Applied Research Presentations
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Bacteriological agreement, predictive values, and gamithromycin susceptibility profiles of nasopharyngeal swabs and bronchoalveolar lavages in calves diagnosed with bovine respiratory disease

Sarah Capik

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Although commonly practiced, the utility of antemortem testing for Bovine Respiratory Disease (BRD) bacterial pathogens such as *Mannheimia haemolytica* and *Pasteurella multocida* and the best method for obtaining diagnostic samples remain controversial. Therefore, our objective was to compare the gamithromycin susceptibility, agreement, and predictive values of bilateral nasopharyngeal swabs (NPS) and bronchoalveolar lavages (BAL) for *M. haemolytica* and *P. multocida* from calves treated for BRD. Bilateral NPS and blind BAL samples were obtained from 28 mixed-breed calves with clinical BRD 5 days post-treatment with gamithromycin. Up to 12 *M. haemolytica* and 6 *P. multocida* isolates were selected for gamithromycin susceptibility testing from each positive NPS or BAL sample; although prevalence was low, kappa values and predictive values for organism presence were calculated. Kappa values for BAL vs. NPS for *M. haemolytica* and *P. multocida* 5 days post-treatment were 0.71 and 0.81 respectively. When BAL culture results were used as the gold-standard for organism status, the positive and negative predictive values of NPS were: 66.7% and 100% for *M. haemolytica* and 75% and 100% for *P. multocida*. Although high in this study, the agreement of paired samples and the negative predictive value of NPS were affected by the low organism prevalence and would vary in other populations according to prevalence. Gamithromycin susceptibility phenotypes were not always consistent between isolates from paired NPS and BAL samples, indicating that although organisms of the same species may be concurrently isolated from the upper and lower respiratory tract, phenotypic diversity may be observed.

Development of large animal model for Schmallenberg virus

Abaineh D. Endalew (PhD student)

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Schmallenberg virus (SBV), an *Orthobunyavirus* in Simbu serogroup, emerged late 2011 in sheep and cattle in Germany associated mostly with mild transient disease. It causes abortion, still birth & congenital defects in naïve pregnant animals. It is transmitted by biting midges (*Culicoides*). To establish an animal model in the US that can be used to evaluate SBV diagnostics and vaccines, two separate studies were conducted in sheep and cattle using various infectious inocula: (i) infectious cattle serum (ii) cell culture-derived virus, and (iii) infectious lamb brain homogenate. Virological and serological responses were assessed in both animal species throughout the course of the experiment. SBV RNA in serum (RNAemia) was detected as early as day 2 (in sheep) and day 3 (in cattle) post-infection (pi) and the level peaked on day 4 pi in both species. Cattle showed higher level of RNAemia. Experimental infection with infectious serum inoculum resulted in the highest level of RNAemia in both species followed by cell culture-derived virus. SBV neutralizing antibodies were first detected at day 14 pi in both species and neutralization antibody titers were much higher in cattle than in sheep. Higher levels of SBV RNAemia and SBV antibody response make cattle a preferable animal model to study SBV infection, pathogenesis, diagnostics and vaccine development.

Key words: Schmallenberg virus, RNAemia, PRNT, animal model

Effect of topical 0.5% proparacaine hydrochloride on yield of bacterial isolates from infected corneal ulcers

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Bacterial culture and sensitivity is imperative for appropriate management of infected corneal ulcers. Application of a topical anesthetic prior to sample collection is commonly performed to alleviate patient discomfort; however, there is some evidence that topical anesthetics inhibit bacterial growth. The objective of this study was to determine if application of topical 0.5% proparacaine hydrochloride impacts yield of bacterial cultures obtained from naturally occurring infected corneal ulcers. Samples were obtained from 32 eyes with infected ulcers (25 dogs, 4 horses, 2 alpacas). A mini-tip culturette swab was used to collect a sample from the ulcer. Following initial sample collection, one drop of 0.5% proparacaine hydrochloride with 0.01% benzalkonium chloride was placed on the affected eye. After a period of one minute, a second culture sample was collected in the same manner from a different area of the same ulcer. Both samples were submitted for culture and the results were analyzed using a paired T-test. There was no significant difference between the number of bacterial species isolated before administration of proparacaine and the number of bacterial species isolated after administration of proparacaine ($p=0.1839$). A Pearson's correlation analysis was also performed, showing that there was a significant positive correlation between a positive culture prior to proparacaine application and a positive culture following application of proparacaine ($p<0.001$, $r=0.9295$). In conclusion, the application of topical 0.5% proparacaine hydrochloride prior to sample collection for bacterial culture does not affect the number of isolates identified.

