2018 Summer Research Abstracts

Optimization of methods to study the effect of cannabinoids on immune responses in stimulated canine PBMCs
Clare Brown, Evangel Kummari, Todd Archer, Barbara Kaplan

Current canine autoimmune disease treatments are not ideal due to limited efficacy or adverse side effects. Cannabinoids, such as cannabidiol (CBD) and tetrahydrocannabinol (THC), are compounds from the marijuana plant (Cannabis spp.) that have gained attention as potential treatments for autoimmune diseases. In mice it has been established that they are immunosuppressive and provide some benefit in autoimmune models, but in most other species there exists little data. The aim of this project was to examine the potential of cannabinoids as effective treatments for canine autoimmune diseases by determining whether they are immunosuppressive in canine peripheral blood mononuclear cells (PBMCs) in vitro. PBMCs were isolated from canine blood and treated with CBD or THC, followed by stimulation with ConA, LPS, or PMA/ionomycin (PI). Carboxyfluorescein succinimidyl ester (CFSE) staining was used in conjunction with flow cytometry to assess lymphocyte proliferation. RNA was also isolated to determine gene expression of cytokines interleukin-2 (IL-2) and interferon gamma (IFNγ) via qPCR. After assessing PBMC responsiveness under various conditions, the studies focused on PI as it was the most effective stimulant. Results from both flow cytometry and qPCR indicated that CBD and THC could affect proliferation and
cytokine production, but that it was dog-specific; some dogs exhibited no effect, while other dogs exhibited modest suppression by CBD and THC. IFNγ consistently exhibited a higher magnitude of change in expression than IL-2. These findings provide important preliminary insight into the potential of these compounds for canine autoimmune diseases, and establish a knowledge base of effective methods for future studies.

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Characterization of Receptor Binding Properties of Subtype H6 Avian Influenza A Viruses from North America
Andrew Cox, Minhui Guan, Xiaojian Zhang, Liyuan Liu, and Xiu-Feng Wan

Influenza A viruses (IAVs) have affected more lives than any other single agent throughout history. Just over the past 100 years there have been four documented influenza pandemics killing over 50 million people. Yet, new strains have been emerging and the threat of another pandemic still looms. To date, 16 hemagglutinin and 9 neuraminidase subtypes of IAVs have been recovered from wild water birds, the natural reservoirs for IAVs. In addition to alpha2,3-linked sialic acids (SA2,3Gal), some avian IAVs can also bind to alpha2,6-linked sialic acids (SA2,6Gal), and can infect humans directly. H6 avian IAV is one of the most frequent subtypes detected in avian species worldwide. In addition, sporadic spillovers of H6 IAVs from avian to both swine and humans were reported. However, the receptor binding properties of H6 avian IAVs have not been well characterized. We hypothesize that there would be variations in receptor binding abilities for H6 avian IAVs and that a small portion of H6 IAVs could bind to SA2,6Gal. We focused on H6 avian IAVs recovered from wild birds in North America, and their binding affinities to three glycan analogs, including one SA2,3Gal analog, SA2,6Gal analog, and sialyl Le^X (one of SA2,3Gal analog derivative). Among six tested H6 viruses, all had strong binding affinities to SA2,3Gal and sialyl Le^X and only three could bind to SA2,6Gal, but with different binding affinities. Future studies need to be carried out to assess the risks of these North American H6 IAVs to public health. By integrating earlier studies showing that H6 avian IAVs can be spilled over to swine and humans, this study suggests continual surveillance and monitoring of subtype H6 avian IAVs are needed.

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Enhancing Wild Animal Semen Quality Through Nanotechnology Tools
Casey Durfey, Trisha Rowlison, Pierre Comizzoli, Charles Lagu, Celsus Sente and Jean Feugang

