and humidity on Bovine Respiratory Disease in Preweaned Calves on California Dairies; S.A. Dubrovsky, A.L. Van Eenennaam, B.M. Karle, Paul V. Rossitto, T. W. Lehenbauer, S. S. Aly

Scientific presentations:

Bittar J.H.J, Hoyos-Jaramillo A, Hurley DJ, Stoskute A, Hamrick B, Lauber K, Norton N, Adkins M, Saliki JT, Havenga LJ, Palomares RA. Onset of protection from Bovine viral diarrhea virus infection induced by modified-live virus vaccination concurrent with injectable trace minerals in newly received beef calves. Academy of Veterinary Consultants. August 2018, Denver CO

Hoyos-Jaramillo A, Bittar JHJ, Rodríguez A, González EA, Kirks SJ, Stanley SI, Urdaneta J, Gutierrez A, Wall S, Miller K, Finley M, Skrada K, Lopez D, Hurley DJ Havenga L, Palomares RA. T cell populations in calves infected with BVDV + IBR after intranasal vaccination and trace minerals injection. 99th Annual Meeting of the Conference of Research Workers in Animal Disease and BVDV Symposium, December 2018, Chicago, IL.

Palomares RA. Intranasal vaccination against bovine respiratory viruses and strategies to enhance its efficacy. Academy of Veterinary Consultants. Denver CO, August 2018.

Palomares R.A. Strategies to enhance the immune response to vaccines against respiratory and reproductive disease in cattle. Zoetis Food Animal Symposium. Ohio State University, Columbus OH, April 2018.

BRD Scoring System Development. BRD Symposium. Animal Health Research Institute, Taiwan 2017.
BRD Scoring System validation. BRD Symposium. Animal Health Research Institute, Taiwan 2017.

Epidemiology of BRD, the BRD 10K and BRD 100 studies. BRD Symposium. Animal Health Research Institute, Taiwan 2017.

BRD Scoring system app. BRD Symposium. Tainan City Animal Health Inspection and Protection Office, Taiwan. 2017.

Development of a BRD Scoring system and its application uses. Boehringer Ingelheim BRD Meeting China. 2017.

Betsy M. Karle, Sharif S. Aly, Deniece R. Williams, Jeffery W. Stackhouse, Alison L. Van Eenennaam, Terry W. Lehenbauer. Bovine Respiratory Disease prevalence estimation in pre-weaned dairy calves using a mobile application American Dairy Science Association Pittsburg, Pennsylvania, 2017

Karle, Betsy M., Gabriele Maier, Sasha A. Dubrovsky, William J, Love, Deniece R. Williams, Jeffery W. Stackhouse, Randall J. Anderson, Alison L. Van Eenennaam, Terry W. Lehenbauer, Sharif S. Aly. Management practices and prevalence of bovine respiratory disease in pre-weaned dairy calves in California. American Dairy Science Association (2017) Pittsburg, Pennsylvania, 2017

Dubrovsky S, Van Eenennaam A, Karle B, Lehenbauer TW, Aly SS. Epidemiology of Bovine Respiratory Disease in Pre-weaned Dairy Calves in California, American Dairy Science Association (2017) Pittsburg, Pennsylvania, 2017

Gabriele Maier, Dubrovsky S, Van Eenennaam A, Karle B, Lehenbauer TW, Aly SS. Development of a risk assessment tool for prevention of bovine respiratory disease in pre-weaned calves on California dairies, American Association of Bovine Practitioners (2017) Omaha, Nebraska, 2017

Outreach presentations:
Amy Young: Simplified Scoring System to Identify Respiratory Disease in Dairy Calves, eXtension, http://articles.extension.org/pages/73380/simplified-scoring-system-to-identify-respiratory-disease-in-dairy-calves, January 28, 2017

Karle, B.M. 2017. There's an App for that! Respiratory Disease in Dairy Calves. UCCE California Dairy Newsletter. 9:1 January 2017.

Management strategies for calf pneumonia: BRD scoring system and app. UCCE Calf Management Round Table Discussion workshops. Orland, Petaluma, Eureka. 2017.

Bovine respiratory disease in preweaned dairy calves and use of the CA scoring system as a diagnostic tool. UC Davis School of Veterinary Medicine Winter Conference. 2017.

Calf Management practices in California and using the BRD scoring system & App to estimate herd prevalence. California State University, Chico Dairy Production and Management Class. Chico, CA. 2017.

Translating bovine respiratory disease (BRD) genomic information to industry outcomes. Meat Animal Research Center, Clay Center, NE.

Genetics of Disease Resistance. Snyder Livestock Co. Bull Test Sale, Yerington, NV.

BRD Scoring system and epidemiology in CA. Pacific Veterinary Conference, Zoetis. Avila Beach, CA.

Sobraske J, Abdelsalam K, Chase CCL. The effects of bovine herpes virus 1 on monocyte-derived dendritic cell cytokine production and surface marker expression. Abstract P112. 98th annual meeting of the Conference for Research Workers in Animal Diseases, Chicago IL, December 4, 2017.

Abdelsalam K, Elmowalid G, Braun L, Sobraske J, Chase C. The indirect effect of bovine viral diarrhea virus (BVDV) on macrophage inflammatory function and lymphocyte apoptosis. Abstract 214. 98th annual meeting of the Conference for Research Workers in Animal Diseases, Chicago IL, December 4, 2017.

Chase C. The good, the bad and the ugly of the immune system: How can we maximize the vaccine response. Faculty of Veterinary Medicine, University of Sao Paulo, Sao Paulo, Brazil, January 22, 2018.

Chase C Understanding Immunology: How to Use the Immune System to Our Advantage. Danish Association for Dairy Herd Health, Kolding Denmark, March 21, 2018
Chase C. Understanding Immunology- the bad and the ugly- when it comes to immunology can we have too much of a good thing. Danish Association for Dairy Herd Health, Kolding Denmark, March 21, 2018

Chase C. Immunology-The Important Stuff You Forgot From Vet School
Understanding the Good of Immunology. Merck Animal Health Technical Service Meeting, April 19, 2018, Southlake , TX

Chase C. Immunology- Avoiding the Ugly Side of the Immune Response. Merck Animal Health Technical Service Meeting, April 19, 2018, Southlake , TX.

Theses:

UW-Madison

- Ismail Boukahil, PhD 2017
- Jose Rivera-Rivas, PhD 2017

SDSU

- Jacob Sobraske, MS 2018, Thesis title- The effects of bovine viral diarrhea virus and bovine herpesvirus type 1 on monocyte-derived dendritic cells

Collaborations, Fund leveraging:

The Czuprynski Lab has collaborated with Drs. Ollivett and Suen at UW-Madison as well as with investigators at Washington State (Srikumaran), Oklahoma State (Confer), and NADC (Briggs).

MS: Collaborations with researchers at GA has allowed us to assess the impact of parasitism while also assessing host immune response in our trials to test the impact of on-arrival vaccination and deworming on health and growth of high-risk stocker cattle.