**Abstracts**

**Annual Student Research Day**

**March 19, 2021**

1. **Review: The Role of Host Cell Calcium in Viral Propagation and Pathogenesis**  
   **Nicholas E. Anderson**, Ronald Harty and Bruce D. Freedman, Department of Pathobiology, University of Pennsylvania School of Veterinary Medicine, Philadelphia, PA.  
   VMD

Viruses are obligate intracellular pathogens that utilize and depend upon host machinery for propagation. The high rates of evolution and adaptation by viruses has challenged anti-viral therapeutic development by producing a moving target for strategies such as vaccination and virus targeted therapeutics. Thus, approaches that may initially be effective often do not produce durable protection. By contrast, therapeutics designed to target host mechanisms and pathways required by viruses to enter, replicate, or leave a host cell are potentially immutable and thereby durable targets to disrupt viral replication and disease transmission. One key host signal that is required by viruses is intracellular calcium. Calcium is a “second messenger” that dynamically regulates host mechanisms hijacked by viruses for their entry, trafficking, replication, and egress from the cell. Recent efforts have established that calcium pumps,
transporters, and ion channels in the plasma membrane, mitochondria endoplasmic reticulum, and endolysosomal system play a vital role in propagation of many viruses. Consequently, these represent host therapeutic targets for broad spectrum control of a range of virus families and family members. This review of the role of calcium in virus-host interactions seeks to clarify recent research into the underlying mechanisms that govern viral propagation and seeks to highlight areas for future study.

2. Exploring the gene expression profiles of *Dirofilaria immitis* in susceptible and refractory strains of *Aedes aegypti* mosquitoes

Camille S. Andrews, Elizabeth B. Edgerton, Michael Povelones. Department of Pathobiology, University of Pennsylvania School of Veterinary Medicine, Philadelphia, PA. VMD

The parasite *Dirofilaria immitis* causes heartworm disease, a parasitic infection threatening dogs worldwide. Although the disease can be prevented by chemoprophylaxis, the disease remains a widespread problem due to owner non-compliance, significant animal reservoirs, and the emergence of drug-resistant parasite strains. Therefore, it is important to identify new ways to prevent the spread of this potentially life-threatening disease. One possibility being explored is the prevention of dog infection by disrupting *D. immitis* in mosquitoes. A detailed understanding of the molecular interactions between the parasite and its mosquito host would greatly facilitate this effort. Thus, the purpose of this study is to compare gene expression profiles of *D. immitis* developing in infection-susceptible and -refractory strains of mosquitoes to understand the basis of compatibility with the insect host. This study took advantage of well-established laboratory strains of *Aedes aegypti* with strong differences in their *D. immitis* infection susceptibility. In this infection model, *D. immitis* are developmentally arrested in the Malpighian tubules of the refractory strain within the first three days of infection. Malpighian tubule total RNA from the first three days of infection of refractory and susceptible *A. aegypti* was used to map *D. immitis* transcripts. Consistent with their developmental profiles in the different mosquito strains, we found that the total number of *D. immitis* transcripts increased each day in the susceptible mosquitoes but remained relatively constant and low in the refractory ones. Principal component analysis suggests that mosquito susceptibility has a large impact on *D. immitis* gene expression profiles. We found 11,354, and 508 differentially regulated genes between the susceptible and refractory mosquitoes from days 1, 2, and 3, post infection, respectively. Using a gene ontology analysis, we found that the *D. immitis* in the susceptible strain at day 3 post infection showed upregulated genes for metabolic pathways and DNA replication. However, this same time point in the refractory strains showed upregulated genes for ion and transmembrane transport suggesting that differences the extracellular environment of the worms may contribute to their inability to develop. This knowledge could be used to develop novel approaches to prevent *D. immitis* from successfully completing its mosquito life cycle ultimately blocking the transmission of this pathogen.

3. A Tumor-restrictive Role for Type III Collagen in the Breast Cancer Microenvironment

Ashton C. Berger¹, Bassil Dekky¹, Becky K. Brisson¹, Susan W. Volk¹², ¹Department of Clinical Sciences and Advanced Medicine, ²Department of Biomedical Sciences, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA. VMD

The development and progression of cancer is influenced by interactions with non-cancer cells and extracellular matrix components, such as fibrillar collagens, in the tumor microenvironment (TME). Our group has previously shown important regulatory roles for type III collagen (Col3) in healing tissue and in laboratory and companion animal models of breast cancer. Using a murine orthotopic model of triple negative breast cancer (TNBC), our data revealed increased primary and recurrent tumor growth with a higher rate of metastasis in Col3 haploinsufficient (Col3+/-) mice compared to wild type (Col3+/+) mice. We further evinced that Col3 suppresses formation of a tumor-permissive microenvironment as Col3+/- mice tumors had increased desmoplasia, fibrillar alignment, and myofibroblast density relative to Col3+/+ mice. Notably, application of Col3 biomaterials hindered orthotopic tumor growth compared to control.
Our recent preliminary studies immunolabeling human TNBC samples demonstrate elevated Col3 deposition in non-invasive versus invasive regions (p<0.01). To further characterize patterns of Col3 expression and identify molecular signatures of at-risk patients, we are using in silico analyses of over 1000 breast cancer patients from The Cancer Genome Atlas BRCA cohort. Survival analyses indicate that patients with higher Col3:Col1 bulk tumor expression had improved disease-free and progression-free survival relative to those with higher Col1:Col3 expression (p<0.05). Furthermore, within the subset of tumors with a basal molecular subtype, higher Col3:Col1 expression associated with better disease-free, progression-free, disease-specific, and overall survival than higher Col1:Col3 expression (p<0.05). The combination of these data not only reaffirm a tumor-restrictive role for Col3 in breast cancer, but also support pursuing tissue engineering strategies to promote a Col3-rich TME and potentially improve clinical outcomes by limiting tumor recurrence or progression in patients. Research was supported by grants from the NIH (R21CA216552 and R01GM124091) Student Support: Student support was provided by a grant from the NIH (5T35OD010919-23).

4. Identifying Sow Lameness Utilizing Time-of-Flight Cameras
Matthew Boulanger and Thomas Parsons, Department of Clinical Studies, New Bolton Center, University of Pennsylvania School of Veterinary Medicine. VMD

Growing public concern about the welfare of gestating sows is driving changes in husbandry practices as producers are being asked to move away from individual to group housing of sows. However, increased incidence of sow lameness is often reported with group housing. Ambulation can make lameness more noticeable, and agonistic behavior associated with the establishment of a social hierarchy can also lead to increased leg injuries and lameness. Lameness is both a production and welfare problem, but early detection remains challenging as existing metrics are manual and notoriously unreliable. Thus, reliable early detection of lameness promises improved therapeutic outcomes, increased productivity, and improved welfare. This investigation uses time of flight cameras (ToF) to look for correlations between lameness and features of a sow’s dorsum as she walks. ToF cameras generate a physical topography, unlike a visual camera. Allowing curvedness to successfully and automatically identify the spine. Twenty-five sows were trained to traverse a 13-meter course and were recorded with a ToF camera from above as well as a visual camera. While traversing, pigs were graded using the visual camera on a tagged visual analog scale (tVAS) to indicate a clinical lameness. The tVAS is not practical for industrial use and requires a clinician to grade videos. However, it serves as an excellent reference diagnostic. This was then correlated to scalar coefficients generated by fitting the topography above the spine to second order polynomials. Using these polynomial coefficients as index screening tools and tVAS as the reference standard, a logistic regression classification model was trained using supervised machine learning to predict lameness. The developed model achieved a sensitivity of 85%, a specificity of 81%, and an overall accuracy of 84%. This model only utilized the B coefficient. However, permutations of A,B, and C were tested- B alone yielded both the highest sensitivity and accuracy. As a potential screening tool for lameness, it was important to identify a model with high sensitivity. This ensures that farmers and veterinarians have the ability to identify and treat these conditions in their earliest stages and promises the best possible therapeutic outcomes.

5. Development of a RT-LAMP Assay for the Detection of SARS-CoV-2 in Companion Animal Samples at Ryan Veterinary Hospital
Eli Braun, Jaelyn Dietrich, Stephen Cole, and Shelley Rankin, Department of Pathobiology, University of Pennsylvania School of Veterinary Medicine. VMD

In December 2019 Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), an enveloped single-stranded RNA virus, was detected in Wuhan China and has caused a pandemic of Coronavirus Disease 2019 (COVID-19) in humans. Due to their close contact with humans, and the worldwide distribution of the virus, domestic pets can be exposed to high numbers of virus particles. It has been observed that up to 47% of cats
and 15% of dogs that live with a COVID-positive human can have a positive PCR test or serology test. Pets infected with SARS-CoV-2 can suffer from diarrhea, vomiting, coughing, lethargy, and shallow breathing, among other clinical signs. Research is still limited on the zoonotic ability of the virus to transmit from companion animals to humans. Therefore, it is important to develop and validate tests to detect SARS-CoV-2 in specimens specifically from cats and dogs. Using a novel rapid technology, an assay was developed, with the long-term aim of determining the prevalence of the virus in domestic companion animals in the community. The technology chosen uses reverse transcription loop-mediated isothermal amplification (RT-LAMP) to detect a viral gene. The isothermal nature of the reagents will also allow this rapid (20 minute) test to be further developed for use at the point-of-care. This test has now been used to screen for the presence of SARS-CoV-2 in over 400 specimens from animals that presented to the Ryan Veterinary Hospital at the University of Pennsylvania. The assay was verified as fit-for purpose in December 2020 when it detected the SARS-CoV-2 virus in feces, saliva, oropharyngeal, and nasopharyngeal specimens from a dog at Ryan VHUP. The CDC RT-qPCR SARS-CoV-2 assay was also positive with these samples and the test results have since been confirmed by the USDA National Veterinary Services Laboratory and reported to the World Organization for Animal Health (OIE) as the 3rd companion animal case in Pennsylvania. The RT-LAMP assay is a promising step to quick, sensitive, and cost-effective testing for SARS-CoV-2 in domestic pets when compared to current gold-standards. Screening pets with no known risk factors will better gauge the potential for zoonotic exposure to humans.

