Posters
Phi Zeta Research Day
March 1, 2016, 1:00-5:00pm, Mara Conference Center, 4th Floor, Trotter Hall
(Posting from 1:00 – 5:00pm; Q&A for Judging 3:00 – 3:30pm)

Poster Number **Presenter – Title** **Abstract**

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Predicting bull mounting behavior in a multiple-sire pasture with classification algorithms

Kaitlynn Abell

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Parentage data from beef calves has shown that in multiple-sire pastures a disproportionate number of calves are born from a single bull. The reason(s) for the variability in the number of calves sired is unknown. Successful investigation requires accurate ways of quantifying mounting behavior by each sire in the pasture. The study objective was to assess accelerometer data and various classification algorithms to accurately predict bull mounting events in a multiple-sire pasture.

Accelerometers were attached to two bulls in four difference locations. Bulls were placed in a pasture with 10 estrus synchronized cows for four days. Video data were watched and logged to quantify the exact time and duration of each mounting event. Video data were matched with accelerometer data for each second of the study. Data were analyzed as single and multiple datasets and sub-grouped based on different tag locations. Data were partitioned into training, testing, and validation datasets. Datasets were trained using multiple classifiers to identify specific mounting events. Validation data were used to determine accuracy, and accuracies were compared between the different classifiers for each sub-group.

Dataset accuracies varied based on individual classifiers. The overall prevalence of a mounting event ranged from 0.6% to 0.7% of the total time available for the different sub-groups. The best performing classifier had accuracies that ranged from 74% to 78% within datasets. Further studies are necessary to determine differences in successfully parenting offspring between bulls in multiple-sire pastures as related to bull mounting behavior.

Pulmonary surfactant significantly inhibits cell penetrating peptide nanoparticle-based gene transfection in lung epithelial cells

Jennifer Delzeit

Author(s): Jennifer Delzeit, Susumu Ishiguro, Tagae Lloyd, Masaaki Tamura

Targeted gene delivery remains a challenge for effective cancer gene therapy. We have previously demonstrated a highly effective lung cancer gene therapy using a pulmonary aerosol delivery of apoptosis inducer genes by a modified HIV-1 TAT peptide (dTAT) nanoparticle vector (Kawabata *et al*., 2012). Although the therapeutic efficacy in mice and the material safety have been carefully studied, cancer tissue-targeting mechanism and off-target effect on normal lung epithelium have not been rigorously addressed. Accordingly, the aim of this study was to investigate the effect of pulmonary surfactant on the efficacy of gene transfection in lung cancer cells and normal lung epithelial cells. Green fluorescent protein (GFP) gene was transfected to the cells in the presence of different concentrations of two kinds of surfactants; a bovine pulmonary surfactant and rat lung lavage, which was analyzed to be dipalmitoylphosphatidylcholine (DPPC)-rich pulmonary surfactant. Both pulmonary surfactants dose-dependently inhibited GFP expression by dTAT and polyethylenimine NP vectors in A549 human lung adenocarcinoma and BEAS-2B normal bronchial epithelial cells. Considering the major component of the pulmonary surfactant, DPPC alone did not inhibit GFP gene transfection by the dTAT NP vector, it is suggested that the pulmonary surfactant specifically inhibits dTAT NP-based gene transfection. Since, a large portion of human non-small cell lung cancers do not express surfactant specific proteins, it is suggested that lung cancer cells are not covered by the lung surfactant, thereby dTAT NP-based gene delivery causes lung cancer-targeted gene transfection of therapeutic genes; whereas normal lung epithelium is protected.

Feed and ingredient type impact on Porcine Epidemic Diarrhea Virus (PEDV) infectivity using polymerase chain reaction analysis and bioassay

Jordan T. Gebhardt

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Feed and feed ingredients have been shown to be potential vectors of Porcine Epidemic Diarrhea virus (PEDV). Therefore, understanding PEDV infectivity characteristics in various feed matrixes is critical for development of potential mitigation strategies. Thus, our objective was to evaluate feed matrix and time effects after inoculation with PEDV using real time quantitative reverse transcriptase polymerase chain reaction (qRT-PCR) and bioassay. Swine complete feed (FEED) and spray-dried porcine plasma (SDPP) were inoculated with PEDV (USA/IN/2013/19338, passage 8). Samples were analyzed by qRT-PCR on day 0, 1, 3, 7, 14, 21, and 42 after inoculation. The day 0, 3, and 21 samples were also evaluated in a 10 day old pig bioassay to assess viral infectivity. There was a feed matrix × day interaction ($P < 0.001$) in which the PEDV qRT-PCR cycle threshold (Ct) increased over time in FEED, whereas little increase over time was observed in SDPP. On day 0, Ct was similar in SDPP (Ct = 28.8) and FEED (Ct = 29.8). On day 42, SDPP had a lower Ct ($P < 0.001$; Ct = 29.7) compared to FEED (Ct = 40.7). The day 0 samples were demonstrated to be infectious for both FEED and SDPP. However, only SDPP was infectious at day 3 after inoculation. In summary, degradation of PEDV RNA was observed in FEED, whereas infectious capability was extended in SDPP. This data supports the concept that PEDV infectivity can be enhanced depending on the matrix contaminated.

Keywords: swine, PEDV, feed, bioassay

Developing a multiplex oligonucleotide bead assay to detect *Ehrlichia* and *Anaplasma* infections in dog serum

Chantal Girard

Author(s): Chantal Girard, Melinda Wilkerson, Anushka George, Marta Perea, Bhumika Sharma, Kathryn Gibson, Diane Stone. Arathy Nair, Roman Ganta

The prevalence of Ehrlichiosis and Anaplasmosis, zoonotic tick-borne serological infections, depends on the inhabitant tick species in a given region. Grenada, an island inhabited by a population of dogs known as pothounds, has a high prevalence of canine *Ehrlichia* and *Anaplasma* infections when screened with a commercial ELISA, indicating continuous exposure to the infectious ectoparasite, *Rhipicephalus sanguineus*. This ELISA is able to recognize positive exposure for *Ehrlichia* and *Anaplasma*, but is unable to differentiate species and unable to detect DNA of the organisms. A multiplex fluorescent oligonucleotide bead assay is being developed to detect *Ehrlichia* and *Anaplasma* DNA in canine serological samples. The hypothesis is that this high-throughput, species-specific testing method will provide an efficient tool to accurately identify single and co-infections, thus improving knowledge to further investigate methods of disease prevention, control, and treatment. The infectious bacterial species investigated in this study are *Anaplasma phagocytophilum, Anaplasma platys, Ehrlichia canis, Ehrlichia chaffeensis*, and *Ehrlichia ewingii.* Ribosomal 16S oligonucleotides reported to be specific to each of the five species were coupled to five unique sets of MagPlex® beads and confirmed using the MAGPIX® instrument. Positive control samples for each species were analyzed, each producing a unique fluorescence on their respective beads, thus validating the specificity of the assay. DNA extracted from whole blood samples of 104 Grenadian dogs will be tested in this multiplex assay and the results will be compared to those of a published nested PCR assay.