Longitudinal evaluation of serum symmetric dimethylarginine (SDMA) and creatinine (sCr) in dogs and cats with early CKD

Sarah Guess

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Our objective was to compare SDMA and sCr for detection of early CKD in a prospective, longitudinal study of older dogs and cats.

SDMA and traditional serum and urine clinicopathologic tests were measured biannually for four years in 43 dogs and 33 cats.

CKD was documented in 23 dogs (53%) by US abnormalities (n=13), decreased GFR (> 40% reduction) (n=13), persistent renal proteinuria (UPC $\geq$ 0.5) (n=6), or renal histology (n=6) (12 dogs had multiple abnormalities). CKD was documented in 13 (39%) cats by renal US abnormalities (n=10), decreased GFR (> 40% reduction) (n=1), persistent renal proteinuria (UPC $\geq$ 0.4) (n=3), or renal histology (n=3) (4 cats had multiple abnormalities).

9 of 23 dogs had increased SDMA ($\geq$ 14µg/dl) without hypersthenuria concurrent or subsequent to CKD diagnosis. Conversely, only 2 of the 23 dogs had increased sCr (> 1.8mg/dl) at any point and both of these dogs had concurrent/prior SDMA increases. Increased SDMA without hypersthenuria had 39% sensitivity and 100% specificity for CKD whereas increased sCr without hypersthenuria had 9% sensitivity but 100% specificity (p=0.024). 7 of 13 cats had increased SDMA ($\geq$ 14µg/dl) without hypersthenuria concurrent or subsequent to CKD diagnosis. Conversely, 1 of the 13 CKD cats had increased sCr (>2.3mg/dl) and this cat had a concurrent increase in SDMA. Increased SDMA without hypersthenuria had 54% sensitivity and 95% specificity for CKD whereas increased sCr without hypersthenuria had 8% sensitivity and 100% specificity (p=0.042).

SDMA is a sensitive biomarker for early detection of CKD in dogs and cats.

Comparison of Arthroscopic Lavage and Needle Lavage Techniques, and Lavage Volume on the Recovery of Colored Microspheres From the Tarsocrural Joints of Cadaver Horses

Megan Guyan

Author(s): Patrick G. Loftin, Warren L. Beard, Megan E. Guyan, and Brad J. White

Septic arthritis in horses can be life threatening making early and aggressive therapy essential. The mainstay of therapy is thorough joint lavage. Arthroscopic lavage is generally advocated as more effective than needle lavage and lavage with copious amounts of fluid is recommended. Both recommendations are based on clinical impression and historical protocols. There is no data directly comparing the 2 methods of joint lavage, nor have there been studies to determine what volume of lavage is considered sufficient. Needle joint lavage does not require special equipment and can be performed in the standing patient, making it more practical for general practitioners, reducing cost, and decreasing the risk associated with anesthesia and recovery in the patient.

The purpose of this study was to compare the efficacy of arthroscopic and needle lavage of the tarsocrural joint. Colored polysterene microspheres were injected intra-articular to quantify the efficiency of each method in removing particulate matter from the joint. Egress lavage fluid was collected and analyzed by liter to determine total microsphere recovery per each liter of lavage fluid. Tarsocrural joint lavage using 14g needles with 2 egress needles was more effective at removing colored microshperes in a normal cadaveric joint than arthroscopic lavage with a single egress cannula. Recovery of microspheres decreased significantly after 1 L of lavage and there was no significant change in recovery of microspheres in subsequent liters. Results of this study question the conventional wisdom that arthroscopic lavage is superior and that large volumes of lavage fluid are required.

The effects of β-adrenergic agonists and different cattle handling intensities on performance and physiological response in finished beef cattle.