6. Web-Resource on Available DNA Variant Tests for Hereditary Diseases and Genetic Predispositions in Dogs and Cats

Julia Canino, Jennifer L. Rokhsar, Karthik Raj, Scott Yuhnke, Jeffrey Slutsky, and Urs Giger.
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Vast progress has been made in the clinical diagnosis and molecular basis of hereditary diseases and genetic predisposition in companion animals. The purpose of this report is to provide an update on the availability of DNA testing for hereditary diseases and genetic predispositions in dogs and cats utilizing the WSAVA-PennGen DNA Testing Database web-resource (URL: http://research.vet.upenn.edu/WSAVA-LabSearch). Information on hereditary diseases, DNA tests, genetic testing laboratories, and afflicted breeds added to the web-based WSAVA-PennGen DNA Testing Database was gathered. Following verification through original research and clinical studies, searching various databases on hereditary diseases in dogs and cats, and contacting laboratories offering DNA tests, the data was compared to the resource reported on a decade ago. The number of molecularly defined Mendelian inherited diseases and variants in companion animals listed in the WSAVA-PennGen DNA Testing Database in 2020 drastically increased by 112% and 141%, respectively. The number of DNA variant tests offered by each laboratory has also doubled for dogs and cats. While the overall number of laboratories has only slightly increased from 43 to 47, the number of larger corporate laboratories increased, while academic laboratories have declined. In addition, there are now several laboratories that are offering breed-specific or all-breed panel tests rather than single DNA tests for dogs and cats. This unique regularly updated searchable web-based database allows veterinary clinicians, breeders and pet owners to readily find available DNA tests, laboratories performing these DNA tests worldwide and canine and feline breeds afflicted and also serves as a valuable resource for comparative geneticists. Rokhsar JR*, Canino J*, Raj K, Yuhnke S, Slutsky J, and Giger U; Human Genetics, in press, 2021.
*these two authors contributed equally.

Speaker:
Collagen V Haploinsufficiency Results in Delayed Healing and Altered Wound Matrix Post-Injury in Murine Tendons
Carlson JA, Sun M, Adams SM, Weiss SN, Birk DE, Soslowsky LJ. University of Pennsylvania Perelman School of Medicine and School of Veterinary Medicine. VMD PhD
Patients with Classic Ehlers-Danlos syndrome (cEDS), a disorder characterized most commonly by COL5A1 haploinsufficiency, suffer from tissue hyperelasticity, tendon/ligament fragility and abnormal wound healing. Collagen V (ColV) haploinsufficiency leads to abnormal tissue development and altered collagen assembly, and mechanical loading of the mouse patellar tendon shows a delay in healing and alterations in stiffness and dynamic modulus post-injury (PI). Therefore, the objective of this study was to determine the effect of ColV deficiency in female mice on wound matrix formation and resultant structure-function relationships when mechanical load is applied post-injury. We hypothesized that ColV deficiency will have effects post-injury, resulting in increased fibril diameter and cellularity, decreased mechanical properties and leading to a delayed healing response when compared to wild-type tendons. Col5a1 expression was significantly increased in WT tendons at 1- and 3w PI compared to uninjured controls, with no significant changes in Col5a1 expression seen PI in Col5a1+/- tendons. TEM analysis showed Col5a1+/- fibrils PI were larger and more broadly distributed than WT fibrils. Further, mechanical testing showed that WT tendons realigned through 5% strain, with Col5a1+/- tendons continuing to realign through 6% strain. Lastly, Col5a1+/- tendons had a significant increase in cellularity persisting to 6w PI when compared to uninjured tendons, that was not seen in WT tendons. Without the initial increase in ColV following injury, fibrillogenesis is less regulated, resulting in an altered fibril diameter distribution of Col5a1+/- tendons PI. Additionally, increased cellularity in Col5a1+/- tendons would alter the matrix alignment and architecture, weakening the tissue and affecting mechanical properties. This study indicates that the lack of an early increase in Col5a1 expression PI in Col5a1+/- tendons influences matrix architecture, alignment, and cellularity throughout tendon healing, demonstrating altered and delayed healing compared to WT tendons. This study was supported by NIH/NIAMS AR065995 and the Penn Center for Musculoskeletal Disorders (AR069619).

7. Does Genetic Sex Impact Gene Expression of ACE2 and TMPRSS2 in WT and NPC Cats?

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Immune responses are stronger in females, who have enhanced cytokine production, higher levels of circulating antibodies, and are more likely to reject allografts than males. This strong immune response causes females to exhibit superior survival, including in response to the global pandemic COVID-19 caused by the virus SARS-CoV-2. Strong immune responses in females are proposed to have two etiologies: genetic and hormonal. X chromosome inactivation (XCI) is an epigenetic process that ensures dosage compensation between the sexes. The X is enriched for immune related genes, and it has been shown that some of these genes can escape XCI, resulting in bi-allelic overexpression in females, contributing to an enhanced immune response. Hormones also play a role, as immunity-related genes with hormone response elements in their promoter region are expressed differentially between the sexes. SARS-CoV-2 enters alveolar type II cells (AT2) cells through the ACE2 receptor, aided by TMPRSS2. Increased ACE2 expression seen in females is protective due to its anti-inflammatory properties that protect against lung injury. Cats are a representative model for SARS-CoV-2 infection in humans, as they are infectible, shed virus orally and nasally, and produce a neutralizing antibody response that protects against reinfection. In this study we will investigate sex differences between male and female cats in gene expression of ACE2 and TMPRSS2, two proteins involved in cellular entry of SARS-CoV-2.

8. Platelet-endothelial associations may promote cytomegalovirus replication in the salivary gland in mice

Platelet decline is a feature of many acute viral infections, including cytomegalovirus (CMV) infection in humans and mice. Platelet sequestration in association with other cells, including endothelium and circulating leukocytes, can contribute to this decline and influence the immune response to and pathogenesis of viral infection. We sought to determine if platelet-endothelial associations (PEAs) contribute to platelet decline during acute murine CMV (mCMV) infection, and if these associations affect viral load and production. Male BALB/c mice were infected with mCMV (Smith strain), euthanized at timepoints throughout acute infection and compared to uninfected controls. An increase in PEA formation was confirmed in the salivary gland at all post-inoculation timepoints using immunohistochemistry for CD41+ platelets co-localizing with CD34+ vessels. Platelet depletion did not change amount of viral DNA or timecourse of infection, as measured by qPCR. However, platelet depletion reduced viral titer of mCMV in the salivary glands while undepleted controls demonstrated robust replication in the tissue by plaque assay. Thus, platelet associations with endothelium may enhance the ability of mCMV to replicate within the salivary gland. Further work is needed to determine the mechanisms behind this effect and if pharmacologic inhibition of PEAs may reduce CMV production in acutely infected patients.

**Speaker: Qualitative Assessment of Fracture Configuration and Subchondral Bone Pathology in Horses with Lateral Condylar Fractures of MC/MT3**

Justine Cianci, Kathryn Wulster, Dean Richardson, and Kyla Ortved. Department of Clinical Studies, New Bolton Center, University of Pennsylvania School of Veterinary Medicine.  VMD

Objective: To evaluate fracture configuration and subchondral bone pathology using computed tomographic images in Thoroughbred racehorses with fractures of the lateral condyle of MC/MT3 and investigate the effects of these factors on long-term prognosis. Study Design: Retrospective case series. Sample Population: Thoroughbred racehorses (n=54) with lateral MC/MT3 condylar fracture that had a preoperative CT performed. Methods: Medical records of horses with lateral MC/MT3 condylar fractures were reviewed for age, sex, breed, limb and surgical treatment. Computed tomography scans were evaluated to determine fracture configuration and characteristics. Palmar osteochondral disease (POD), articular comminution, fracture length, location, and displacement and whether the fracture was incomplete or complete was recorded. Racing performance data was obtained from an online database including racing postoperatively, preoperative and postoperative earnings, and speed ratings. Univariable and multivariable analyses determined associations between independent variables and outcomes. Results: Thirty-three (66%) horses raced postoperatively in a median time of 296 days (range 172-590). On univariate analysis, postoperative speed rating was significantly decreased if the horse had multiple screws, a concurrent sesamoid fracture or fractured through their lateral condylar POD lesion. Postoperative racing earnings were significantly decreased in horses who fractured through their lateral POD lesion. The final multivariable analysis assessing racing postoperatively found intact male horses were 22 times more likely to race postoperatively when compared to female horses (p=0.015); geldings did not differ from females. Horses with complete fractures were significantly less likely to race postoperatively when compared to horses with incomplete fractures (p=0.007). Conclusion: The prognosis for racing postoperatively after lateral condylar fracture of MC/MT3 is as favorable as previously reported. Horses with complete fractures are less likely to return to racing when compared to horses with incomplete fractures. Additionally, horses with concurrent lateral POD lesions or sesamoid fractures can be expected to have decreased racing performance if they return to racing. Assessment of lateral condylar fractures using cross-sectional imaging allows for more detailed evaluation of fracture configuration and concurrent bone pathology.