Rahul Nandre

A novel MEFA (multiepitope fusion antigen) strategy for developing broadly protective vaccines against enterotoxigenic *Escherichia coli* (ETEC) associated diarrhea.

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Enterotoxigenic *Escherichia coli* (ETEC) strains are the most common bacterial cause of diarrhea to young animals and children. ETEC bacteria attach to host small intestinal epithelial cells by using bacterial adhesins. It is regarded that anti-adhesin vaccines against bacterial attachment to host cells serve as the first-line of defense against ETEC diarrhea. But ETEC strains produce different adhesins to attach host cells, and conventional strategies have been unable to develop an effective vaccine against ETEC diarrhea. In this study, we applied the novel multiepitope fusion antigen (MEFA) strategy to construct a single polypeptide carrying representative epitopes from adhesin tips of the 9 most important ETEC adhesins associated with diarrhea in humans, CFA/E, CFA/II (CS1, CS2, CS3), CFA/IV (CS4, CS5, CS6), CS21, and EtpA, and evaluated immunogenicity of the constructed tip MEFA and potency in developing an effective anti-adhesin vaccine against ETEC diarrhea. Data showed that mice intraperitoneally immunized with this tip MEFA developed strong immune responses to all 9 ETEC adhesins. Moreover, induced anti-adhesin antibodies had significantly inhibited adherence of ETEC or *E. coli* strains expressing these 9 adhesins to Caco-2 cells. These results suggested that the constructed adhesin tip MEFA can be used for developing a broadly protective anti-adhesin vaccine. More importantly, this innovative MEFA strategy can be used in general for multivalent vaccine development.

Double mutant heat-labile toxin (dmLT, $LT_{R192G/L211A}$) of enterotoxigenic *Escherichia coli* (ETEC), an effective adjuvant for parenteral vaccines

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Cholera toxin (CT) of *Vibrio cholera* and heat-labile toxin (LT) of enterotoxigenic *Escherichia coli* and their derivatives have been studied as adjuvants to enhance antigen-specific mucosal and systemic antibody responses. Recently, double mutant LT (dmLT, $LT_{R192G/L211A}$), which possesses much reduced enterotoxicity but LT immunogenicity, is reported an effective mucosal adjuvant in oral or intranasal immunization. But adjuvant effect of dmLT in parenteral immunization has not been investigated. In this study, we intraperitoneally (IP) immunized mice with toxoid fusion antigen $3xSTa_{N12S}$-dmLT by using dmLT or Freund's adjuvant as the adjuvant, or subcutaneously (SC) with dmLT or ISA51 as the adjuvant, and then examined adjuvanticity of dmLT in immunoregulating mouse antigen-specific immune responses. Data from this study showed that the IP immunized mice with dmLT adjuvant developed higher anti-STa and anti-LT IgG antibody responses compared to the mice immunized with the same antigen but with Freund's adjuvants. Data also revealed that the SC immunized mice using dmLT or ISA51 as the adjuvant developed similar levels of systemic anti-STa and anti-LT IgG antibody responses. These results indicate dmLT is equally or more effective to immunoregulate stimulation of systemic immune responses against ETEC toxic antigens in IP and SC routes, and suggest that dmLT can be an effective adjuvant for parental vaccines against ETEC and perhaps other infectious agents.

Development and validation of a real-time PCR assay, based on the clustered regularly interspaced short palindromic repeat sequence polymorphisms (CRISPR), for serotype-specific detection and quantification of Enterohemorrhagic *Escherichia coli* O157:H7 in cattle feces.

Lance Noll

Author(s): Lance Noll, Jianfa Bai, T.G. Nagaraja

Enterohemorrhagic *Escherichia coli* (EHEC) colonize the hindgut of cattle and are shed in the feces, which serves as a source of contamination of food products. Among EHEC, O157:H7 is the most frequent serotype associated with human illnesses. Several quantitative PCR (qPCR) assays have been developed for detection and quantification, generally based on targeting genes for serogroup specific O-antigen (*rfb*E_{O157}), H7 antigen, and one or more major virulence factors (Shiga toxins 1 and 2, and intimin). The limitation of such assays is that detection of genes in a sample does not mean the H7 and virulence genes are associated with the O serogroup. CRISPR loci are highly conserved within *E. coli* serotypes, including O157:H7, and have been used as a typing method. Our objective was to develop a qPCR assay, based on the CRISPR loci, for the detection and quantification of *E. coli* O157:H7 in cattle feces. Concentrations of primers and probes were optimized with extracted DNA from a strain of *E. coli* O157:H7. Sensitivity of the assay was determined with extracted DNA from serially diluted pure cultures and feces spiked with pure cultures. In pure culture, the minimum detection limit of the assay was $2.1x10^2$ CFU/mL. The detection limit of the qPCR assay for *E. coli* O157:H7 with DNA extracted directly from cattle feces was $2.1x10^3$ CFU/g. However, after enrichment, sensitivity increased to $2.1x10^0$ CFU/g. The assay targeting the CRISPR loci has the potential to be a high-throughput method for detecting and quantifying *E. coli* O157:H7 serotype in cattle feces.

INDICATORS OF PERSISTENT HYPERTONICITY IN DIABETIC DOGS

Cristian Perez

Author(s): Cristian Perez, Tara Brandt, Thomas Schermerhorn

Hypertonicity is a state that is associated with common human conditions (geriatric age, obesity, and diabetes) and may affect disease progression and prognosis. However, it is not known if hypertonicity has consequences for similar canine disorders. Sodium (Na) and glucose (GLU) are the major endogenous solutes that affect serum tonicity (Osm_E) and previous work has shown that both contribute substantially to Osm_E in diabetic dogs. The study objective was to evaluate dogs with diabetes for evidence of persistent hypertonicity.

A retrospective study was performed to compare pertinent solute concentrations and other indicators of tonicity between diabetic dogs (DD; n=37) dogs and non-diabetic dogs (NDD; n=164). Compared to NDD, Osm_E (315 vs 311 mOsm/L; p=0.02) and GLU (295 vs 110 mg/dl; p=0.0001) were elevated despite significantly lower sodium and chloride concentrations in DD. Overall, hypertonicity ($Osm_E \geq 315$ mOsm/L) was more frequent in DD (45.9% vs 31.1%). The mean corpuscular volume difference (dMCV), a marker for hypertonicity, was significantly increased in DD (3.9 vs 1.7 fL for NDD). A dMCV value > 3 fL (indicating hypertonicity) predicted an elevated fructosamine (>450 umol/L) with 82.6% sensitivity and 42.8% specificity. The data shows that hypertonicity is associated with diabetes in dogs. GLU was found to be the major contributing factor for the high Osm_E in DD. An increase in dMCV is a modest predictor of high fructosamine, suggesting the latter is an indirect marker for hypertonicity in DD. Abnormal hypertonicity markers in DD suggest the possibility that these dogs experience persistent serum hypertonicity.