Assisted reproductive techniques (ARTs) have been widely used in wildlife species for conservation of threatened species. However, approaches to obtain high quality semen through purification are not reliable in all wildlife species. Thus, there is a

need to develop a multi-species compatible technique for efficient preservation of viable spermatozoa. This study aims to identify the compatibility of a sperm nanopurification technique in wildlife species through applications with domestic feline and threatened cattle species. Domestic feline epididymal sperm and ejaculated Ankole and Sahiwal bull semen samples were mixed with magnetic nanoparticles designed to interact with non-viable acrosome-reacted spermatozoa. Mixed semen was placed under an electromagnetic field trapping moribund sperm, enriching the dose with viable cells. Motility characteristics were analyzed, and felid samples cryopreserved and utilized for in vitro fertilization and microscopic analysis. Comparable motility characteristics were found in all species with microscopic analysis of felid nanopurified sperm showing an increased proportion of acrosome intact cells. In vitro fertilization of nanopurified felid sperm showed improved 8-cell embryonic growth 3 days post-fertilization. Findings indicate sperm nanopurification is compatible with feline and bovine species. Additionally, sperm nanopurification does not interfere with sperm viability, cryopreservation, or in vitro fertilization potential. Additional research is being conducted to confirm current findings for future implementation in conservational reproductive breeding programs.

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Canine gastrointestinal histamine receptor-1 expression in normal and inflammatory bowel disease biopsies
Anika Eidson, Alicia Olivier, Ryan Taylor, Sharon Yang, John Thomason, Alyssa Sullivant

Histamine is released upon the degranulation of several different cell types, particularly mast cells, which are abundant in the gastrointestinal (GI) tract. After its release, histamine binds to histamine receptors (H1-H4) located throughout the body. Upregulation of histamine and its' receptors is well documented in various GI disease in human medicine. In dogs, based on full thickness biopsies collected via surgery, H1-H4 receptors were identified throughout the entire canine GI tract. In this study, our aim was to identify and score the expression of H1 receptors in pinch biopsies of the GI tract of dogs collected via endoscopy. Our study utilized immunohistochemistry (IHC) to investigate the expression of H1 receptors in the GI tract of healthy dogs and dogs diagnosed with inflammatory bowel disease (IBD). Our hypothesis was that expression of the H1 receptor would be more abundant in the GI tract of dogs with IBD compared to healthy dogs. Biopsies were collected from four dogs with no clinical signs of IBD (control) and archived samples from 17 dogs diagnosed with mild or moderate/severe IBD. IHC staining was performed on endoscopic samples from the stomach, small intestine and colon. IHC staining was scored on abundance and intensity on a scale of 0 to 3. H1 receptor expression varied by anatomic site and tissue location (superficial vs deep) in the control and IBD dogs, with the most abundant expression in the stomach. There were no significant differences between control and IBD (mild vs moderate/severe) H1 receptor expression. The results of our study suggest that the H1 receptor can be identified using endoscopically collected biopsies, and that dogs with IBD do not have increased H1 receptor expression.

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Antimicrobial-loaded poloxamer 407 hydrogel for the treatment of osteomyelitis
Bethany Foust, Leah Horstemeyer, Keun Seo, Elizabeth Swanson, Joo Youn Park, Seongbin Park, and Lauren Priddy

The most common etiologic agent of osteomyelitis is *Staphylococcus aureus (S. aureus)*, which often causes chronic biofilm formation in or around bone. Failure to resolve osteomyelitis may be due to an inability to reach effective antimicrobial concentration at the infection site or the resistance of pathogens to antimicrobials. Thus, the aim of this study was to evaluate vancomycin-, fosfomycin-, and bacteriophage-loaded hydrogels for localized treatment against *S. aureus*. Poloxamer 407 (P407) hydrogels loaded with 128 μg of fosfomycin or vancomycin were added to transwells, submerged in PBS, and incubated for 48 hours at 37°C. *S. aureus* (ATCC 6538-GFP) was treated with the eluents for growth curve analysis to estimate concentration and confirm bioactivity of antibiotics (in triplicate). Additionally, biofilms were treated with fosfomycin (0-128 μg/ml) in P407 or PBS (n =1-2). An *in vivo* pilot study establishing infection in the femur diaphysis of rats was used to compare the efficacy of fosfomycin and bacteriophage (n=1-2) in PBS or P407 for the treatment of chronic osteomyelitis. Approximately 8-16 μg of the loaded vancomycin eluted from P407 over 48 hours, while the fosfomycin concentration could not be determined. Vancomycin bioactivity was maintained, while fosfomycin efficacy and bioactivity was unclear. Treatment of biofilms with fosfomycin in P407 and PBS showed a decrease in colony counts as antibiotic concentration increased. The pilot *in vivo* results showed a transient reduction of

bacteria in rats treated with bacteriophage, with no observable differences between delivery vehicles. Overall, the findings suggest P407 may serve as an effective local delivery vehicle for the treatment of chronic osteomyelitis.

