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Twenty-First Annual Research Symposium*

November 20, 2009
Virginia-Maryland Regional College of Veterinary Medicine

Table of Contents

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Virginia-Maryland Regional College of Veterinary Medicine
21st Annual Research Symposium
Program Sessions

Friday, November 20, 2009

7:30 **Registration and Continental Breakfast**

8:00 **Welcome & Introduction**
Dr. Gerhardt Schurig, Dean VMRCVM
Dr. Roger Avery, Senior Associate Dean for Research and Graduate Studies, VMRCVM

Session I: Graduate Student Presentations
Chair: Dr. Ansar Ahmed

8:15 Anya Hawthorn (PhD Student, Pathology Resident), **Abstract 1**

A STAT-1 KNOCKOUT MOUSE MODEL FOR THE HEPATITIS E VIRUS INFECTION. A.C. Hawthorn, X.J. Meng, T. LeRoith. Department of Biomedical Science and Pathobiology, Virginia-Maryland Regional College of Veterinary Medicine, Blacksburg, Blacksburg, Virginia.

8:30 Burouj Ajlouni (PhD Student), **Abstract 2**

POLYMORPHISMS IN THE FLT1 GENE AND THEIR RELATION TO EXPRESSION OF THE SECRETED FLT1 VARIANT. B. Ajlouni and W.R.Huckle. Department of Pathobiology and Biomedical Sciences, Virginia-Maryland Regional College of Veterinary Medicine, Blacksburg, Virginia.

8:45 David Goodwin (PhD Student), **Abstract 3**

EFFECTS OF DOPAMINE ON THE DEVELOPMENT OF *TOXOPLASMA GONDII* IN CELL CULTURES. David Goodwin[1], Jeannine Strobl[2], Terry Hrubec[1,2], Brad Klein[1], Anne Zajac[1], and David S. Lindsay[1] [1]Department of Biomedical and Veterinary Science. Virginia Polytechnic Institute. [2]Edward Via Virginia College of Osteopathic Medicine. Blacksburg, Virginia.

9:00 Parthiban Rajasekaran (PhD Student), **Abstract 4**

EFFECT OF DIFFERENT GROWTH CONDITIONS ON GENE EXPRESSION OF *BRUCELLA SUIS*. P. Rajasekaran[1], S. Mane[2], S. Halling[3], N. Sriranganathan[1], C. Evans[2], B. Sobral[2], O. Crasta[2], Y. He[4], G. Yu[5], S. M. Boyle[1]. [1]Virginia-Maryland Regional College of Veterinary Medicine, Virginia Tech, Blacksburg, VA, [2]Cyberinfrastructure Group, Virginia Bioinformatics Institute, Virginia Tech, Blacksburg, VA, [3]Bacterial Diseases of Livestock Research Unit, NADC/ARS/USDA, Ames, IA, [4]Unit for Laboratory Animal Medicine, School of Medicine, University of Michigan, Ann Arbour, MI, [5]Boise State University, Boise, ID.

9:15 Pergentino Balbuena (PhD Student), **Abstract 5**

NEUROTOXICANTS MALATHION AND LEAD ACETATE INCREASE GENE EXPRESSION OF SCAFFOLD PROTEINS ZO1 AND ZO2, AND CALCIUM CHANNEL PROTEIN TRPC1 IN ENDOTHELIAL CELLS. P. Balbuena, W. Li and M Ehrich, Virginia-Maryland Regional College of Veterinary Medicine, Virginia Tech.

9:30 Deena Khan (PhD Student), **Abstract 6**

ESTROGEN UPREGULATES IL-17 SECRETING CELLS AND LEVELS IN C57BL/6 AND LUPUS-PRONE NZB/W MICE. Deena Khan*, Rujuan Dai*, S Ansar Ahmed*. *Department of Biomedical Sciences and Pathobiology, Virginia-Maryland Regional College of Veterinary Medicine, Virginia Polytechnic Institute and State University, Blacksburg, Virginia.

9:45 Neeraj Singh (PhD Student), **Abstract 7**

MOLECULAR MECHANISMS REGULATING INNATE IMMUNE CELL'S FREE RADICAL GENERATION IN RESPONSE TO LPS CHALLENGE. Neeraj Singh1 and Liwu Li1,2. 1 Department of Biomedical Sciences and Pathobiology, VMRCVM, Blacksburg, VA,2Laboratory o Innate immunity and inflammation, Department of Biology, Virginia Tech, Blacksburg, VA.

10:00-11:00 **Student Poster Presentations and Break**

Keynote Speaker

Pfizer Award for Research Excellence Recipient

11:00 Nammalwar Sriranganathan (Professor of Bacteriology)

NANOMEDICINE: DRUG DELIVERY AGAINST INTRA-CELLULAR PATHOGENS. N. Sriranganathan. November 20th, 2009. Pfizer Award Presentation.