Jacob Hagenmaier

Author(s): Jacob A. Hagenmaier; Daniel U. Thomson, DVM, PhD; Chris Reinhardt, PhD; Matt Ritter, PhD; Carl Guthrie, DVM; Gary Vogel, PhD; Michelle Calvo-Lorenzo, PhD; Rob Starkey

One-hundred twenty-eight beef cattle (BW = 654 ± 60 kg) were used to examine the effects of ractopamine hydrochloride and cattle handling intensities on performance and physiological responses in finished beef cattle. Cattle were blocked by sex, stratified by weight and randomly assigned to one of four treatments: 1) CONLSH (no β-agonist (CON) and low-stress handling (LSH)); 2) CONHSH (CON and high-stress cattle handling (HSH); 3) RACLSH (fed a β-agonist (BA) and LSH) or 4) RACHSH (BA and HSH). Cattle assigned to BA treatment were fed 400 mg/hd/d ractopamine hydrochloride for 28 d. Cattle were walked (LSH) or kept at a trot (HSH) for 1,500 m prior to shipment for slaughter. Blood measurements were recorded prior to handling (BASELINE), following handling (POSTHAND), and during exsanguination (EXSANG). Cattle fed BA had similar DMI intake, greater ADG and improved F:G than CON cattle ($P \leq 0.05$). The only BA x HI interaction observed was an increase in POSTHAND norepinephrine ($P \leq 0.05$) in BA cattle exposed to HSH. Feeding BA to cattle had no effect on POSTHAND variables. Cattle on HSH treatments had greater lactate, epinephrine, norepinephrine and cortisol concentrations POSTHAND ($P \leq 0.05$) than LSH cattle. Effects of RAC and HSH had additive effects on EXSANG creatine kinase ($P \leq 0.05$) in cattle. There were no other effects of BA or HI on cattle at EXSANG. Cattle fed BA have greater responses in norepinephrine to HSH. Metabolic acidosis, a precursor for fatigued cattle syndrome, was observed in CON and BA cattle.

A deterministic model of cow-calf production over 10 years

Douglas Shane

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The long term impacts of management factors in cow-calf enterprises are difficult to assess. We developed a deterministic 10-year model of cow-calf production using a system modelling software package (Stella ®) with the outcome of pounds of calf weaned. Four theoretical 1000-head herds were compared (A, B, C, and D). Herds started breeding heifers 45 days prior to cows (A and B) or concurrent with the cows (C and D). The heifer breeding season was 42 days (A and C) or 63 days (B and D). The length of the cow breeding season was 63 days (A and C) or 84 days (B and D). Herd A and Herd C were assumed to have right skewed population distributions by 21-day calving interval with 67.9%, 23.7%, and 8.3% of the population in the first, second, and third intervals, respectively. Herd B and Herd D were assumed to have a population distribution by 21-day calving intervals with 37.3%, 36.8%, 17.6 and 8.2% of the population in the first, second, third and fourth intervals, respectively. The total number of calves weaned for herds A, B, C, and D were 8,856, 9,330, 8,784, and 9,272, respectively. The average pounds weaned per cow exposed for herds A, B, C, and D was 352.56, 364.98, 346.13, and 356.12, respectively. These results indicate the long term differences in total production which may arise due to differences in management factors in cow-calf production enterprises.

DETECTION OF ANTIBODY RESPONSE TO VACCINES IN HORSES USING A MULTIPLEX MICROSPHERE-BASED ASSAY

Kaitlin Haukos

Author(s): Kaitlin Haukos, Dr. Melinda Wilkerson, Dr. Susan Moore, Kaori Knight, Anushka George, Dr. Elizabeth Davis

In an effort to protect horses from infectious disease, equine veterinarians utilize a vaccine protocol that includes an initial primary vaccination followed by a booster vaccination 3-4 weeks later to induce a secondary (amnestic) immune response. Subsequent boosters are given at 6-12 month intervals depending on the pathogen of interest. Despite wide-spread protocol acceptance, duration of effectiveness of vaccines in protecting horses from disease is unknown. It was hypothesized that horses vaccinated annually will have serum antibody concentrations that remain present for extended periods of time, but that serum antibody concentrations wane when booster immunization is needed. We used a novel multiplex-bead-based indirect immunoassay to screen sera from adult, previously vaccinated horses to measure antibody levels in prior to and following vaccination for routine core and risk-based pathogens. The antigens tested were West Nile Virus, Eastern Equine Encephalitis, Western Equine Encephalitis, Equine Influenza Virus, Equine Herpes Virus 1 and 4, Tetanus, and 7 different Rabies antigens (3 lab and 4 wild strains). The assay is a 14-plex capture antibody, which quantifies equine IgG targeting viral antigens derived from purified vaccine antigens and rabies virus strains. A standard curve was developed to quantify the viral-antigen reactive IgG detected in vaccinated horse serum. Vaccinated horses had increased serum antibody concentration for each antigen post-vaccination with the percent increase ranging between 34.0% for Equine Herpes Virus 4 and 257.3% for Equine Influenza Virus. Use of the novel assay may provide veterinarians with the ability to measure serum antibody concentrations against routine vaccine antigens.