Expression of the Schmallenberg virus glycoprotein Gn and its assessment as a diagnostic antigen

Dustin Renken

Author(s): Dustin R. Renken, Abaineh Endalew, Bonto Faburay, Juergen A. Richt

Schmallenberg virus (SBV) is a new emerging pathogen which is associated with birth defects and abortions in ruminants. In 2012, Schmallenberg virus infection was classified as an "emerging animal disease" with the potential to become a global animal health epidemic and a threat to United States agriculture. The virus belongs to the *Bunyaviridae* family, genus *Orthobunyavirus*. SBV carries two surface glycoproteins, Gn and Gc, which are necessary for binding and entry of virions into susceptible cells. The objective of this work was to express recombinant SBV Gn protein and to examine its potential use as a diagnostic antigen. Using the Bac-to-Bac expression system, a recombinant baculovirus containing the ectodomain of the SBV Gn was created and used to express the Gn in *Spodoptera frugiperda* cells. The Gn protein was purified from infected cells and Western blot analysis showed expression of a 26 kDa recombinant protein. Immunoreactive analysis using enzyme-linked immunosorbent assays (ELISAs) demonstrated that the Gn and N antigens (the latter was used as a reference antigen) were recognized by SBV-specific antibodies in sera from cattle experimentally infected with wild type SBV; however, Gn reactivity was lower when compared to N reactivity. These results suggest that both, the recombinant Gn and N proteins can be used for serological diagnosis of SBV infections in ruminants. In addition, the Gn/N ELISA system might be useful to differentiate infected from vaccinated animals (DIVA) with the development of novel SBV vaccines consisting solely of SBV glycoproteins.

A survey to describe current outdoor facilities used to finish cattle in the United States.

Jorge C. Simroth

Author(s): D.U. Thomson, C.D. Reinhardt, S.J. Bartle, and C.K. Larson

Feedlot managers (n = 43) participated in a survey to obtain a current description of outdoor cattle feeding facilities in the U.S. Participants answered questions on general feedlot information, shipping and receiving areas, finishing pens, and hospital areas. Feedlots had one-time capacities of: >20,000 animals (53%); 10,000 to 20,000 animals (26%); and < 10,000 animals (21%). Most (95%) feedlots have receiving pens providing pen space of 9.3 m^2/animal. Crowding tubs (74%) are the most commonly used type of cattle gathering facility in the processing barn, followed by Bud Boxes (19%). Most feedlots (68%) provide pen space of 13.9 to 18.6 m^2/animal and 50% of participating feedlots provide bunk space of 22.9 to 30.5 cm/animal for calves at elevated risk of respiratory disease. Most feedlots (66%) provide pen space of 9.4 to 23.2 m^2/animal and 55% of feedlots provide 22.6 to 30.5 cm/animal of bunk space for finishing cattle. Twenty-seven percent of feedlots allow 7.6 to 15.2 cm/animal of linear water tank space for finishing cattle. Windbreaks are used in finishing pens by 43% of the feedlots surveyed. Most (71%) feedlots provide mounds within the finishing pens and all feedlots use concrete aprons adjacent to the feed bunk. A minority (17%) of feedlots provide shade for cattle in feeding pens although 50% provide shade in the hospital pens. Most (66%) of feedlots have dedicated health treatment facilities which are distinct from the post-arrival processing facility. These data benchmark outdoor cattle feeding facilities utilized in the High Plains of the United States.

Validation of alpaca (*Vicugna pacos*) genome with physical maps using massively parallel imaging

Stephanie Skinner

Author(s): Stephanie M Skinner, Michelle Coleman, Rebecca Biswell, Ha Le, Susan J Brown

The alpaca industry is a relatively new and growing market in the United States. Unfortunately, alpacas are plagued by many different congenital diseases, therefore it is important to gain a more complete picture of the alpaca genome to better understand these congenital issues. The alpaca genome was recently sequenced using a whole genome shotgun approach, resulting in a genome assembly with over 75,000 contigs (N50 = 213,649) and 52,000 scaffolds (N50 = 5,303,709). Ideally, there would be one contiguous sequence for each of the 37 chromosomes contained within the alpaca genome. The purpose of this study is to create a whole genome restriction map using a new technology based on imaging ultralong single molecules of DNA. These maps can be used to validate the current sequence assembly, and create larger scaffolds to generate a more complete genome. Blood was obtained from a healthy female adult Huacaya alpaca. White blood cells were harvested and embedded in agarose, and high molecular weight (HMW) DNA was extracted from the plugs. The DNA was labeled by treating with a restriction enzyme that was adapted to create single strand nicks (NEB), incorporating florescent nucleotides at the nick and repairing the strand. Labeled HMW DNA was imaged in 13,000 massively parallel nanochannels (40nms in diameter). The images were converted into molecules and analyzed to produce overlap layout consensus maps. These maps are visualized as "barcodes" which are than compared with the existing genome. Using this method, over 45x coverage was achieved.

Coating of animals to manage solar radiation: Reduction of surface heating by sunlight in cattle by applying reflective pigment to the dorsal surfaces.

Elsie J. McCoy

Author(s): E.J. McCoy, D.U. Thomson, D. van der Merwe, C.D. Reinhardt, S.J. Bartle

Feedlot heifers (n = 30, 29 black and 1 red; 269 kg +/- 27.6 kg) were used to evaluate a reflective coating applied to the dorsal midline for the mitigation of heat stress. Heifers were randomly assigned to control or painted treatments. Paint was applied with an electronic airless sprayer coating a 30 to 40 cm width along the midline, except for the region over the dorsal anterior midline, which served as a control. Reflectance from the dorsal surface was measured with a suspended modified digital camera. Hide surface temperature was measured with a suspended infrared thermal imaging sensor; panels of white, black, and grey were placed in the surface temperature image to act as references with known temperatures. Vaginal thermometers attached to blank CIDRs were inserted into 10 heifers in each treatment to record internal body temperature. Reflectance in the green color zone includes the majority of irradiation and was found to be 8.8 times greater for the coated areas of cattle than the uncoated, indicating that heat waves were reflected rather than absorbed by the animals. The uncoated control areas of animals averaged of 3.3 ^{o}C higher than the coated areas ($P < 0.001$). Uncoated cattle had a 0.7 ^{o}C greater ($P < 0.01$) increase in body temperature than coated cattle over 2 to 3 h of natural solar radiation exposure. Reflective coating applied to the dorsal midline of feedlot cattle shows great potential for decreasing heat absorbed and reducing heat stress.