Student Support: Boehringer Ingelheim Veterinary Scholars Program and Mississippi State University College of Veterinary Medicine

Detection of *Hepatozoon americanum* in Gulf Coast Ticks from Oktibbeha County, MS
Alexandra N. Frankovich, Shaira L. Rivera Martinez, John V. Stokes, Andrea S. Varela-Stokes

American Canine Hepatozoonosis (ACH) is a protozoal disease caused by *Hepatozoon americanum*. This organism is transmitted to canines when they ingest the definitive host, the Gulf Coast tick (*Amblyomma maculatum;* GCT) or a paratenic host (rodents and rabbits). There is currently no treatment for eliminating protozoa in the infected canine. The main source of infection, tick or paratenic host, has not yet been identified for ACH making it difficult to develop preventative measures. Data on disease prevalence and distribution are reliant on detection in the diseased canine. Geographical distribution of ACH is assumed to overlap GCT distribution. Currently, there is no data detailing *H. americanum* prevalence in the tick vector. This study aimed to fill the gap in knowledge by investigating GCT infection prevalence. We collected 129 adult GCTs from 3 different sites in Oktibbeha Co., MS and extracted DNA from half or whole ticks. We used a TaqMan quantitative PCR assay to test for *H. americanum*. No half tick extracts were positive using conservative threshold levels; two whole tick extracts had low copy numbers but could not be confirmed. Thus, prevalence of *H. americanum* in these GCTs was 0% which supports the null hypothesis. To evaluate potential false negative extracts, ticks with amplifiable DNA were tested with conventional PCR. Thus far, results suggest GCTs in this area may not be a source of *H. americanum* infection. Future studies targeting areas with ACH cases and investigating infection rates of nymphal GCTs and various paratenic hosts will offer more insight on the main transmission route for ACH here. This information could be crucial for the development of improved methods for prevention.

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Positive reinforcement of a foundation behavior to reduce perceived anxiety of kenneled dogs: A pilot study
Tobi N. Ku , Christine D. Calder, Lucy H. Senter

Measurement of animal welfare and establishment of protocols to reduce fear, anxiety and stress have become a research priority in recent years. Anxiety and stress in research dogs can impact lifespan, life quality and physiological parameters that may be significant to experiments, in addition to damaging the bond between dogs and their caretakers. The purpose of this pilot study was to evaluate the use of positive reinforcement training in the open kennel environment of research dogs and determine if training would decrease observed clinical signs of stress in dogs with a history of fearful behavior. Five purpose-bred research dogs with history of fearful behavior in the kennel environment were taught “nose touch targeting” using positive reinforcement clicker training over the course of two to four weeks. Dog behavior in response to a stranger was recorded before, immediately after, and two weeks after the training period to observe possible changes in stress signs and determine whether these changes were retained two weeks post-training. Three observers were instructed to use a standardized list of stress-related behaviors produced in a previous study to conduct a randomized, blind-order video review. All five dogs met the target mastery level for “nose touch targeting” during the training period. Weaknesses in the methods for analyzing dog behaviors were identified, as there was only moderate agreement between observers and no significant relationship was found between successful training and changes in observed stress behavior. This study serves as a foundation for future positive reinforcement and cooperative care training with dogs in the laboratory and other open kennel environments, including animal shelters.

Student Support: Boehringer Ingelheim Veterinary Scholars Program and Mississippi State University College of Veterinary Medicine

Role of catfish B cells in innate and adaptive immune responses to *Edwardsiella ictaluri* live attenuated vaccines
Kara Majors, Adef Kordon, Hannah Pray, Lesya Pinchuk, Attila Karsi