**Speaker: Remodeling the Electron Transport Chain in Macrophages by a Peptide-miRNA Axis**

Megan Clark, Walter Mowel, Jasmine Wright, Sam McCright, Jorge Henao-Mejia. University of Pennsylvania Perelman School of Medicine and School of Veterinary Medicine.  VMD PhD
Macrophages are critical innate immune cells necessary for anti-pathogen immunity, cancer immunosurveillance and wound healing. Powering these diverse responses in macrophages is the dynamically regulated electron transport chain (ETC) within the mitochondria. An important regulator of ETC function is complex IV (CIV), which is critical for oxygen consumption, ATP generation, and cell survival. Humans with loss-of-function mutations in CIV subunits present with severe immunodeficiencies characterized by recurrent bacterial and viral infection, highlighting the importance of CIV in immune responses. Intriguingly, CIV is the only respiratory complex which remodels its protein subunit composition in response to environmental stimuli, such as hypoxia, to modulate its function. This is achieved through the upregulation of subunit isoforms which replace highly homologous core CIV subunits that function to improve cellular fitness. However, whether immune cells remodel CIV subunit composition during immune responses and whether this remodeling is important for myeloid cell function is unknown. Since inflammatory responses are powerful external stimuli that result in mitochondrial reprogramming, we hypothesized that CIV subunit composition is remodeled in macrophages and functions to regulate innate immune responses. To this end, we identified a transcript induced in pro-inflammatory macrophages encoding a miRNA and a highly conserved peptide with striking homology to a core CIV subunit. Intriguingly, our preliminary data indicate that the peptide and miRNA work together to replace a core CIV subunit in response to interferons (IFN). Preliminary data in vitro demonstrated that this subunit switch played a critical role in regulating inflammatory cell death induced by NLRP3 inflammasome activation, as macrophages deficient in this transcript were resistant to pyroptosis. To understand the role of this peptide-miRNA axis in vivo, we explored the impact of remodeling CIV within the tumor microenvironment, where IFN signaling has been shown to be critical. Excitingly, recent preliminary data indicate that mice deficient in this transcript demonstrated increased tumor growth, whereas mice deficient in both the transcript and core CIV subunit displayed significantly reduced tumor burdens. Overall, we hypothesize that this novel peptide and miRNA remodel CIV subunit composition to control innate immune responses in the tumor microenvironment by regulating cellular fitness and survival.

9. The Role of the Lateral Habenula in Central Itch Processing
Suna L. Cranfill and Wenqin Luo. University of Pennsylvania School of Veterinary Medicine and Perelman School of Medicine. VMD PhD

Itch is a complex sensory experience that also encompasses affective and behavioral components, as evidenced by the common presentation of comorbid depressive/anxiety symptoms in chronic itch patients. However, the neural circuits processing these different components of itch remain poorly understood. Here, we identify a novel role for the lateral habenula (LHb), a brain region linked to negative valence and aversive behaviors, in mediating acute itch behavior. Using Fos immunohistochemistry in mice, we found that the LHb is highly activated by both itch and pain stimuli. Activation of itch-responsive LHb neurons during itch stimulation using optogenetic or chemogenetic approaches increases passive behavior and suppresses active itch-related behaviors. However, activation does not affect pain-related behaviors, raising the possibility that LHb ensembles engaged in processing itch are context specific. Our findings thus far suggest a role for the LHb in promoting a passive behavioral state during acute itch, and in linking chronic itch with the maladaptive behavioral states that characterize psychiatric disorders.

10. Characterization of Antimicrobial Prescriptions Administered at Pennsylvania Race Tracks
Katherine Dorph, Joanne Haughan, Mary A Robinson, and Laurel E Redding. Department of Clinical Studies New Bolton Center, University of Pennsylvania School of Veterinary Medicine. VMD

Racehorses stabled at racetracks are frequently administered antimicrobials, but the types, frequency and indications for these treatments have yet to be characterized. Antimicrobial resistance is an increasingly
urgent problem in horses, especially in the case of respiratory disease, which can be highly infectious and spread rapidly through horses housed close together. The purpose of this study was to examine antimicrobial prescribing at four of six racetracks (two Standardbred and two Thoroughbred) in Pennsylvania during the summer of 2018 (peak racing season) to investigate the frequency of antimicrobial use, drug choice, variation across tracks, variation across time, and reasons for administration. Treatment sheets documenting daily prescribed medications at each racetrack were collected and manually transcribed. Information on all antimicrobial treatments was then extracted and characterized. The percentage of total treatments that were antibiotics ranged from 7% to 11% across tracks. Enrofloxacin was the most frequently prescribed antimicrobial (32% of prescriptions), followed by gentamicin (21%) and ceftiofur (14%). The proportion of treatments that were antibiotics varied significantly by track, trainer, and veterinarian. The most common indication for treatment with antibiotics was “infection”. However, because listed “reasons for treatment” on treatment sheets were often vague (e.g., “sick”, “infection”), illegible or sometimes missing, our ability to assign a precise indication to most dispensations was limited. For this reason, it was impossible to assess the judiciousness of antimicrobial use on the racetracks. Nevertheless, this study yielded valuable information on antimicrobial prescribing patterns and is the first to document antimicrobial prescriptions in the racetrack setting, where the risk of infection transmission is increased due to the high population density. We found that a high priority critically important antimicrobial (enrofloxacin) was the most frequently used antimicrobial, which could have implications for the selection and spread of antimicrobial resistance. Future directions include correlating antimicrobial use patterns with the presence of antimicrobial resistant organisms and more precise elucidation of indications and durations of therapy to assess judiciousness of antimicrobial use in the racetrack setting.

11. Prognostic Value of Neutrophil-to-Lymphocyte and Platelet-to-Lymphocyte Ratios in Critically Ill Canine Patients

Lisa H. Dourmashkin; Bridget Lyons; Rebecka S. Hess; Koranda Walsh; and Deborah C. Silverstein. Department of Clinical Sciences and Advanced Medicine, University of Pennsylvania School of Veterinary Medicine. VMD

Objective: To evaluate whether the neutrophil-to-lymphocyte ratio (NLR) and platelet-to-lymphocyte ratio (PLR) are accurate prognostic indicators and correlate with illness severity scores in critically ill canine patients. Design: Prospective observational study from December 2016 to May 2017. Setting: Intensive care unit at a veterinary teaching hospital. Animals: Seventy-two client-owned dogs admitted to the ICU with CBCs and abbreviated and complete acute patient physiologic and laboratory evaluation (APPLEfast and APPLEfull) scores were enrolled. Measurements and main results: The NLR, PLR, APPLEfast, and APPLEfull scores were calculated for each patient on the day of admission. Patients were followed from admission to discharge and diagnosis, survival, and length of hospitalization were recorded. The patient population was assessed as a whole and as subcategories of patients with neoplastic disease, infectious disease, sepsis, and severe hemorrhage. Dogs with non-septic disease processes (n=52) that died had a significantly lower median PLR (p=0.04) compared to those that survived. The PLR was predictive of ICU length of stay in dogs with severe hemorrhage (p= 0.03, Spearman’s rho 0.84). The NLR was positively correlated with APPLEfull (p= 0.04, Spearman’s rho 0.24) and PLR was negatively correlated with APPLEfast scores (p= 0.02, Spearman’s rho -0.27). Conclusions: The PLR correlated with length of ICU stay and survival for certain populations of critically ill canine patients, and PLR and NLR correlated with illness severity as measured by APPLE scores. Future studies with larger sample sizes are warranted to further support the use of NLR and PLR as indicators of morbidity and illness severity.
12. Effects on Longevity and Productivity for Sows in Pen Gestation Removed for Lameness
Ashley Hallowell and Meghann Pierdon, Department of Clinical Studies, New Bolton Center, University of Pennsylvania School of Veterinary Medicine.

Lameness has been a problem in swine production for many years and becomes more important as the use of pen gestation increases. Our objective was to determine the impact of lameness on sow productivity and lifespan in herds using pen gestation. Production records were collected from 11 farms using pen gestation for the removal of 109,672 sows over 5 years (2014-2019). Farms were enrolled if recording reasons for over 80% of removals. Cox proportional hazard analysis was performed to determine differences in survival time. All data were non-normal and Mann Whitney U-test was used to determine the difference in productivity. Pearson Chi Square test was used to determine the odds ratios. Results are reported as median and interquartile range. P<0.05 was the level of significance. Top removal reasons included reproduction (54.5%), age (17.3%), lameness (12.5%), body condition (3.9%), and sudden death (2.8%). Lameness had significantly shorter days in the herd (219) compared to sows removed for other reasons (504) resulting in fewer litters for lame sows (1+/-2) compared to others (3+/-5) (p<0.001). Lameness in dynamic pens had fewer days in the herd (192) compared to static pens (259) (p<0.001). Lameness mixed prior to pregnancy detection lived fewer days (213) compared to post pregnancy detection (263) (p<0.001). Nonproductive days per parity were higher for lame sows (44.0+/-44) compared to others (24.8+/-60.7) (p<0.001), increasing the cost of lameness. Born alive per litter was lower for lame sows (12.7+/-3.0) compared to other reasons (12.8+/-2.8) (p<0.001), meaning lame sows are having fewer piglets. The odds of being removed as a gilt were higher for lame animals compared to other reasons (OR=2.28; CI 2.20-2.37). The odds of a lame animal being euthanized or dying were higher than animals removed for other reasons (OR=6.82; CI 6.56-7.09) thus removing the salvage value of the animal. Recognizing the cost of lameness is important because it helps swine producers and veterinarians understand how much and where they can invest in fixing the problem.