Production and characterization of monoclonal antibodies against emerging swine pestivirus

Fangfeng Yuan

Author(s): Fangfeng Yuan, Zhenhai Chen, Yin Wang, Pengcheng Shang, Benjamin M. Hause, Ying Fang

Atypical porcine pestivirus (APPV) has been reported to emerge in the US swine herd recently. To prevent its potential outbreak, specific diagnostic reagents and assays are urgently needed. In this study, we generated a panel of monoclonal antibodies (mAbs) against putative E2 glycoprotein of APPV. E2 antigen was expressed as the recombinant protein in E. coli expression system. Western blot result confirmed that the antigen was specifically recognized by field serum samples from APPV-infected pigs. Subsequently, BALB/c mice were immunized with the E2 antigen and splenocytes of hyperimmunized mice were extracted and fused with mouse NS-1 cells. Hybridoma cells were established under the HAT selecting medium. Specific hybridoma clones secreting E2-specific antibodies were initially screened by immunofluorescence assay and ELISA, and monoclones were obtained by subsequent single cell cloning. A total of five mAbs against APPV E2 were obtained. **Cross-reactivity with other pestivirus E2 antigens, including BVDV E2 and CSFV E2**, was not detected. These mAbs provide a powerful tool for development of rapid diagnostic assays for early detection of APPV infection.

Cochlear Pendrin Contributes to Recovery of Hearing after Noise Exposure

Laura Constance

Author(s): Laura Constance, Leah Freilich, Joel Sanneman, Tracy Miesner, Philine Wangemann

Chapter 1 -
Pendrin mutations are a common cause of progressive hearing loss associated with enlargement of the vestibular aqueduct. During development, pendrin expression is required in the endolymphatic sac but not in the cochlea. It is not known whether pendrin expression in the cochlea has a function. The goal of our study was to determine whether cochlear pendrin expression confers protection against noise-induced hearing loss.

Chapter 2 -
Tg(-);*Slc26a4*$^{\Delta/+}$ mice that express pendrin in the cochlea and Tg(+);*Slc26a4*$^{\Delta/\Delta}$ mice that lack cochlear pendrin expression were generated in a mixed background and were backcrossed four generations into the 129S6 strain. These mice cochlear, renal and adrenal pendrin expression. Hearing thresholds were determined from auditory brain stem recordings before and after noise exposure. Cochlear nerve conduction was evaluated by analysis of wave I. Enlargement of the vestibular aqueduct and pigmentation of stria vascularis were evaluated by histology. Blood pressure was measured with a tail-cuff.

Tg(-);*Slc26a4*$^{\Delta/+}$ and Tg(+);*Slc26a4*$^{\Delta/\Delta}$ mice had similar hearing thresholds before noise exposure and immediately after noise exposure, however, permanent threshold shifts were elevated in Tg(+);*Slc26a4*$^{\Delta/\Delta}$ mice. Cross-sectional areas of the vestibular aqueduct were similar and no hyperpigmentation of stria vascularis was seen after noise exposure. Tg(-);*Slc26a4*$^{\Delta/+}$ and Tg(+);*Slc26a4*$^{\Delta/\Delta}$ mice did not differ in blood pressure.

In conclusion, the data suggest that cochlear pendrin expression supports recovery after noise-induced hearing loss. The data suggest that restoration of cochlear pendrin expression may be beneficial to slow progression of hearing loss in humans carrying mutations of *SLC26A4*.

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Multiplex Serology for Common Viral Diseases in Sera from Feral Pigs (Sus scrofa) in Kansas.

Brandon Bell

Author(s): Brandon Bell, Nicholas Monday, Izabela Ragan, Benjamin Trible and Bob Rowland

Viral diseases adversely affect the health and production of animals in the swine industry. Preventing disease outbreaks requires constant surveillance and prompt diagnosis. Despite biosecurity efforts, reservoirs of disease remain with the potential to spread pathogens. Feral pig (*Sus scrofa*) populations may be one such reservoir. Traditional serological assays are costly as each assay tests for one pathogen. Advances in multiplex technology allow for more economical surveillance. Fluorescent microsphere immunoassay (FMIA) is a high-throughput multiplex format for the detection of up to 50 analytes in one sample. The aim of this study was to use FMIA to evaluate the presence and prevalence of several common porcine viruses in feral pigs in Kansas. The microsphere (bead) sets included two antigens each for Porcine Reproductive and Respiratory Syndrome Virus (PRRSV), Swine Influenza Virus (SIV), and Porcine Circovirus Virus Type 2 (PCV2), and two negative controls. Feral pig sera (n=188), as well as positive and negative control sera for each virus, were plated in duplicate in 96-well plates and combined with the bead panel. Bead-bound antibodies (Abs) were labeled with biotinylated goat anti-swine Ab and Streptavidin-Phycoerythrin. Beads were run in the Luminex MagPIX analyzer to identify Ab-bound and unbound beads by fluorescence. Signals were reported as Median Fluorescent Intensity (MFI) and used to compute Sample-to-Positive (S/P) ratios for each sample. Abs to PCV2 were the most prevalent (36/188 = 19%), while SIV and PRRSV Abs were less prevalent (3.7 and 1.0%, respectively). These results indicate that feral populations in Kansas are a potential pathogen reservoir.

"Escherichia coli and coliforms on hide and pre-intervention carcasses in beef processing plants"

John Brandsma

Author(s): J. F. Brandsma, M. W. Sanderson, N. Cernicchiaro, A. McKiearnan

Shiga toxin-producing *Escherichia coli* (STEC) are foodborne pathogens that can cause illness and death in humans. These organisms are shed by cattle, their potential reservoir, via feces. Hide removal may be a point of carcass contamination during beef processing. To assess contamination, indicator organisms *E. coli*/coliforms (EC) are quantified, as proxies of STEC. The objective of this study was to determine prevalence and concentration of contamination of fecal-origin (EC) of hide-on and pre-intervention hide-off carcasses during commercial beef processing. Samples were collected from four commercial beef processing facilities in TX, KS and NE from June 8th to 30th, 2015 on 8 sampling days. Collection occurred at two points on the processing line, hide-on and pre-intervention hide-off. Sponges were used to sample an area of 2,700 cm^2 from brisket to umbilicus. Twenty samples were collected at each processing point, on each plant visit. Serial dilutions were prepared and samples were plated on EC 3M Petrifilms. Samples were incubated and colony forming units (CFU) enumerated. Prevalence of coliforms for hide-on and hide-off samples was 99.4% and 70.6% respectively. For hide-on samples the concentration of *E. coli* had a range of 0 to 1.12x10^6 CFU/100cm^2 with a mean and median of 8.6x10^{4}CFU/100cm^2 and 3.5x10^4 CFU/100cm^2 respectively. Hide-off carcass concentration of *E. coli* was reduced with a range of 0 to 329 CFU/100cm^2, mean 14.8 CFU/100cm^2 and median 2.6 CFU/100cm^2. This study will be used in a quantitative microbial risk analysis model understanding the risk of human illnesses due STEC along the beef chain.