Relationship between trauma sustained at unloading and carcass bruise prevalence in finished cattle at commercial slaughter facilities

Tiffany Lee, DVM

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Bruising in cattle is an indicator of poor welfare, as well as a significant cause of economic loss due to decreased carcass value. Vehicle design, transport conditions, and transport time/distance are considered sources of carcass bruising, however have not been explored extensively. The objective of the current study was to determine whether a relationship exists between trauma incurred during unloading and prevalence of carcass bruising in finished beef cattle at commercial slaughter facilities. Carcass bruises were categorized by location and size, according to the Harvest Audit Program™ Carcass Bruise Scoring System. Bruising episodes were observed as cattle exited trailers onto the unloading docks, and were categorized as "back", "shoulder", or "hip/rib" episodes. Average carcass bruise prevalence per lot was 67.60% (± 1.16%). Average prevalence of bruises along the dorsal midline was 53.52% (± 1.12%). Bruising along the left and right sides of the carcass averaged 19.98% (± 1.04%) and 26.49% (± 1.10%), respectively. Average bruising episode prevalence per lot was 20.75% (± 1.12%). Average prevalence for small, medium, and large bruises were 28.64% (± 1.31%), 41.77% (± 0.97%), and 29.58% (± 1.81%), respectively. Correlation analysis revealed an *r* of 0.28 when comparing overall carcass bruise prevalence with bruising episode prevalence. While the correlation found in the current study is lower than expected, it is understood that bruising can occur at any point during the transportation process, including loading and transport, and such areas should be explored to determine all causes of bruising in beef carcasses to help implement prevention practices.

Newcastle Disease Virus-Based Influenza Vaccine Completely Protects Chickens from Lethal Challenge with highly pathogenic H5N2 Avian Influenza Viruses

Jingjiao Ma

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Since December 2014, Eurasian-origin, highly pathogenic H5 avian influenza viruses including H5N1, H5N2 and H5N8 viruses (called H5Nx viruses), which belong to the H5 clade 2.3.4.4, have been found in U.S. wild birds. Subsequently, the highly pathogenic H5N2 and H5N8 viruses have caused outbreaks in U.S. domestic poultry. Vaccination is one of most effective ways to control influenza outbreaks and to protect animal and public health. Newcastle disease virus (NDV)-based influenza vaccines have been demonstrated to be efficacious and safe in different species. In this study, we developed an NDV-based H5 vaccine candidate (NDV-H5) that expresses a codon-optimized ectodomain of the hemagglutinin from A/chicken/Iowa/04-20/2015 (H5N2) virus. This vaccine candidate was evaluated in chickens. Our results showed that both live and inactivated NDV-H5 vaccines induced HI antibodies against the H5N2 virus; the inactivated NDV-H5 with adjuvant induced a higher HI titer in immunized chickens after booster than the live NDV-H5 vaccine. Both NDV-H5 vaccines completely protected chickens from lethal challenge with the highly pathogenic A/turkey/Minnesota/9845-4/2015 (H5N2) virus. No clinical signs and minimal virus shedding was observed in both vaccinated groups. In contrast, all mock-vaccinated chickens shed virus and died within 5 days post challenge. Taken together, our results indicate that killed and live NDV-based H5 vaccines are able to protect chickens against intercontinental H5Nx viruses and could be used to protect the U.S. poultry industry.