13. Evaluation of Meropenem Prescribing Practices at a Veterinary Teaching Hospital Prior to an Outbreak of Carbapenem Resistant E. coli
Stephen D. Cole, Ashley Hallowell, Dayana Perez-Bonilla, Shelley C. Rankin, and Laurel Redding
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Carbapenems are a critically important class of antimicrobials that are used to treat multidrug resistant Gram-negative infections. Use of carbapenems in veterinary medicine is off-label and should be reserved for cases with limited therapeutic alternatives and where animals have a high likelihood of survival and should be guided by culture and susceptibility (C&S) results. The specific aim of this study was to describe the use of meropenem by prescribers at a referral veterinary teaching hospital. Pharmacy records between 2013 and 2018 were reviewed for prescription of a carbapenem. Patients prescribed meropenem were characterized by species, signalment, primary clinical service responsible for their management and underlying disease. Culture and susceptibility testing results were also reviewed. Seventy-eight animals met inclusion criteria for the study. Species prescribed meropenem were 61 dogs and 17 cats across 104 and 31 visits respectively. The mean (SD) age was 9.8 (4.6) years with a sex distribution of 47% female and 53% male. Visits were primarily to the medicine (n=65; 48.1%) and intensive care (n=39; 28.9%) services. The remaining visits were to surgery (n=13; 9.6%), emergency (n=9; 6.7%) and oncology services (n=9; 6.7%). C&S was performed at 99 visits (82%). Bacteria were isolated in 83 cultures (83.8%). A single gram negative organism was isolated from 55 cases (55.6%): Escherichia coli (n=41), Enterobacter spp (n=7) and Pseudomonas aeruginosa (n=7). A single gram positive organism was isolated from 12 cases (12.1%): Staphylococcus spp. (n=5), Enterococcus spp. (n=5), Streptococcus spp. (n=2). Two organisms were isolated from the 16 remaining cases (32.3%). Overall use was uncommon and used to treat appropriate types of infections. Areas for improvement include increasing of submission of C&S and more targeted use towards Gram negative infections. Studies such as...
this will contribute to the refinement of prescribing practices for a critically important antibiotic in veterinary medicine.

14. Dissection of Bandavirus Entry Pathways with Fluorescent Vesicular Stomatitis Pseduovirus Particles

Philip Hicks, Tomaz Manzoni, and Paul Bates, University of Pennsylvania Perelman School of Medicine and School of Veterinary Medicine. VMD-PhD

Bandavirus is a newly recognized genus within the viral order Bunyavirales that contains seven members, all of which are tickborne. One member of the genus, severe fever with thrombocytopenia syndrome virus (SFTSV, newly reclassified as Dabie bandavirus) emerged in 2007 and has since caused >8000 cases globally with an approximate fatality rate between 10 and 16%. The genetically similar heartland bandavirus (HRTV) emerged in Missouri in 2009 and has also caused fatal human infections. Despite the severity of infections caused by bandaviruses and the rapidly expanding geographical range of their tick vectors, no FDA-approved vaccines or therapeutics exist to combat disease. A better understanding of bandavirus replication cycles may elucidate targets for antiviral therapeutics. Vesicular stomatitis virus (VSV) is a useful tool to study viral entry pathways due to its propensity to include heterologous viral glycoproteins into its virions. These pseudotypes enter target cells following the biology of the glycoproteins they harbor and are safe for use under BSL2 conditions. In addition, a plasmid recovery system for VSV exists which allows for genetic manipulation of any viral component. To study the entry pathway of SFTSV and HRTV under BSL2 conditions, I cloned a pseudotype system in which the phosphoprotein of VSV was fused by its N-terminus to the fluorescent mNeonGreen protein. Virions created by this system are fluorescent and are suitable for live cell microscopy. SFTSV and HRTV pseudotypes (SFTSV-mNeon and HRTV-mNeon) were endocytosed by A549 cells and could be found in Rab5+ endosomes within 5 minutes of entry. By 15 minutes post-infection, most particles were still found in Rab5+ endosomes while only a small fraction of each pseudotype occurred within Rab7+ endosomes. In contrast, pseudotypes harboring the cognate VSV glycoprotein were abundant in Rab7+ late endosomes 15 minutes post-infection, which is the consensus fusion site for VSV entry. Each pseudotype diverged early during their entry time-courses from the model antigen cholera toxin subunit-B, which progressed from Rab5+ endosomes to Golgin-97+ structures by 15 minutes post-internalization. These findings are in accordance with other entry studies conducted in the field and validate the use of fluorescent VSV pseudotyped particles to study the entry pathway of highly pathogenic bandaviruses.

15. Imaging Filovirus VP40-mediated VLP Egress from the Plasma Membrane via TIRF-SIM

Justin M. Hoffman, Gordon Ruthel, Bruce D. Freedman and Ronald N. Harty. Department of Pathobiology, University of Pennsylvania School of Veterinary Medicine. VMD

Total Internal Reflection Fluorescence (TIRF) microscopy provides a clear view of cellular processes that occur at or close to the plasma membrane by limiting fluorescence excitation to a narrow region of approximately 100 nm from the coverglass on which the cells are grown. By combining TIRF with Structured Illumination Microscopy (TIRF-SIM), imaging is further enhanced by increasing lateral resolution to approximately 120 nm. This technique is a valuable tool to observe interactions of filovirus VP40 with the plasma membrane and the surrounding cytoskeleton scaffold to form budding VLPs. TIRF-SIM can therefore reveal changes in these interactions when the function of host proteins involved in VP40 trafficking to, insertion at or near the plasma membrane is altered. Together these principles make TIRF-SIM a valuable tool for imaging live virus-host interactions at the level of the plasma membrane for both filoviruses and other pathogens.
16. The Microbiota Regulates the Progression of Obesity and Inflammation Through a Highly Conserved Family of MicroRNAs
Jimenez MT, Virtue AT, McCright SJ, Wright J, Mowel WK, Kotzin JJ, Joannas L, Thaiss CA, Clark ML, Micheletto M, and Henao-Mejia J. University of Pennsylvania School of Veterinary Medicine and Perelman School of Medicine VMD PhD

The gut microbiota is a key environmental determinant of mammalian metabolism. Regulation of white adipose tissue (WAT) by the gut microbiota is a critical process that maintains metabolic fitness, while dysbiosis contributes to the development of obesity and insulin resistance (IR). However, how the gut microbiota controls WAT functions remains largely unknown. Herein, we show that tryptophan-derived metabolites produced by the microbiota control the expression of the miR-181 family in white adipocytes to regulate energy expenditure and insulin sensitivity. Moreover, we show that dysregulation of the microbiota-miR-181 axis is required for the development of obesity, IR, and WAT inflammation. Thus, our results indicate that regulation of miRNA levels in WAT by microbiota-derived metabolites is a central mechanism by which host metabolism is tuned in response to dietary and environmental changes. Strikingly, the role of this microRNA family in regulating inflammatory processes is not limited to WAT as we have recently discovered that miR-181 is protective against colitogenic insult by its activity in neurons and intestinal epithelial cells (IECs). Finally, as we also show that MIR-181 is dysregulated in WAT from obese human individuals and IECs from ulcerative colitis patients, the MIR-181 family may represent a potential therapeutic target to modulate WAT and intestinal function in the context of metabolic and inflammatory disorders.