Porcine reproductive and respiratory syndrome virus utilizes nanotubes for intercellular spread

Rui Guo

Author(s): Rui Guo, Benjamin B. Katz, John Tomich, Thomas Gallagher, Ying Fang*

Intercellular nanotube connections have been determined as an alternative pathway for cellular spreading of certain viruses. In cells infected with porcine reproductive and respiratory syndrome virus (PRRSV), nanotubes were observed to connect two distant cells with contiguous membranes, and the core infectious viral machinery (viral RNA, certain replicases and structural proteins) was observed being present in/on the intercellular nanotubes. Live-cell movies tracked the intercellular transport of a recombinant PRRSV that expresses green fluorescent protein (GFP)-tagged nsp2. In MARC-145 cells expressing PRRSV receptor, GFP-nsp2 moved from one cell to another through nanotubes in the presence of viral neutralizing antibody. Intercellular transport of viral proteins did not require the PRRSV receptor, as it was observed in receptor-negative HEK-293T cells after transfection with a full-length cDNA infectious clone of GFP-PRRSV. In addition, GFP-nsp2 was detected in HEK293-T cells co-cultured with recombinant PRRSV-infected MARC-145 cells. The intercellular nanotubes contained filamentous actin (F-actin) with myosin associated motor proteins. The F-actin and myosin-IIA were identified to be co-precipitated with PRRSV nsp1β, nsp2, nsp2TF, nsp4, nsp7-8, GP5 and N proteins. Drugs inhibiting actin polymerization or myosin-IIA activation prevented nanotube formations and viral clusters in virus-infected cells. These data lead us to propose that PRRSV utilizes cytoskeletal machineries inside nanotubes for efficient cell-to-cell spread. This form of virus transport represents an alternative pathway for virus spread, which is resistant to the host humeral immune response.

Systemic and pulmonary deliveries of apoptosis inducer gene by polylysine nanoparticles inhibit mouse lung carcinoma allograft growth

Susumu Ishiguro

Author(s): Susumu Ishiguro, Nabil A. Alhakamy, Deepthi Uppalapati, Cory J. Berkland, and Masaaki Tamura

Transfection efficiency and toxicity concerns remain a challenge for gene therapy. Nanoparticle-based gene delivery technique potentially overcomes these concerns and may be applicable to cancer gene therapy. Cell penetrating peptides (CPPs) have been broadly investigated to improve the transfection of genetic material (e.g., pDNA and siRNA). Here, we report a newly synthesized polylysine CPP (K9 peptide)-based gene therapy for lung cancer treatment. The apoptosis inducer gene, angiotensin II type 2 receptor-encoded plasmid DNA (pAT2R) and K9 peptide (K9-pAT2R) complexes were condensed using calcium chloride (K9-pAT2R-Ca^{2+}). The resulting complexes were small (~150 nm) and showed high levels of gene expression *in vitro* without any cytotoxicity in several different human and mouse cell lines. Additionally, this K9-pDNA-Ca^{2+} complex demonstrated cancer targeted gene delivery when administered *via* intravenous (IV) injection or intratracheal (IT) spray into LLC cell orthotopic allograft-bearing mice. In the K9-pAT2R-Ca^{2+} IT and the K9-pAT2R-Ca^{2+} IV treatment groups, tumor growth in the lung was significantly smaller than those of the control PBS, K9-pLUC-Ca^{2+} IT or K9-pLUC-Ca^{2+} IV groups, These results suggest that LLC tumor growth was attenuated by apoptosis inducer gene, pAT2R, delivery. Immunohistochemical analysis confirmed that the complex effectively delivered pAT2R to the cancer cells, where it was expressed mainly in cancer cells along with bronchial epithelial cells. A single administration of these complexes markedly attenuated lung cancer growth offering preclinical proof of concept for a novel non-viral gene delivery method exhibiting effective lung tumor gene therapy *via* either IV or IT administration.

Mutations in a Highly Conserved Motif of nsp1β Protein Attenuate the Innate Immune Suppression Function of Porcine Reproductive and Respiratory Syndrome Virus

Yanhua Li

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Porcine reproductive and respiratory syndrome virus (PRRSV) nonstructural protein 1β (nsp1β) is a multifunctional viral protein, which is involved in suppressing the innate immune response and activating a unique -2/-1 programmed ribosomal frameshifting (PRF) signal for the expression of frameshifting products. In this study, site-directed mutagenesis analysis showed that the R128A or R129A mutation introduced into a highly conserved motif (123GKYLQRRLQ131) reduced the ability of nsp1β to suppress interferon beta (IFN-β) activation and also impaired nsp1β's function as a PRF transactivator. Three recombinant viruses, vR128A, vR129A, and vRR129AA were characterized. In comparison to the wild-type (WT) virus, vR128A and vR129A showed slightly reduced growth abilities, while the vRR129AA mutant had a significantly reduced growth ability in infected cells. Pigs infected with nsp1β mutants had lower levels of viremia than did WT virus-infected pigs. Compared to the WT virus in infected cells, all three mutated viruses stimulated high levels of IFN-α expression and exhibited a reduced ability to suppress the mRNA expression of selected interferon-stimulated genes (ISGs). In pigs infected with nsp1β mutants, IFN-α production was increased in the lungs at early time points postinfection, which was correlated with increased innate NK cell function. Furthermore, the augmented innate response was consistent with the increased production of IFN-γ in pigs infected with mutated viruses. These data demonstrate that residues R128 and R129 are critical for nsp1β function and that modifying these key residues in the GKYLQRRLQ motif attenuates virus growth ability and improves the innate and adaptive immune responses in infected animals.

Characterization of the heat inactivation profiles of arthritogenic alphaviruses

So Lee Park

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Plaque reduction neutralization test (PRNT) is the gold standard of serological diagnosis that demonstrates neutralizing capacity of serum samples. Heat inactivation at 56^{o}C for 30 minutes is required prior to the assay in order to eliminate adventitious viruses and complement activity. Although this procedure is well accepted for the serological diagnosis of flaviviruses and alphaviruses, recent studies have determined that longer heat inactivation procedures are required for some alphaviruses. Incomplete inactivation can potentially pose a serious risk to laboratory personnel and cause false negative results in laboratory diagnosis and serological surveillance. Recently, large outbreaks and rapid increase in disease burden of alphaviruses have been documented worldwide. Whilst significant amount of resources have subsequently been dedicated for the surveillance systems of various alphaviruses epidemics, it is critical to determine the thermostability of alphaviruses in order to ensure the high sensitivity of PRNT. In this study, the thermotolerance of Ross River, Barmah Forest, and o'nyong-nyong viruses were investigated. Our results indicate that thermostability may be a phenotype that is virus-specific in this genus. Therefore, evidence-based heat inactivation procedures for alphaviruses will be recommended for serological diagnosis.