Enteric septicemia of catfish (ESC) is a devastating disease within the catfish farming industry, resulting in large economic losses and high mortality rates annually. The causative agent of ESC is *Edwardsiella ictaluri*, an intracellular, Gram-negative bacterium. There are previously established methods of treatment for ESC, such as medicated feed, however live attenuated vaccines (LAVs) are currently being studied as a viable form of prevention. Two LAVs of *E. ictaluri*, *Ei*Δ*evpB* and ESC-NDKL1 that showed strong protection in catfish fry and fingerlings have been recently developed in our laboratory. The aim of this study was to determine the effects of the LAVs on innate and adaptive immunity, with specific focus on antibody production, phagosome formation and bacterial killing properties of catfish B cells. We showed that IgM levels in the sera of challenged fish peaked at 14 days, followed by significant decreases at 21 days, with additional increases at 28 and 35 days post-exposure suggesting development of protective adaptive immune responses. Furthermore, the phagosome formation was observed in IgM^+ B cells purified by magnetic sorting and exposed to both LAVs, and wild-type *E. ictaluri in vitro*. The bacterial killing assay did not show significant decreases in bacterial numbers throughout the 48-hour incubation, suggesting that although catfish B cells were capable of phagocytosis, they lacked the bactericidal ability at 4°C. These findings support the hypothesis that catfish B cells are able to respond to *E. ictaluri* LAVs by eliciting protective innate and adaptive immunity.

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IgE concentrations in bronchoalveolar lavage fluid from horses with severe pasture-associated equine asthma
Sydney McConnell, Bettina Wagner, Jacquelyn Bowser, Cyprianna Swiderski

Equine asthma is the most common respiratory disease of horses. Horses with severe asthma demonstrate respiratory distress at rest. Stall-associated severe equine asthma predominates in horses housed in continental climates and has been clearly associated with exposure to moldy hay. However, the etiology of a pasture-associated form of severe equine asthma (equine pasture asthma, EPA) affecting horses during conditions of high heat and humidity is unclear. We identified Basic Helix Loop Helix 40 (BHLHE40) as differentially expressed in the lung transcriptome of EPA horses. BHLHE40 is a TH2 signature gene in human asthma that is central to IgE production. This is curious because eosinophilic airway infiltrates typify Th2 responses. However, horses with EPA demonstrate neutrophilic airway inflammatory infiltrates. In human asthma, neutrophilic airway infiltrates with strong antigen-specific IgE responses are diagnostic of asthma with fungal sensitization. Accordingly, we hypothesize that increases in BHLHE40 in the lung transcriptome of EPA horses will be reflected in increased IgE in their bronchoalveolar lavage fluid (BALF). To examine this hypothesis, total IgE was quantified in BALF of EPA (N=8) and non-diseased control horses (N=11) using an equine IgE-specific ELISA. Horses were sampled during timing of seasonal asthma exacerbation and remission in the diseased horses. Significant differences in total IgE concentrations were not identified in comparisons between diseased and control horses, nor in comparisons within groups by season. Efforts to quantify antigen-specific IgE are warranted in order to determine the significance of increased BHLHE40 in BALF samples of horses with pasture-associated asthma.

Student Support: Mississippi State University College of Veterinary Medicine

CRISPR/Cas9 phage and antibiotic combination therapy against *Staphylococcus aureus* biofilm
Katelynn Nelson, Joo Youn Park, Seunghyun Yoon, and Keun Seok Seo

Infections with Methicillin-resistant *S. aureus* often cause biofilm-associated infections such as diabetic foot ulcers and osteomyelitis, which are refractory to current vancomycin treatment. Biofilm is a sessile microbial community in which bacterial cells are closely associated with polysaccharides, proteins, and other nutrients, which forms physical barriers against host immune response and penetration of antibiotics. As a result, bacteria in biofilm are 1000 times resistant to antibiotics than their free-living counterparts. Since bacteriophages produce enzymes that disintegrate biofilm structure that will aid penetration of small molecular weight antibiotic, such as fosfomycin, we hypothesized that combination therapy using fosfomycin and bacteriophage could be better therapeutic approach against biofilm-associated *S. aureus* infection. The treatment of vancomycin alone partially reduced the biofilm structure (~50%) but has no effect on viable

cell counts. The treatment of fosfomycin alone showed better reduction of biofilm (~75%) and viable cell counts (~2 log scale). The treatment of phage alone completely removed biofilm (~ 95-99%), but still remained viable cell counts (4 log scale). The combination of phage and vancomycin therapy did not improve the effect, but the combination with phage and fosfomycin significantly reduced the viable cell count more than 6 log scale. These results demonstrate that the combination of phage and fosfomycin showed greatest effect against *S. aureus* biofilm. Currently, we are integrating CRISRP/Cas9 system into the phage genome to improve the effect of phage therapy.