17. Clinical Biomarkers of Cancer Cachexia in Cats
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Cancer cachexia (CC) is a multifactorial syndrome that occurs in human cancer patients, and is associated with involuntary weight loss, anorexia, sarcopenia, and increased serum levels of inflammatory cytokines and acute phase proteins (APP). No effective interventional treatments currently exist. Some cats with cancer present with clinical signs similar to humans with CC; however, specific clinical biomarkers in cachectic cats have not been investigated. In this study, we aim to characterize abnormalities indicative of CC in cats by comparing body weight, body condition, complete blood count, serum chemistry, and inflammatory cytokine concentrations in cachectic and non-cachectic cats with cancer, and healthy cats. Additionally, we aim to evaluate clinical follow up data to determine prognostic value of measured biomarkers. Initial analysis reveals mean body weight and BCS were not significantly different, demonstrating the importance of measuring muscle mass in clinical evaluation of CC. Cachectic cats had significantly lower hematocrit and albumin compared to non-cachectic (p<0.0001, p<0.0001) and healthy cats (p<0.0001, p=0.0003). Cancer-bearing cats had significantly higher white blood cell counts compared to healthy cats (p<0.0001). Cachectic cancer-bearing cats had significantly higher serum amyloid A compared to both non-cachectic and healthy cats (p=0.0086, p=0.05). Preliminary data also demonstrate that cancer-bearing cats have elevated serum cytokine concentrations of IL-12p40, Flt-3L, KC, RANTES, and IL-8 compared to healthy cats. Analysis of alpha1-acid glycoprotein is in progress; we hypothesize cachectic cancer-bearing cats will have elevated alpha1-acid glycoprotein, and cats with elevated APP will have shorter overall survival. Results thus far indicate the clinical and inflammatory profiles of feline CC is similar to humans, signifying potential for a feline model to further research of CC in human cancer patients.
18. 2020 Survey of Non-Human Primate Enrichment Practices in the United States
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Environmental-enrichment activities are vital for the well-being and welfare of laboratory-housed non-human primates (NHPs). However, NHP enrichment practices have not been thoroughly categorized since a survey circulated in 2014 (Baker 2016). A widespread understanding of common practice is beneficial in aiding facilities to evaluate and forecast necessary improvements in their own programs. It can also inform the need to continue improving standards for NHP behavioral management programs as a whole. This paper describes the feedback from a 2020 survey distributed to University, private, and government facilities around the country that house NHPs. We will present our survey findings that include questions that explore the development, implementation, structure, and management of NHP enrichment that are used in both behavioral and non-behavioral research. The areas of enrichment that are queried include social housing, positive reinforcement and human interaction, structural enrichment including housing amenities and exercise enclosures, and an array of different types of enrichment devices. We also analyze facilities’ rationale for increasing enrichment management and when limitations on enrichment may be needed. Because in recent years, technology has become more pervasive in all areas of our society, the research world included, an exploration of how technology has been incorporated into enrichment programs is necessary to establish standard practices. Thus, we will also present findings that explore the utility of digital enrichment through the use of music, television, or tablets. Support: NIH/Boehringer Ingelheim Summer Research Program

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The role of computers has expanded from acquisition of images and transferring data, to aiding in the detection of abnormalities and forming diagnoses for patients. Computer-Aided Detection (CAD) programs have been designed for various uses in human medicine, including detection of lung nodules, but very few have been developed for use in veterinary medicine. The overall goal of this experiment is to develop a computer-aided detection program, requiring minimal user input, to identify lung nodules (such as pulmonary metastases) in thoracic Computed Tomography scans of cats and dogs. Pulmonary nodules in CT are recognized visually by their generally finite spherical shape and soft tissue attenuation of X-rays, which separates them from low (gas) attenuation of the surrounding lung. This program uses attenuation differences to focus explicitly on the lung field, and utilizes Watershed Segmentation to detect potential nodules and analyze them individually. Nodules are selected based on criteria including shape and attenuation, using empirically derived boundaries. The program utilizes Maximum Intensity Projections (MIP) and slice differences to mitigate the quantity of false-positive detections, while ensuring a high sensitivity. While in development, our program has been able to successfully identify nodules with a high false-positive rate. Therefore, future areas of development should aim at decreasing the false positive rate to allow veterinarians to make more accurate diagnoses and prognoses, which can heavily influence the treatment for patients.
Aggression directly affects sow quality of life. Many farms house pigs in large dynamic groups during gestation, but sows are removed to farrow individually. This cycle necessitates periodic reintroduction of sows to the large dynamic group after rebreeding. Swine naturally form social groups when housed together and display aggression toward newly introduced members. The goal of our study was to determine whether pre-mixing - housing sows together for 7 days post weaning prior to mixing, and method of introduction - individually through the electronic sow feeder or in a batch through the side gate of the pen, affect aggression at the time of mixing. One hour of behavior immediately post-mixing was video recorded and coded using Noldus Observer 11.5 and aggression was measured by biting, chasing, displacing, and side pressing behaviors. We found the likelihood that a sow displays aggression was significantly lower for sows that were pre-mixed and introduced as a batch. We also found that the duration of aggressive activity was significantly increased, and the duration of inactivity (periods of rest) significantly decreased, for unmixed sows. However, we did not observe a significant change in productivity measures. This information could help farmers reduce sow aggression at post-gestation mixing resulting in improved sow quality of life without impacting productivity.

20. Stabilization of an Iodinated Muscle Acetylcholine Receptor Chimera by Tyrosine Replacement

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Not for Oral Presentation

The muscle nicotinic acetylcholine receptor (nAChR) is a prototypical member of the Cys-loop family of ligand-gated ion channel that plays a pivotal role in neuromuscular transmission and the pathology of myasthenia gravis (MG), a debilitating antibody-mediated autoimmune disease. In MG, autoantibodies target and destroy nAChRs at the neuromuscular junction, impairing neuromuscular transmission. A serological test for autoantibodies to the nAChRs is currently used as a diagnostic test for MG, but lacks correlation with disease severity. More than half of AChR-specific autoantibodies in MG are directed to the main immunogenic region (MIR), a small region of overlapping epitopes on the alpha1 subunit of muscle nAChR. Autoantibodies against the MIR exhibit better correlation with disease manifestations in MG. A chimera of the MIR sequence in Aplysia ACh binding protein (AChBP), a soluble homologue of the extracellular domain of nAChRs, binds autoantibodies from patients with MG, allowing the detection of pathological autoantibodies in these patients. We found that the MIR/AChBP chimera, upon iodination for use in radioimmunoassay, is destabilized and decays rapidly, limiting its potential clinical practicality. Using computational analysis, we identified specific tyrosine residues of the chimera that were eligible for iodination and mutated them to phenylalanine. Two of these mutants showed improved stability after iodination, probably due to the removal of steric strain caused by the binding of iodine-125. Studies are currently underway to further pin down those tyrosine residues directly responsible for the instability and generate a stable mutant of the chimera, ideally for use in radioimmunoassay, by replacing these residues with phenylalanine.
Speaker. IL-1β Alters Cytoskeletal Networks and Reduces Cell Contractility in Meniscal Fibrochondrocytes

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The meniscus, a dense connective tissue located between the femur and tibia, serves a critical load-bearing role in the knee. Meniscus injuries are common and often require surgical intervention to restore pain-free function. The pro-inflammatory cytokine IL1β is found at higher levels post-injury and prevents anabolic meniscal remodeling partially by inhibiting cell migration to the injury site. We evaluated the effects of IL1β on meniscus fibrochondrocyte (MFC) migration in 2D and 3D. Then, we tested whether IL1β affects cell contractility, solidity, and adhesion through focal adhesion (FA) and yes-associated protein (YAP) nuclear/cytoplasmic ratio analyses. Lastly, we investigated the duration of the MFC migratory deficit after an inflammatory insult. MFCs exposed to IL1β show a dose-dependent decrease in 2D migration. IL1β exposure results in persistent attenuation of 2D migration through day 3. By day 7, MFCs return to control levels. Cell morphological analysis reveals that MFCs treated with IL1β have an increased number of FAs, increased solidity, and decreased YAP nuclear localization compared to control cells. Together, these findings suggest that MFCs exposed to IL1β are less contractile and more firmly attached to the substrate than control cells. Results from the 3D migration study show IL1β reduces the number of cells migrating from a meniscus explant to a de-vitalized substrate. These findings support the concept that IL1β inhibits the migratory capacity of MFCs. Our data provide new insight into the mechanism of this migratory deficit, suggesting that IL1β attenuates cell contractility and spreading abilities by interfering with YAP nuclear localization. These insights may contribute to novel therapeutic interventions that rescue the migratory ability of MFCs after injury and consequently promote endogenous anabolic meniscal repair.

21. A Systematic Review of the Potential Implication of Infectious Agents in Myasthenia Gravis

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Myasthenia gravis (MG) is an autoimmune disorder of unknown etiology in most patients, in which autoantibodies target and destroy components of neuromuscular junctions. Objective: To provide a synthesis of the evidence examining infectious agents associated with the onset of MG. Hypothesis: We hypothesized that microbes play a pathogenic role in the initiation of MG. Methods: We searched PubMed and Web of Science. Papers captured by a search algorithm (n = 415) were assessed, yielding a total of 42 publications meeting the inclusion and exclusion criteria. An additional 6 papers were retrieved from the reference lists of relevant articles. For each pathogen, an integrated metric of evidence (IME) value, from minus 8 to plus 8, was computed based on study design, quality of data, confidence of infectious disease diagnosis, likelihood of a causal link between the pathogen and MG, confidence of MG diagnosis, and the number of infected patients. Negative IME values corresponded to studies providing evidence against a role for microbes as triggers of MG. Results: One hundred and sixty-nine myasthenic patients infected with 21 different pathogens were documented. Epstein-Barr virus (median = 4.71), human papillomavirus (median = 4.35), and poliovirus (median = 4.29) demonstrated the highest IME values. The total median IME was 2.63 (mean = 2.53; range -3.79 to 5.25), suggesting a general lack of evidence for a causal link. Conclusions: There was a notable absence of mechanistic studies designed to answer this question directly. The question of the pathogenic contribution of microbes to MG remains open.
22. Do Veterinarians Want Increased Reporting of Equine Strangles?