Escherichia coli and coliforms on beef carcasses prior to and after evisceration in beef processing plants

Austin Pauly

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Shiga toxin-producing *E. coli* (STEC) and other coliforms cause foodborne illness and are of public health importance. When cattle are harvested, bacteria could be transferred from the gastrointestinal organs onto the carcass during evisceration. The objective of the study was to determine the potential effect of evisceration on bacterial contamination of carcasses during commercial beef processing. On each sampling day, surface sponge samples were collected from 20 carcasses both before and after evisceration. Each of four large commercial beef processing plants in Texas, Kansas, and Nebraska were sampled three times in June and July 2015. Samples were cultured for indicator organisms, *E. coli* and coliforms, using Petrifilm plates for bacteria enumeration. A total of 160 carcasses (320 samples pre- and post-evisceration) were collected. Pre-evisceration, most carcasses had no *E. coli* (61%) or coliforms (54%); concentrations for enumerable samples ranged between 237 to 379 and 2120 to 6210 CFU/100cm^2, respectively. Post-evisceration, most carcasses again had no *E. coli* (67%) or coliforms (46%); concentrations for enumerable samples ranged between 23 to 429 and 437 to 2970 CFU/100cm^2, respectively. Results show contamination of beef carcasses with *E. coli* and coliforms was not common either pre- or post-evisceration. However, these data are only from four weeks of a larger, year-long study. This research provides information about potential risks of bacterial transfer during evisceration in commercial processing plants, and these data will be used in a quantitative microbial risk assessment model to improve management of potential risks of STEC human illnesses attributed to beef.

Epitope Mapping of ASFV p72 Capsid Protein using Polyclonal Swine Sera and Monoclonal Antibodies

Mallory Phillips

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African swine fever is a hemorrhagic disease of domestic pigs caused by African Swine Fever Virus (ASFV), belonging to the family *Asfarviridae*. Its multilayer virion is composed of more than 34 proteins, with p72 being the major capsid protein. A single conformational neutralizing epitope has been identified on p72, but information on the other antigenic regions (epitopes) is lacking. The objective of this study was to identify p72 epitopes using polyclonal swine sera and a panel of monoclonal antibodies (mAbs). The p72 protein, from amino acid (aa) 1 to 345, was divided into 5 overlapping fragments. Fragments, produced by PCR or commercially synthesized, were cloned into pHUE expression vector, and transformed into *E. coli* competent cells. The recombinant proteins were expressed *in vitro*, purified and used as antigens in indirect ELISA and Western Blotting (WB) to test mAbs and polyclonal swine sera. The mAbs were produced against a p72 protein based on the ASFV Georgia/07 strain. The polyclonal sera were from pigs immunized with a defective alphavirus replicon particle, RP-sHA-p72, expressing a recombinant protein composed of the extracellular domain of the ASFV HA protein together with the whole p72 protein. The polyclonal sera reacted to the p72 region between aa 1 and 83 and aa 250 and 280. The mAbs reacted with the p72 regions between aa 100 and 171; 180 and 250; 280 and 345. Studies are in progress to perform the fine mapping of the identified epitopes.

Loss of TGFβ-signaling in dendritic cells leads to severe epididymitis, antisperm antibodies and no orchitis.

Fernando Pierucci-Alves

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Sperm are immunogenic and can induce severe immune responses in males and females. Human reproductive pathologies that lead to infertility such as antisperm antibodies (ASAs) and aseptic epididymo-orchitis have been thought to unfold as consequences of disruptions in mechanisms that maintain immunotolerance to sperm. However, causal factors remained unidentified. In this study, we determined that mice lacking transforming growth factor β (TGFβ)-signaling in dendritic cells (*Tgfbr2*$^{\Delta DC}$ mice) exhibit epididymal leukocyte infiltrations that reach sperm in the lumen, and circulating ASAs. Epididymal leukocytosis progresses to formation of large sperm granulomas, while the testes exhibit no histopathology. These data suggest that dendritic cell-specific TGFβ-signaling supports immunotolerance to sperm physiologically present in the lumen of the epididymal duct, and that the *Tgfbr2*$^{\Delta DC}$ male is a potentially unique animal resource to model human antisperm antibodies (ASAs), aseptic epididymitis and aseptic leukocytospermia. To broaden our understanding of mechanisms leading to pathology in the *Tgfbr2*$^{\Delta DC}$ epididymis, epididymides with mild to undetectable leukocytosis and control tissues were subject to total RNA isolations and microarray analyses (Mouse Gene 2.0 ST; Affymetrix). The *Tgfbr2*$^{\Delta DC}$ epididymis revealed 656 transcripts regulated by a fold change (FC) greater or smaller than + or - 1.5, respectively. Among the 50 genes with greatest upregulation (2.7 ≤ FC ≤ 12.0) were 9 interferon-induced GTPases: guanylate-binding proteins (Gbp) 2, 2b, 3, 4, 7 and 10; immunity-related GTPase family M (Irgm) proteins 1 and 2; and interferon-induced GTPase 1 (a.k.a. as Iigp or Irga6).

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Chronic wasting disease: in vitro analysis identifies and distinguishes resistant prion protein polymorphisms

Rachel Rielinger

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Despite high homology among mammals, the amino acid sequence of the prion protein (PrP) has subtle allelic variations depending on the species. Some of these polymorphisms have been shown to promote resistance, while others contribute to prion disease susceptibility. Previous studies have concluded that amino acid variability in white-tailed deer at codons 95, 96, 116, and 226 can have an impact on resistance, with resistance commonly characterized as a delayed progression of chronic wasting disease (CWD). Elk are known to have a resistant codon at position 132, while other cervids and conspecifics (e.g. fallow deer and antelope) seem to be naturally resistant to CWD. Various polymorphisms, either individually or in combination, were examined for amplification efficiency in vitro using real time quaking-induced conversion (RT-QuIC) to assess whether multiple codons may act in synergy to promote resistance. Each PrP allele was tested against eight different CWD isolates, representing a range of host species, genotypic backgrounds, and geographic origins. It was hypothesized that the allele with the greatest number of previously identified resistant genes would exhibit the lowest amplification efficiency. The results indicate that the greatest determinants of CWD resistance lie at codons 95, 96, and 226, with some evidence of synergy between multiple resistant codons. These findings will be beneficial to both cervid farmers and wildlife professionals, who can use this information to predict *in vivo* susceptibility to establish selective breeding programs and evaluate local susceptibility and CWD enzootic risk, respectively.