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Excisional wound healing in diabetic and nondiabetic mice treated with a amnion-derived matrix- Pilot Study
Pedro J. Olivencia-Morell, Elizabeth A. Swanson

Human amniotic tissues enhance wound healing through anti-inflammatory, antimicrobial, and tissue regeneration properties. Porcine-derived amniotic tissues may be a source for noninvasive, larger and less expensive wound treatment products. The objective of this pilot study was to evaluate wound healing of full-thickness excisional skin wounds treated with a novel porcine amnion-derived acellular matrix (PAAM) in diabetic and nondiabetic mice. Our hypothesis was that full-thickness excisional skin wounds treated with PPAM would heal quicker and with less scarring than untreated wounds in diabetic and nondiabetic mice. Two excisional wounds were created on the cranial dorsal midline of 10 diabetic (Group D) and 10 nondiabetic (Group ND) mice. One wound was treated with PAAM and one was an untreated control. Wound surface area (SA) was measured biweekly and wounds were harvested weekly from 2 mice per group for histologic evaluation. Mean initial wound SA was larger on Day 0 for treated than untreated wounds in group D, while treated and untreated wounds in group ND were similar in size. In group D, treated wounds were larger than untreated wound, but both were healed by day 21. In group ND, treated wounds were smaller than for untreated wounds, but both were healed by day 17. No inflammation was grossly present in treated and control wounds. In conclusion, treated and untreated wounds showed a similar rate of healing in both diabetic and nondiabetic mice, with treated wounds possibly healing faster. No adverse effects were noted. Disparity in wound size in diabetic mice may be the result of refining the wounding technique. Additional larger studies to examine wound healing properties when treated with PAAM are warranted.

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Transfection of bovine kidney cells with mRNA for the human cathelicidin, LL-37
Leslie Reed, Amelia Woolums, Merrilee Thoresen, Peres Badial, Philip Santangelo, and Pooja Tiwari

Bovine respiratory disease (BRD) is the most common cause of morbidity and mortality in feedlot cattle in the U.S. Methods to improve host immunity could help cattle resist BRD. This study investigated the feasibility of mRNA therapy to induce expression of an immunotherapeutic, the human cathelicidin LL-37, for eventual use to treat or prevent BRD. The objectives were: 1) to transfect Madin Darby bovine kidney (MDBK) cells with exogenous mRNA encoding the reporter protein luciferase; 2) to measure expression of LL-37 in MDBK cells transfected with LL-37 mRNA and 3) to determine whether supernatants or lysates from MDBK cells transfected with LL-37 mRNA induced antiviral effects against bovine herpesvirus-1 (BHV-1) or bovine respiratory syncytial virus (BRSV). MDBK cells were transfected with luciferase mRNA in Viromer® Red (VR) or Lipofectamine® 3000 (L3K) transfection reagents. Results from the bioluminescence assay confirmed luciferase expression. 46.62 relative light units (rlu) were measured for VR transfected cells vs 0.01 rlu in VR transfected control cells. MDBK cells were transfected with LL-37 mRNA using L3K or VR; expression was assessed using immunocytochemistry (ICC) but was not confirmed. Supernatants and lysates from MDBK cells transfected with LL-37 mRNA for 6 or 24 hours were assessed for antiviral effect against BHV-1 and BRSV using TCID50 assays to quantify virus in treated and control cultures. Supernatants and lysates from LL-37 transfected cells did not have significant antiviral effects against BHV-1 or BRSV. Tests are ongoing to determine whether modified culture conditions lead to LL-37 expression and induction of antiviral effect by transfected MDBK cells.

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Effects of Liver Cell Surface Glycoconjugates on Invasion by *Plasmodium berghei*
Aumbriel M. Schwirian and Jonas King