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Although equine strangles is reportable in all states, synchronous reporting of this disease does not occur across the country. States have variable regulations on reporting (actionable, notifiable, and monitored) and no mandatory comprehensive databases exist for tracking prevalence. We hypothesized that veterinarians would prefer increased synchronous reporting of the disease nationally. A questionnaire was disseminated via social media and email to veterinarians across the United States to solicit their opinions on reporting of strangles and factors influencing their opinion. A total of 250 veterinarians participated: 84 participants (34%) believed that strangles should continue to be nationally monitored and that individual states should have jurisdiction over laboratory confirmed positive cases; 58 (23.2%) believed strangles should become nationally monitored with mandatory notification of positive cases to a central forum; 24 participants (10.5%) thought strangles should become notifiable nationally; and 44 (19.2%) thought strangles should become notifiable and actionable. Veterinarians who already reported strangles cases were 87% more likely (P = 0.054) to want increased reporting, as did veterinarians who ranked strangles as “very important” or “important” relative to other infectious disease (OR 3.77, P = 0.037). Veterinarians practicing in the southwest (P=0.01) and west (P = 0.04) were significantly less likely than northeast practitioners to rank strangles of higher importance. Opinions on equine strangles and desire for increased reporting were varied in the sampled veterinary community.

23. In vitro expansion of canine T regulatory cells

William Smallridge, Renee Cotton, Megan Kim, Raimon Duran-Struuck. Department of Pathobiology, University of Pennsylvania School of Medicine. Not for Oral Presentation

The use of regulatory T cells (Treg) has been shown to prevent graft rejection, autoimmunity, and GVHD in murine animal models. Recent studies using non-human primates (NHPs) as a pre-clinical model have been promising whereby the use of polyclonal Tregs promoted renal tolerance. The goal of this study was to build from our NHP protocols and expand in vitro polyclonal Tregs isolated from canine peripheral blood mononuclear cells (PBMC) and assess their suppressive functions. PBMCs were isolated from donor canine blood and sorted using flow cytometry selecting the top 1% of the CD4+ CD25hi cells. Cells were cultured with IL-2, anti-CD3, and K562 cells expressing CD80 and CD86. Cultures were re-stimulated every 7 days for 21-28 days. The cultured cells were then phenotyped and a CFSE suppression assay was performed using PBMCs isolated from the same canine donor. Responder PBMCs were bead stimulated and co-cultured in vitro with the expanded Tregs. Treg expanded up to 1000-fold and maintained expression of CD25 and FoxP3 at the end of the culture period. Tregs exhibited at least a 50% suppression at (or above) a 1:4 ratio (Tregs:bead stimulated PBMCs). To summarize, our preliminary studies show that we can successfully expand in vitro canine Tregs. These show similar suppression activity to NHP and human polyclonal Tregs. We aim to develop an “off the shelf” canine Treg cellular therapy that can be used for the treatment of autoimmunity or renal allograft rejection in dogs. Additional studies are being performed to optimize the survival of Tregs after cryopreservation while maintaining their phenotype and suppression activity.
Conjugated linoleic acid (CLA) improves oxidative stress and mitochondrial biogenesis in various species but has not been thoroughly investigated in horses. We collected blood and muscle samples from lightly exercising horses before and 6 and 12 wk after receiving either soybean oil (CON; n=5) or CLA (CLA; n=5) supplementation. Samples were analyzed for markers of mitochondrial characteristics, antioxidant status, oxidative stress, and muscle damage. Data were analyzed using a repeated mixed model ANOVA. In the triceps brachii (TB), citrate synthase (CS) activity was higher in CON than CLA horses (P=0.003) but was unaffected by diet in the gluteus medius (GM). Integrative (relative to mg protein) cytochrome c oxidase (CCO) activity was higher in TB than in GM (P<0.0001), while intrinsic (relative to CS) CCO was lower in TB than GM (P=0.02) and tended to be lower in CON than CLA horses (P=0.06). Neither CS nor integrative CCO activities were affected by time. In the GM, superoxide dismutase activity tended to increase in CON through wk 12 (P=0.10). Over both muscle groups, glutathione peroxidase activity tended to be higher in CON compared to CLA at wk 12 (P=0.06). Malondialdehyde was higher in the TB than the GM (P=0.0004) but was unaffected by diet, while serum creatine kinase activity tended to be lower in CLA than CON (P=0.07). These results suggest that CLA supplementation may lead to mitochondrial adaptations and prevent myofiber perturbation in skeletal muscle of young, exercised horses.

SPEAKER Non-canonical NF-κB Signaling in Lymphatic Vessels Regulates Immune Homeostasis in the Lung

Julianne Nolte, Michelle Cully, Kelly McCorkell, Athena Patel, Nipun Jayachandran, and Michael May. Department of Biomedical Sciences, University of Pennsylvania School of Veterinary Medicine.
25. **The novel innate immune-antagonistic effects of the multifunctional ectromelia virus C15 protein**  
**Elise Peauroi, Katherine S. Forsyth, and Laurence C. Eisenlohr, University of Pennsylvania Perelman School of Medicine and School of Veterinary Medicine. VMD-PhD**

The success of poxviruses as pathogens depends upon their extensive antagonism of host immune responses by a large arsenal of immunomodulatory proteins. The C15 protein of ectromelia virus (ECTV, the agent of mousepox) is the largest of the ECTV immunomodulatory proteins and is a member of a well-conserved poxviral family previously studied as inhibitors of T cell activation. We have recently determined that C15 also facilitates viral spread in vivo as early as 3 days post infection, suggesting a second non-adaptive function of C15. Accordingly, we sought to further investigate this new function and identify the cellular target. We first found that C15 maintains its replication-promoting effect in RAG KO but not RAG IL2RG double KO mice, implying the targeting of NK cells. Nevertheless, the impact of C15 is drastically less in RAG KO than WT mice, suggesting that C15 also targets a RAG-dependent immune component. Preliminary data suggests this can be attributed to CD8 T cells, which is unique given the very early timing. Further studies have suggested that C15 is both necessary and sufficient to inhibit NK cell mediated cytolysis in vitro. Additionally, C15 appears to negatively impact NK cell and CD8 T cell number and function in vivo. These results prompt further investigation of the mechanism used by C15 to inhibit both cell types, particularly an early, likely non-antigen specific CD8 T cell response.

26. **In vitro Generation and Expansion of Rodent Acetylcholine Receptor-Specific Chimeric Antigen Receptor Regulatory T Cells**  
**Yiyun Peng, Gavin Ellis, Jim Riley, Jennifer Punt, Oliver Garden, and Jie Luo. Department of Clinical Sciences and Advanced Medicine. University of Pennsylvania School of Veterinary Medicine. Not for Oral Presentation**

Myasthenia gravis (MG) is a chronic autoimmune disease in which T-cell dependent autoantibodies target and destroy muscle nicotinic acetylcholine receptors (AChRs) at the neuromuscular junction, causing muscle weakness and fatigue. Current treatments for MG, including symptomatic treatments and general immunosuppression, can help many but not all patients and are often associated with serious adverse effects. Regulatory T cells (Treg) play a protective role in experimental autoimmune MG (EAMG) by maintaining peripheral immune tolerance. Adoptive chimeric antigen receptor (CAR) T cell therapies, which have achieved remarkable success in treating patients with hematologic cancers, are rapidly expanding to the treatment of autoimmune diseases. Aiming to test our hypothesis that CAR-engineered AChR-specific Tregs are significantly more potent than polyclonal Tregs in suppressing the autoimmune response to muscle AChRs in EAMG rats, we have generated a lentiviral construct that encodes an AChR-specific CAR targeting AChR extracellular domain and lentivirally transduced purified rat CD4+CD25hi T cells. The transduced cells were expanded and analyzed by flow cytometry for CAR and FoxP3 expression. Studies are currently underway to evaluate the suppressive function of transduced Tregs and to generate a distinct CAR that is directed at the cytoplasmic domain of AChR. Our ultimate goal is to establish therapeutic approaches to the amelioration of EAMG in rats by adoptively-transferred CAR-engineered AChR-specific Tregs.

27. **The Odor of SARS-CoV-2: a Community Based Study**  
**Victoria Plymouth, Alyssa Nguyen, Amritha Mallikarjun, Sarah Kane, Annemarie DeAngelo and Cynthia Otto. Penn Vet Working Dog Center, Department of Clinical Sciences and Advanced Medicine, University of Pennsylvania School of Veterinary Medicine. VMD**
Working dogs with their superior olfactory capabilities have been utilized in a variety of environments to detect explosives, narcotics, cadavers, missing persons and a variety of diseases. Dogs are then able to discriminate the target odor in environments full of distracting odors making them ideal candidates for screening methods. With this knowledge there is a current interest in research if dogs can detect SARS-CoV-2 to combat the global pandemic. We hypothesized that dogs can be trained to identify people with COVID-19 based upon the smell of their sweat. SARS-CoV-2 positive and negative participants complete an internet-based survey to determine eligibility. Participants who have been tested within 48 hours are provided a cotton T-shirt to wear for one night and then ship back for screening by the dogs and an electronic sensor. A health survey of enrolled participants provides data to determine demographics, sleep patterns, symptomology and stress levels. Preliminary results show that SARS-CoV-2 positive participants sleep longer ($x$ vs $y$) and report higher stress levels (3.4 vs 4.1; $p=0.037$). These differences may confound the specificity of the COVID-19 odor signature. Continued investigation and training are underway to determine the dogs’ sensitivity and specificity in detection of SARS-CoV-2. Results will aid in the development of early detection screening methods for the virus.