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Measuring Bovine γδ T Cell Function at the Site of *Mycobacterium bovis* Infection

Rachel Rusk

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The causative agent of tuberculosis in cattle is *Mycobacterium bovis.* The characteristic lesions of bovine tuberculosis are well-organized pulmonary granulomas. γδ T cells are a unique subset of nonconventional T cells that play major roles in both the innate and adaptive arms of the immune system. Bovine γδ T cells have the capacity for multiple immune functions during infection with *M. bovis.* However, the alternative functions of γδ T cells as well as the responses of γδ T cells *in vivo* at the site of infection remain unclear.

Using a bovine model of experimentally induced *M. bovis* infection, tissue samples from granulomatous lesions in the lungs and mediastinal lymph nodes were collected 3 months after infection. mRNA transcripts for γδ T cells, IFN-γ, IL-17, IL-10, and CCL2 were microscopically evaluated within the granulomas using an *in situ* hybridization system, RNAScope (Advanced Cell Diagnostics Inc.). Transcriptomics analysis was completed on RNA isolated from peripheral blood γδ T cells from M. *bovis* infected cattle. Differentially expressed genes were confirmed with real-time PCR.

The novel ISH assay confirmed that bovine γδ T cells accumulate within the granulomas. However, cytokine expression by the γδ T cells varied within the lesions. We demonstrate here that γδ T cells are not a predominant source of IFN-γ, IL-10, or IL-17 *in situ* at this time-point after infection. Interestingly, γδ T cells were determined to express significant levels of CCL2 within late-stage granulomas. Results observed *in vivo* were also confirmed by transcriptomics and qPCR analysis.

Rift Valley Fever Virus Protein Found by Multiple Immunohistochemical Methods

Elizabeth Stietzle

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Rift Valley Fever Virus (RVFV) is a zoonotic arbovirus endemic to the continent of Africa that spread to the Arabian Peninsula in 2001. RVFV causes abortions, malformed neonates and high mortality in young ruminants including cattle and sheep. In humans, RVFV causes an acute febrile illness but can progress to encephalitis, retinitis or hemorrhagic fever. A potential bioweapon, RVFV is classified as a high priority agent due to a lack of antiviral treatments and approved vaccinations for use outside of endemic countries. This work is part of a larger set of studies focused on developing challenge models in sheep and cattle for testing new vaccines and therapies for RVFV.

Here we hypothesized that we could successfully use polyclonal and monoclonal antibodies raised against a variety of RVFV proteins that were previously untested for immunohistochemistry (IHC) to identify viral antigen distribution by bright-field and fluorescence microscopy in multiple tissues. Using formalin-fixed paraffin-embedded tissue samples from acute and chronic post-infection time-points in both sheep and cattle RVFV studies, we identified viral antigen with antibodies raised against RVFV's nucleoprotein, aminoterminal glycoprotein, and carboxyterminal glycoprotein. An avidin biotin complex technique was most successful for IHC and a one-step indirect technique for immunofluorescence. Optimal antibody concentration, chromogen usage and counterstain selection were established for each tissue type and host species. In general, viral antigens were found within lesions already identified by histopathology. These results provide tools for the RVFV studies and a starting point for multi-label IHC studies focused on questions regarding RVFV pathogenesis.

The pharmacodynamics of antimicrobials changes depending on bacterial susceptibility

Dr. Xuesong Wen

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The relationship between drug concentration and antimicrobial effect can be described by a pharmacodynamic model which is a sigmoidal function. The parameters of the model include the baseline bacterial population growth rate in the absence of drug, the maximal inhibitory effect of the drug on bacterial growth (the term growth is inclusive of the population decay at high drug concentrations), and the Hill-coefficient that reflects the sensitivity of the bacterial growth rate to increases in the drug concentration. We hypothesize that the values of Hill-coefficient and the maximal inhibitory effect change depending on bacterial susceptibility. We tested this hypothesis using enrofloxacin, a bactericidal fluoroquinolone antimicrobial that demonstrates a concentration-dependent effect dynamics against fully susceptible bacterial strains. We performed time-kill experiments with enrofloxacin for a set of *Mannheimia haemolytica* and *Pasteurella multocida* strains with different susceptibility to the drug. The susceptibility was measured by the drug minimum inhibitory concentration (MIC). Our preliminary findings indicate that the value of the Hill-coefficient and maximal inhibitory effect did change as a function of the MIC. The lower the bacterial susceptibility (a higher MIC), the less sensitive is the bacterial growth rate to changes in drug concentration (lower Hill-coefficient). These results, the pharmacodynamic shift to time dependence, suggest that infections by bacterial strains with reduced susceptibility to the antimicrobial could require changes in the dosing interval to ensure successful treatment.

Role of Nitric Oxide on Capillary Hemodynamics during Post-Occlusive Reactive Hyperemia

Jennifer L. Wright

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PORH is an accepted diagnostic test used for evaluating endothelial NO function in humans. However, CH during PORH remain unknown despite the microvasculature being the site of its control. CH were measured via intravital microscopy in the isolated spinotrapezius muscle of Sprague-Dawley rats during superfusion conditions of control (CON; Krebs-Hanseleit solution), sodium nitroprusside (SNP; NO donor; 30 μM) and N^G-nitro-L-arginine methyl ester (L-NAME; nonspecific NO synthase inhibitor; 1.5mM) followed by 1 min feed artery occlusion (FAO) and subsequent reperfusion at 30 and 300 secs. The number of flowing capillaries (cap#), red blood cell velocity (RBC_{vel}) and flux (RBC_{flux}) through the capillaries remained unchanged in CON; but where ↑ (+7%, $p<0.03$) and ↓ (-17%, $p<0.03$) in SNP and L-NAME following superfusion, respectively. PORH induced similar ↓s in cap# across CON, SNP and L-NAME (-4 to -8%, $p<0.03$) at 30 secs of reperfusion and were followed by similar ↑s at 300 secs (+6 to +11%, $p<0.05$) of reperfusion suggesting a minimal role for NO. In contrast, RBC_{vel} and RBC_{flux} was ↑ in CON (+40% and +27% respectively, $p<0.03$), ↓ in SNP (-49% and -56% respectively, $p<.05$) and remained unchanged in L-NAME (+3%, both, $p>0.05$) at 30 secs after reperfusion with no further changes after 300 secs of reperfusion supporting a regulatory role for NO for these parameters. We conclude that NO bioavailability plays an important role in the PORH response of the spinotrapezius muscle of the rat as indicated by ↑s in RBC_{vel} and RBC_{flux} following 30 secs of reperfusion after FAO.