A chronic disease model for Classical Swine Fever virus

Luca Popescu

Author(s): Luca Popescu

Classical Swine Fever virus infection can be divided into acute, chronic and persistent forms. Acute CSFV infection causes severe, hemorrhagic fever that is usually fatal within 2 weeks after infection; however, current circulating strains typically cause more moderate, long-term disease. The detection and control of chronic/persistent infections is especially difficult, making these strains perfect candidates for reintroduction into CSFV free regions. This study aims to establish a chronic/persistent disease model and the associated pathogenesis. Ten, 3-week-old pigs were infected with a low dose of the moderately virulent Paderborn strain. Eight different clinical signs were monitored daily, and graded on a 3-point scale; rectal temperature was also measured starting on day 9 post infection, when clinical signs began deteriorating. Based on these observations we were able to identify 2 groups of pigs representing the acute and chronic presentations of CSFV: Group 1 was euthanized at 15 DPI (acute/subacute), and Group 2 was euthanized between 24-31 DPI (chronic). A 3rd Group was not displaying significant clinical signs. This pilot study has allowed us to categorize the wide spectrum of clinical presentations generated by Paderborn infection. This model and the associated samples will be used for the development of new diagnostic tests and control methods.

EVALUATION OF FLUORESCENCE MICROSPHERE IMMUNOASSAY FOR ANTIBODY DETECTION TO RIFT VALLEY FEVER NUCLEOCAPSID PROTEIN AND GLYCOPROTEINS

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Rift Valley Fever Virus (RVFV) is a zoonotic disease that infects ruminants including cattle, sheep, goats, camels and buffalo. A fluorescence microsphere immunoassay (FMIA) was developed for the detection of antibodies towards the RVFV glycoproteins and the immunodominant nucleocapsid (N) protein. The purpose of this study was to validate the FMIA for the detection of antibodies and apply it to the screening of animals for positive or negative disease status. Well-characterized sera from vaccinated and experimentally infected sheep and calf were used for assay validation. Recombinant viral proteins were produced then coupled to polystyrene magnetic beads for analysis using Luminex® xMAP technology. A control bead set coupled to an unrelated protein was added to account for non-specific binding of antibodies to the antigen. Median Fluorescence Intensity (MFI) results were converted to Sample/Positive ratios to standardize test results. Strong MFI values were obtained for the detection of IgG antibodies against the N protein. The FMIA results correlate well (R^2= 0.91) when compared with serum neutralization tests. Preliminary results of the glycoprotein target indicate its potential as a monitoring tool for differentiating vaccinated from non-vaccinated animals for candidate subunit vaccines. The development of additional RVFV targets such as NSs is currently under investigation. The results from this project demonstrate that the FMIA provides a rapid and robust diagnostic screening tool for the detection of antibodies against RVFV. The goal is to provide an early warning target for a proposed multiplex assay that can simultaneously screen for several ruminant diseases.

"Evaluation of drug content and stability of compounded and FDA approved doxycycline formulations used in pets"

Tanner Slead

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Doxycycline is an antimicrobial commonly used for bacterial infections in cats and dogs and is frequently compounded for veterinary patients. Drug content of various compounded formulations of doxycycline from 3 veterinary compounding pharmacies were compared via high pressure liquid chromatography to FDA approved doxycycline formulations; 5 samples per formulation were tested. It was hypothesized that approved formulations would have acceptable content range (90-110%) and compounded formulations may have variable content that may decrease over time. FDA approved formulations (capsule, tablet and liquid) were within appropriate content range. Compounded tablets resulted in measured content of 89% ± 1% (2/5 samples failed), 98% ± 1% (none failed), and 116% ± 5% (4/5 failed). Compounded chews resulted in measured content of 98% ± 5% (1/5 failed), 78% ± 2% (all failed), and 81% ± 4% (all failed). Compounded liquids were all below acceptable drug content (range 50-85%). Of the compounded formulations tested, tablets had content closest to the acceptable range, with one formulation passing 5/5 samples. Compounded chews from 2/3 pharmacies and liquids from all pharmacies failed. Content testing was repeated within 3 weeks of initial testing to determine effect of storage. All FDA formulations contained appropriate content. One of three compounded tablet sample sets fell within the appropriate range. All tested chews (range 67-82%) and compounded liquid formulations (range 39-59%) were outside of acceptable content range. These results indicate compounded doxycycline tablets were variable but closest to the correct content, and compounded doxycycline chews and liquids were most likely to fail.