28. **The impact of the COVID-19 pandemic on rabies reemergence in Latin America**

Brinkley Raynor, Elvis W. Díaz, Julianna Shinnick, Edith Zegarra, Ynes Monroy, Claudia Mena, and Ricardo Castillo-Neyra. University of Pennsylvania Perelman School of Medicine and School of Veterinary Medicine. VMD PhD

In the past decades, enormous strides have been made in Latin America towards regional elimination of canine rabies. The recent COVID-19 pandemic has posed challenges and likely setbacks to rabies control programs across Latin America. In Arequipa, Peru, COVID-19 restrictions have limited rabies control efforts including creating movement restrictions for public health officials to conduct surveillance, respond to reports of rabid dogs, and implement mass dog vaccination campaigns. To explore the extent of the harm these disruptions could cause on rabies control efforts, we constructed a compartment model of canine rabies in the city. We parameterized the model with incidence data shared from collaborators at the Ministry of Health and from population data obtained from longitudinal surveys conducted yearly since 2016. We explored 6 different scenarios: high, medium and low vaccination coverage under both normal and decreased surveillance. We found that if optimal vaccination and normal surveillance were maintained, caseloads in the coming months would be greatly reduced; however, with low vaccination coverage and low surveillance, rabies cases would increase exponentially. Unfortunately, we are already seeing the effects of decreased surveillance and suspended vaccination campaigns from 2020 in preliminary surveillance results from 2021. Despite persistently lower-than-average rabies sample submissions to the Peru Ministry of Health, the highest single count of canine rabies cases per month since rabies re-emerged was January 2021. In order to control canine rabies in Arequipa and Latin America, innovative methods to implement control programs in the face of a pandemic are needed in the short-term future to minimize the rabies burden in the coming years to decades.

29. **Defining the role of hematopoietic stem cell IKKα in lymph node homeostasis**

Faazal Rehman, Nipun Jayachandran, and Michael May, Department of Biomedical Sciences, University of Pennsylvania School of Veterinary Medicine. VMD

Chronic Inflammation is a pathological hallmark of many human autoimmune diseases. Thus, developing novel strategies to reduce inflammation has profound significance to human and animal health. The NF-κB family of transcription factors are essential to lymphocyte development and activation. There are two types of NF-κB signaling pathways: classical and non-canonical. The classical pathway’s role in inflammation has been well defined in lymph nodes while the non-canonical pathway is still not completely understood. A
key component of the non-canonical pathway is the IκB kinase IKKα. In this study we utilized a knockout mouse model in which IKKα<sup>F/F</sup> mice were crossed hematopoietic cell-specific Vav-cre mice to generate IKKα<sup>Vav</sup> mice. This restricted the deletion of IKKα to hematopoietic cells while allowing for normal development of lymph nodes. Results indicate unaffected T-cell numbers in lymph nodes, and while immature B cells are present in the spleen, the lymph nodes of IKKα<sup>Vav</sup> mice show a significant drop in B cell numbers and an absence of B cell follicles. Remarkably, the lymphatic network is also disrupted in the lymph nodes of IKKα<sup>Vav</sup> mice indicating a possible functional relationship between the presence of B cells and / or follicles and lymphatic network establishment. Studies are ongoing to investigate and define the role of B cells in the maintenance of lymph node architecture.

30. Understanding the Role of Individual Methanogens in Methanogenesis and their Interactions with Specific Bacterial Populations in the Rumen of Dairy Cows
Amanda Santos, Veronica Kaplan-Shabtai, Meagan Hennessy, Dipti Pitta. Department of Clinical Studies, New Bolton Center, University of Pennsylvania School of Veterinary Medicine. VMD

Enteric methane emissions from ruminants account for a substantial portion of anthropogenic greenhouse gas emissions and are considered a net energy loss to the ruminant host. This methane is solely formed by archaea taking up hydrogen released by other microbes during microbial fermentation in the rumen. Research efforts to mitigate methane emissions are ongoing, but a thorough understanding of methanogenesis and associations between specific archaea and bacteria is needed in order to advance mitigation efforts. The goal of this study was to understand and quantify individual methanogens and bacteria in the rumen and determine to what extent individual methanogens and associated bacteria are affected by mitigation. First, specific primers were acquired or developed to target methanogens such as Methanobrevibacter species, Methanosphaera, and Methanomassillicoccales representatives. Primers were also acquired or developed to target bacterial populations such as Succinivibrionaceae, Clostridium, Bacteroidales, Bulleidia, Ruminococcus, and Prevotella. Due to the limitations of a virtual experience and the ongoing pandemic, the project was unable to be completed. The plan for the rest of the project would have been the following: Second, the identified primer pairs will be validated in the laboratory and the individual microbes will be quantified in rumen samples. Third, these microbes will be quantified and compared between archived rumen samples from dairy cows that were supplemented with a potent methane inhibitor, 3-nitroxypropanol, and cows that were not supplemented. Finally, the qPCR results will be compared to previously collected 16S, metagenomic, and metatranscriptomic data to gather a comprehensive understanding of these microbes and their functional contribution towards methanogenesis. Conclusions will be drawn about methanogen-bacterial associations and their impact on methane emissions.

31. Variability Found in NSAID Prescription Patterns at Thoroughbred and Standardbred Racetracks in Pennsylvania
Lakshmi Sastry, Laurel Redding, Joanne Haughaun, Mary A. Robinson. Department of Clinical Studies, New Bolton Center, University of Pennsylvania School of Veterinary Medicine. VMD

Phenylbutazone, a non-steroidal anti-inflammatory drug (NSAID), was recently found to be associated with an increased risk for catastrophic injury in racehorses. The frequency of NSAID usage in racehorses has not been previously described. In this study, NSAID prescriptions reported for racehorses at four racetracks in PA were analyzed to investigate the hypothesis that prescription patterns are affected by
multiple covariates, including racetrack, breed (Thoroughbred vs Standardbred), time of year, indication for treatment, and veterinarian. Data were collected from treatment logs from four racetracks in Pennsylvania (two Standardbred and two Thoroughbred) from June 2018-August 2018. The frequency of administration and the NSAIDs prescribed were characterized for all racetracks and for each subgroup of interest. Phenylbutazone was prescribed the most frequently (65.0%), followed by flunixin (26.1%) and ketoprofen (8.8%) at all four racetracks; however, Standardbreds received a higher percentage of other types of NSAIDs (33.4%) than Thoroughbreds (22.9%; p < 0.0001). NSAIDs were prescribed more frequently in July than in June and August (p < 0.001). As expected, the most common indication was musculoskeletal soreness, which was treated primarily with phenylbutazone (63%) and flunixin (30%). It was the only indication that was also treated by multiple other NSAIDs such as ketoprofen, firocoxib, and diclofenac. The percentage of NSAIDs prescribed varied greatly by veterinarian. Ongoing studies are expanding the number of racetracks and years available for analysis and will investigate if specific NSAID regimens are associated with an increased risk of catastrophic injuries.

32. Evaluating Frequency and Phenotype of MDSCs and Tregs in Canines with Myasthenia Gravis and Healthy Controls.
Reshmi Sensharma, Carly Seligman, Surabhi Kumar, Oliver A. Garden, Ying Wu, and Jennifer A. Punt. Department of Pathobiology, School of Veterinary Medicine, The College of Arts and Science and Department of Clinical Sciences and Advanced Medicine, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA, Lawton Chiles High School, Tallahassee, FL. VMD

Myasthenia Gravis (MG) is a T cell-dependent B cell-mediated autoimmune disease caused by autoantibodies against acetylcholine receptors (AChR) at neuromuscular junctions. In both dogs and humans, acquired MG occurs spontaneously suggesting that canine MG may serve as a relevant model for investigating the disease. Multiple factors such as MHC genes, breed, environment, and infection play a role in breaking down immune tolerance. However, the exact cellular pathogenesis of MG remains unknown. Myeloid-derived suppressor cells (MDSCs) reduce inflammation by secreting immunosuppressive cytokines, enzymes, and other molecules that block T cell activation and by recruiting regulatory T cells (Tregs) that suppress immune response. Due to their immunosuppressive capacity, MDSCs and Tregs have the potential to play a therapeutic role in MG. In our study, we compared the frequency and phenotype of MDSCs and Tregs in peripheral blood of healthy dogs, seropositive dogs (i.e. with circulating anti-AChR antibody titers greater than 0.6nmol/L), and seronegative dogs. Our preliminary data suggest MG dogs have significantly higher levels of M-MDSC and PMN-MDSC in their peripheral blood. We recognized that a comparison of the activation state of myeloid and regulatory lymphoid cells could be more revealing. To determine the activation state of each cell type, we examined the expression of CD11b and MHC Class II, both of which are upregulated in inflammatory situations. Specifically, we hypothesized that dogs with MG would have less activated MDSCs as compared to healthy or seronegative dogs. Preliminary analysis suggests differences in myeloid cells, however, further studies are needed to determine differences in subpopulations.