Development of a Sandwich ELISA Specific for EHEC O157:H7 γ-intimin Types

Xuehan Zhang

Author(s): **Xuehan Zhang, Meng Li, Bicheng Zhang, Philip R. Hardwidge**

Enterohemorrhagic *Escherichia coli* (EHEC) O157:H7 is a zoonotic pathogen of worldwide importance that caused foodborne infections in humans. Gamma intimin is one of the most important outer membrane proteins required for EHEC's intimate adhesion to epithelial cells. Here we generated a polyclonal antibody (pAb) and a monoclonal antibody (mAb) against EHEC O157:H7 γ-intimin to develop a double antibody sandwich ELISA (DAS-ELISA) with increased sensitivity and specificity to detect EHEC O157:H7. We used a rabbit pAb as a capture antibody and a mouse mAb as a detection antibody. No cross-reactivity was observed with the other pathogenic bacteria tested. The detection limit of the DAS-ELISA was 1×10^3 CFU/mL for EHEC O157:H7 cultures, 1×10^4 CFU/g before enrichment and 1×10^2 CFU/g after enrichment of contaminated samples. Field samples (n=498) were tested both using a previously established Duplex-PCR method and our newly developed DAS-ELISA. The DAS-ELISA had a specificity of 94.4 %, a sensitivity of 91.5 % and accuracy of 94.0 % as compared with Duplex-PCR. The DAS-ELISA developed here could be applied in EHEC O157:H7 detection from food, animal, and environmental samples.

Rupture of a Right Adrenal Gland Tumor Causing Hemoabdomen in a Dog.

Kyle Pfeifer

Author(s): Kyle Pfeifer, Laura Armbrust

A 13-year-old female spayed English Setter was referred to the Kansas State University Veterinary Health Center for hemoabdomen. On examination, the dog was lethargic, tachycardic, tachypnic and hypertensive. Abdominal ultrasound was performed, showing a left adrenal mass and right cranial abdominal mass. Treatment of hypertension was initiated with phenoxybenzamine for a suspect pheochromocytoma. Three days later the dog developed increased respiratory rate, effort and hypoxemia. Thoracic radiographs were performed, nasal insufflation was placed and heparin was added to the treatment regimen for suspect pulmonary thromboembolism. After two weeks of treatment without improvement, the owners elected to pursue surgical removal of the masses. Contrast enhanced thoracic and abdominal computed tomography was performed immediately prior to surgery to assist planning. Images of the thorax showed small areas of atelectasis in the left ventral lung field. Images of the abdomen showed left adrenomegaly, an irregularly marginated spleen with irregular shaped nodules, and a right cranial abdominal mass (liver or adrenal gland origin). Surgical exploration revealed bilateral adrenal masses, with rupture of the right adrenal capsule forming a mass of clotted blood between the liver and right adrenal gland. Bilateral adrenalectomy, and splenectomy were performed. Biopsy samples yielded a histologic diagnosis of diffuse, severe bilateral cortical nodular hyperplasia of both adrenal glands and multifocal infarctions in the spleen. The right adrenal gland was determined to be the most likely source of the dog's hemoabdomen, an occurrence reported rarely in dogs.

Ocular Involvement in a Dog with Sterile Neutrophilic Dermatosis

Emily Sharpe, DVM

Author(s): Emily Sharpe

An 8 year old Bichon frise presented to Kansas State University Veterinary Health Center with a one week history of periocular swelling, hyporexia, and multiple cutaneous papules. On presentation the patient was febrile, exophthalmic, and the right eye had a superficial corneal ulcer secondary to exposure. There were multifocal to coalescing erythematous papular to pustular eruptions on the skin of the distal limbs, base of the tail, and the right periocular tissue. Complete blood count revealed a mature neutrophilia. Ocular ultrasound and computed tomography revealed moderate soft tissue thickening surrounding the right globe, mild lateral displacement of the right globe, and compression of its nasal aspect. There was effacement of the margins of the extraocular muscles and vasculature and loss of retrobulbar fat visualization. Histopathologic evaluation of the skin and periocular lesions revealed a sterile neutrophilic dermatitis resembling Sweet's syndrome in humans. Sweet's syndrome, also known as acute febrile neutrophilic dermatosis, is characterized by the presence of fever, neutrophilia, and painful, raised cutaneous plaques that are characterized by severe neutrophilic inflammation of the dermis on histopathology. The condition responded rapidly to corticosteroid therapy. In the absence of an infectious etiology or neoplastic process, sterile neutrophilic dermatosis should be considered as a differential diagnosis in dogs with cutaneous neutrophilic infiltrates.

A Case of Idiopathic Eosinophilic Pneumonia in a Horse

Amanda Trimble

Author(s): Amanda Trimble

A 16 year old Quarter Horse gelding presented to the VHC for an acute onset of diffuse, non-pruritic, generalized and coalescing urticaria. Low dose dexamethasone resulted in apparent disease resolution within twenty-four hours. However, at approximately 48 hours after onset, colic signs were observed. The patient was febrile and thoracic ultrasound was abnormal, evidenced by bilateral pleural roughening, mild pulmonary consolidation and slight unilateral pleural effusion. Transtracheal wash was performed, and antimicrobials were initiated. Poor clinical response to therapy resulted in the necessity for bronchoalveolar lavage which revealed a predominance of pulmonary eosinophils. Peripheral eosinophilia was also present at this time. A diagnosis of Idiopathic Eosinophilic Pneumonia was made. Treatment included a tapering dose of dexamethasone over four weeks. Due to incomplete disease resolution he was moved to a different environment with apparent disease resolution.

An Unusual Case of Cholangiohepatitis in an Adult Horse

Michelle Tucker, DVM

Author(s): Michelle Tucker, DVM

A 12 year old American Paint mare presented for markedly elevated serum hepatic enzymes in association with a three day history of colic. Severely elevated liver enzymes, ileus, free peritoneal fluid and colic were noted on presentation. Abdominal exploratory laparotomy was necessary due to refractory pain. At surgery, a large colon impaction and small liver with rounded borders were identified. Biopsy samples were obtained and submitted for histopathology. Upon recovery, she was maintained on fluids, injectable broad-spectrum antibiotics and anti-inflammatory therapy. Post-operatively she remained comfortable and demonstrated an overall decline in liver enzymes with improved liver function testing. Histopathology revealed cholangiohepatitis evidenced by mild inflammation and bridging, periportal fibrosis consistent with chronic biliary obstruction. Eight weeks following surgery the mare is healthy and has normal hepatic enzyme concentrations. Equine cholangiohepatitis is an incompletely understood hepatic disease. Debate exists as to whether inflammation occurs secondary to choledocholithiasis or biliary obstruction occurs following ascending infection and inflammation of the biliary system. In the reported case, chronic biliary stasis with an overlying acute component is suspected to be the cause of this patient' signs, the combination of surgical and medical management resulted in a favorable outcome.