The initial interaction between malaria sporozoites and host liver cell has become the focus for a novel malaria vaccination. The current recombinant vaccine (RTS,S/AS01) targets the point of hepatocyte invasion, but fails to block parasite transmission reliably. Parasite invasion is still poorly understood. Interaction between the sporozoite circumsporozoite protein and host hepatocyte heparin sulfate proteoglycans (HSPGs) is believed to facilitate invasion; however, other currently unknown redundant pathways involving glycoconjugates targeted by sporozoites for invasion are suspected. To investigate these pathways, HepG2 human hepatic carcinoma cells were grown in the presence of swainsonine, a Golgi α-mannosidase inhibitor, or *HS2ST1* siRNA, a silencer of the heparin sulfate 2-O-sulfotranferase 1 gene. These were chosen to evaluate how glycoconjugates interact with sporozoites. Determining a dose that would yield reduced HSPG / N-glycan levels while maintaining high cell viability was a critical first step. Cells were dosed with a range of 0.02 µg/mL - 10 µg/mL of swainsonine to determine changes in cellular morphology and cell viability. No changes were detected with microscopy at these concentrations. Cells were also incubated with 100 µg/mL swainsonine or the siRNA and stained with fluorescent lectins to evaluate changes in cell surface glycoconjugates. Real time qPCR was used with a suite of genes whose products are known to be involved in sporozoite invasion to observe the global effects of silencing *HS2ST1*. While this project is still in its beginning stages, it is expected that altering the cell surface glycoconjugates will have a negative effect on plasmodium invasion.

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Comparison of in vitro susceptibility of *Tritrichomonas foetus* to metronidazole and fenbendazole
Alexandra M. Varela-Ortiz, Amelia Woolums, Merrilee Thoresen, Heath King, Darcie Sidelinger, Richard Hopper

Tritrichomonas foetus is a sexually transmitted protozoan that causes reproductive failure in cattle. Trichomoniasis does not cause overt disease but causes early abortion and temporary infertility. This results in a significant decrease in the calf crop coupled with a negative impact on calving distribution, resulting in an overall loss in the operation's revenue. Currently, there is no legal efficacious treatment for *T foetus*. Metronidazole has been proven effective against *T foetus* but is currently illegal under the provisions of Animal Medicinal Drug Use Clarification Act of 1994 (AMDUCA) and 21 Code of Federal Regulations (CFR). This study aimed to determine if fenbendazole is as effective as metronidazole in the in vitro inhibition of *T foetus*. The first objective was to characterize a growth curve at various seeding densities of a wild type strain of *T foetus.* The second objective was to compare the susceptibility of *T foetus* to metronidazole and fenbendazole in vitro. Metronidazole or fenbendazole were added at dilutions ranging from 0.5 to 0.0313µg/mL**.** At multiple time points motile trichomonads were counted with a hemocytometer. Results from the growth curve indicated *T foetus* reached peak density at an average of 11 x 10^6 trichomonads per mL. At 0.5 µg/mL metronidazole completely inactivated *T foetus* by 48 hours; 0.25 µg/mL treatment led to a transient decrease in motility that rebounded by day 7. Lower concentrations of metronidazole also suppressed motility but did not completely inactivate *T foetus.* At the same concentrations fenbendazole had much less effect on motility. These findings indicate that under the conditions of this study, fenbendazole is less effective than metronidazole to inhibit *T foetus*.

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PCR identification of specific BRD pathogens and their integrative conjugative elements
Amy Wallace, Merrilee Thoresen, Jonathan Frye, Sushim Gupta, and Amelia Woolums

Mannheimia haemolytica and *Pasteurella multocida* are important pathogens of bovine respiratory disease (BRD). These pathogens have been shown to harbor antimicrobial resistance (AMR) genes to at least three classes of drugs on integrative conjugative elements (ICEs). ICEs have previously been identified through whole-genome sequencing, but a more rapid and affordable method is needed to facilitate field studies of factors that contribute to the dissemination of multidrug resistance (MDR). The purpose of this study was to develop a PCR assay to differentiate between two common BRD pathogens while identifying ICEs they might carry. Two transcriptional regulatory genes (*Pm0762* and *Pm1231*)

were targeted to identify *P. multocida*. Two regions of a leukotoxin gene (*lkt*) were used to identify *M. haemolytica* and rule out related species. Primers were designed to target *M. haemolytica* specific ICE amplification (ICE), the *rpoB* gene in *M. haemolytica* (rpoB), and a gene encoding an integrative conjugative element protein in both pathogens (ICE4). The PCR assay positively identified *M. haemolytica* isolates known to be MDR. A non-MDR *M. haemolytica* isolate tested negative for an ICE based on PCR results. Primer rpoB identified *M. haemolytica* but PCR conditions require further optimization to confirm specificity. The PCR assay detected an ICE in *M. haemolytica* from a calf not treated with antimicrobials, confirming that cattle can harbor bacteria with genetic elements encoding MDR before they receive antimicrobials. Further research is warranted to identify how ICEs are being disseminated in cattle populations, and to determine methods to mitigate their spread.

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