33. Identification of a SARS-CoV-2 E/Host ZO-1 Interaction: Implications for Tight Junction Damage in Human Lung Epithelial Cells
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Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) is a positive strand RNA virus that emerged in 2019 to cause a global pandemic of severe respiratory syndrome in humans. The outbreak of
Coronavirus Disease 2019 (COVID-19) has infected over 91 million people and claimed over 1.9 million lives worldwide between December 2019 and January 2021. Since severe pneumonia and consolidation of the lungs are often symptoms of COVID-19, it is likely that SARS-CoV-2 disrupts lung epithelial cell barriers. Respiratory viruses may damage epithelial barriers in the lung by a specific mechanism involving an interaction between the virus and specific proteins of the lung epithelial tight junction (TJ) complexes. Notably, the C-terminal amino acids of the Envelope (E) protein of SARS-CoV-1 interact with the PDZ domain of host protein PALS1, a TJ associated protein, leading to delayed formation of cellular TJs and disruption of cell polarity in a renal epithelial model. Interestingly, the extreme C-terminal sequence of the E protein of SARS-CoV-2 is similar to that of SARS-CoV-1, suggesting that it may also engage specific host PDZ-domain containing TJ proteins. Indeed, we identified a singular specific interaction between the C-terminal sequence of WT SARS-CoV-2 E protein and the second PDZ domain of human Zona Occludens-1 (ZO-1), a highly expressed, key regulator of TJ formation in the lung. We hypothesize that the E protein encoded by SARS-CoV-2 may interact with ZO-1 in infected lung epithelia to disrupt TJs and induce barrier cell leak. A better understanding of the molecular events of the SARS-CoV-2 viral lifecycle, such as the identification of virus-host interactions that contribute to the severe lung pathology of COVID-19, will be essential for insights into the biology and pathogenesis of this novel virus, as well as for the future development of therapeutics.

34. Here’s the Tea on T cells: Equine Supporting Limb Laminitis (SLL) is Associated with CD3+ T Cell Infiltration

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Supporting limb laminitis (SLL) is a debilitating, and often fatal complication of equine lameness. Altered weight-bearing disrupts the epidermal and dermal lamellae resulting in failure of the suspensory apparatus of the digit within the hoof capsule. The tissue damage triggers inflammation and lesions similar to human psoriasis. As reported, the interleukin (IL)-17 pathway, the major pro-inflammatory effector cytokine in psoriasis, is activated in SLL. Th17 cells, a subset of CD3+ T cells, produce IL-17 in psoriasis. A recently completed study using the same SLL samples as this study demonstrated the activation of the IL-17 pathway in lamellar tissue from moderately to severely affected cases. Herein we show that lamellar CD3+ T cell infiltrates correlate with laminitis histopathological severity using immunohistochemistry (IHC) to identify and quantify T cells using an anti-equine CD3 primary antibody (a pan T cell marker) in archived lamellar tissue from SLL cases and controls. Positive cells were counted as a percentage of all cells a defined region parallel to the primary epidermal lamellae using digital image analysis (QuPath). Samples were assigned to histopathological subgroups (N=7 to 9) based on severity and stage of laminitis: Control, Developmental, Moderate Acute, Severe Acute (SA), or Severe Chronic (SC). Positive cells were also assigned a localization score based on their location relative to blood vessels: intravascular, strict perivascular cuffs, wide perivascular cuffs, dermal infiltration, or epidermal infiltration. Kruskal-Wallis rank sum test and Dunn’s test for multiple comparisons were used to demonstrate significantly higher proportions of CD3+ T cells as well as significant differences in T cell distribution within lamellar tissue in SA and SC cases vs controls. These data support the hypothesis that CD3+ T cells are positively correlated with SLL severity and IL-17 pathway activation.

SPEAKER. Chemogenetic Activation of Orexin Neurons Accelerates Emergence from Isoflurane Anesthesia

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General anesthesia is routinely performed in human and veterinary medicine, yet surprisingly little is known about the neural mechanisms underlying how consciousness returns following anesthesia. Recent evidence suggests anesthesia emergence is not simply the reverse of anesthesia entry. Understanding the distinct neurobiological processes that drive anesthesia emergence – and how they might be modulated by novel therapies – may improve patient care. Hypothalamic orexin neurons represent one subpopulation that may contribute to anesthesia emergence. The orexin system is involved in sleep-wake control and exerts arousal-promoting and arousal-stabilizing effects. Loss of orexin leads to narcolepsy and delayed emergence from volatile anesthetics. How might orexin activation facilitate anesthesia emergence and influence transitions between consciousness and unconsciousness? To address these questions, we used a chemogenetic approach to activate orexin neurons while we assessed sensitivity to and emergence from isoflurane anesthesia using a loss of righting reflex (LORR) assay. We expressed Cre-dependent excitatory DREADDs (hM3DG(q)-mCherry) in bilateral hypothalamus of Orexin-Cre transgenic mice. We achieved selective and reversible neural activation by intraperitoneal injection of DREADD ligand, clozapine-N-oxide (CNO). In experiment 1, we assessed LORR in response to stepwise increases (0%-1.2%) and then decreases (1.2%-0%) in isoflurane concentration. Compared to vehicle, CNO treatment did not change the isoflurane concentration under which LORR occurred. However, mice regained their righting reflex under higher concentrations of isoflurane when treated with CNO. In experiment 2, we measured the time to regaining righting reflex following 2 hours of 0.9% isoflurane. Mice regained righting reflex significantly faster when treated with CNO. In experiment 3, we exposed mice to 0.9% isoflurane and assessed LORR every 3 minutes for 2 hours. The time to first LORR was longer when mice were treated with CNO compared to vehicle. Taken together, these results strongly suggest that orexin activation can accelerate emergence from isoflurane anesthesia. We hypothesize it is less likely that orexin activation enhances resistance to anesthesia entry but instead may stabilize the state of wakefulness. An ongoing experiment tests this hypothesis by serially measuring LORR in response to a fixed, sub-hypnotic isoflurane concentration, an assay that is sensitive for individual stochastic fluctuations between conscious and unconscious states.

35. **Risk Factor Analysis for Colonization with blaNDM-5 Carbapenem-resistant Escherichia coli at a Philadelphia veterinary hospital**

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Infections caused by carbapenem resistant Enterobacteriaceae (CRE) have been sporadically reported from both humans and companion animals in the United States, Europe, and China. Since July 2018, carbapenem resistant E. coli, that contain a blaNDM-5 gene has been identified in over 40 animal patients from the Ryan Veterinary Hospital at the University of Pennsylvania (Ryan VHUP): a veterinary teaching hospital in Philadelphia. A point prevalence study was conducted by testing the feces of companion animal inpatients at Ryan VHUP from June 24 to September 24, 2019 on weekly sampling days. Medical records the patients were used to investigate hospital services and patient factors and their potential associations to blaNDM-5 colonization. 17 colonized animals were identified and these cases were compared to 254 animals with no CRE by simple logistic regression. Results showed that antibiotic use was significantly associated with an increased likelihood of colonization, as were longer stays in the hospital and greater numbers of prior visits to VHUP. Patient-specific factors, such as age, weight, and purebred status were not significantly associated with increased risk of colonization with blaNDM-5. This indicates that the risk of colonization is more related to exposure and veterinary care than to an inherent quality of the animal, and that more research needs to be done to minimize the healthcare-associated risks that can lead to colonization.

36. **Feasibility of Using Low-Cost Sensors to Monitor Personal Exposure to PM2.5 Among People with Asthma**
Rationale: Exposure to fine particulate matter (PM2.5) increases the risk of asthma exacerbations, and thus, improved measurements of personal exposure to PM2.5 may aid in disease self-management. Traditional methods for estimating personal PM2.5 exposure often rely on measurements taken at regulatory monitoring stations, such as those operated by the U.S. Environmental Protection Agency (EPA), which are typically time-averaged and have limited spatial resolution. Low-cost, portable air pollution sensors offer a convenient way to measure personal pollution exposure directly and may improve personalized monitoring.

Methods: We conducted 15 semi-structured interviews with adults with asthma to understand their willingness to use a personal pollution sensor and privacy preferences with regard to sensor data. To assess the feasibility of using personal pollution sensors, student research assistants were asked to use HabitatMap AirBeam devices to take PM2.5 measurements while walking in Philadelphia neighborhoods in May-August 2018. AirBeam PM2.5 measurements were compared to concurrent inverse-distance-squared-weighted interpolated measurements based on data from three nearby EPA monitoring stations.

Results: All patients stated they would use a personal air pollution sensor, though the consensus was that devices should be small (watch- or palm-sized) and light. Patients were generally not concerned about sharing their GPS location or other sensor data with the public. PM2.5 measurements were taken using AirBeam sensors on 34 walks of 1-3 hours duration, with routes extending through 5 Philadelphia neighborhoods. Sensor PM2.5 measurements ranged 0.6-97.6 μg/mL (mean 6.8 μg/mL), compared to 0-22.6 μg/mL (mean 9.0 μg/mL) measured by nearby EPA monitors. Compared to EPA measurements, which were available as 1-hour integrated averages at discrete monitoring stations, sensor measurements were taken in rapid succession, allowing them to resolve fine-scale fluctuations in PM2.5 levels over time and space.

Conclusions: Patients were generally interested in using sensors to monitor their personal exposure to PM2.5 and willing to share personal sensor data with the public. Compared to traditional methods of personal exposure assessment, sensors captured personalized air quality information at higher spatiotemporal resolution. Improvements to current commercially available sensors, including improved Bluetooth connectivity, increased portability and more efficient data transfer would facilitate their use in a general patient population.