12:00-1:00 **Lunch**

Session II: Graduate Student Presentations
Chair: Dr. David Hodgson

1:00 Naveen Surendran (PhD Student), **Abstract 8**

ENHANCED ABILITY OF *BRUCELLA ABORTUS* ROUGH VACCINE STRAINS TO INDUCE INNATE IMMUNITY IN A MURINE MODEL *IN VITRO* AND *IN VIVO*. N. Surendran[1]*, B.Hiltbold[3], B. Heid[1], H. Lawler[1], N. Sriranganathan[2], S. Boyle[2], Kurt Zimmerman[2], M. Makris[2], S. Witonsky[1]. [1]Department of Large Animal Clinical Sciences, [2]Department of Biomedical Sciences and Pathobiology, Virginia-Maryland Regional College of Veterinary Medicine, Virginia Tech, Blacksburg, VA. [3]Department of Microbiology and Immunology, Wake Forest University, Winston-Salem, NC.

1:15 Ronald Tyler (PhD Student, Pathology Resident), **Abstract 9**

***MYCOBACTERIUM AVIUM* SUBSPECIES *PARATUBERCULOSIS* DNA EXTRACTION AND DETECTION BY A COMMERCIAL REAL-TIME PCR ASSAY FROM FORMALIN-FIXED PARAFFIN EMBEDDED TISSUES AND CORRELATION WITH HISTOPATHOLOGY.** R.D. Tyler Jr.†, K. Anklam‡, E. Manning‡, M. Collins‡, N. Sriranganathan†. † Department of Biomedical Science and Pathobiology, Virginia-Marland Regional College of Veterinary Medicine, Blacksburg, VA. ‡ Department of Biological and Pathological Sciences, University of Wisconsin-Madison School of Veterinary Medicine, Madison, WI.

1:30 Alicia Feagins (PhD Student), **Abstract 10**

CONSTRUCTION AND CHARACTERIZATION OF A HEV CHIMERIC VIRUS USING THE GENOMIC BACKBONE OF A GENOTYPE 1 HEV WITH A LIMITED HOST RANGE AND THE CAPSID GENE OF A GENOTYPE 4 HEV WITH AN EXPANDED HOST RANGE. A. R. Feagins[1], T. Opriessnig[2], Y. W. Huang[1], S. U. Emerson[3], and X. J. Meng[1]. [1]Center for Molecular Medicine and Infectious Diseases, Department of Biomedical Sciences and Pathobiology, College of Veterinary Medicine, Virginia Polytechnic Institute and State University, Blacksburg, VA, [2]Department of Veterinary Diagnostic and Production Animal Medicine, College of Veterinary Medicine, Iowa State University, Ames, IA, [3]Laboratory of Infectious Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD.

1:45 Jamie Wearn (MS Student, Large Animal Resident), **Abstract 11**

THE PHARMACOKINETICS AND PHARMACODYNAMICS OF PIOGLITAZONE IN HORSES AND ITS POTENTIAL ROLE AS AN INSULIN SENSITIZING AGENT FOR THE MANAGEMENT OF EQUINE METABOLIC SYNDROME. J.M.G. Wearn[1], L. J. McCutcheon[1], J.L. Davis[2], D.R. Hodgson[1], M. Ashraf-Khorassani[3], R. J. Geor[4], R.S. Pleasant[1], J. K. Suagee[4], M.V Crisman[1], 1. Department of Large Animal Clinical Sciences, Virginia-Maryland Regional College of Veterinary Medicine, Virginia Polytechnic Institute and State University, Blacksburg, Virginia. 2. Department of Clinical Sciences, College of Veterinary Medicine, North Carolina State University, Raleigh, North Carolina. 3. Department of Chemistry, Virginia Polytechnic Institute and State University, Blacksburg. 4. Department of Animal and Poultry Sciences, Virginia Polytechnic Institute and State University, Blacksburg, Virginia.

2:00 Kelly Johnson (MS Student, Small Animal Resident), **Abstract 12**

IN VITRO 3-DIMENSIONAL KINEMATIC EVALUATION OF THE TIBIAL PLATEAU LEVELING OSTEOTOMY THROUGHOUT THE STANCE PHASE OF GAIT IN THE DOG. KA Johnson, OI Lanz, S Elder, RM McLaughlin, TA Harper. Department of Small Animal Clinical Sciences, Virginia-Maryland Regional College of Veterinary Medicine, Blacksburg, Virginia.

2:15 James Brown (MS Student, Equine Medical Center Resident), **Abstract 13**

DOES GROWTH ON COLLAGEN-COATED PLATES ENHANCE MESENCHYMAL CELL DIFFERENTIATION TOWARD TENDON? J.A. Brown, J.G. Barrett, L.A. Dahlgren* and N.A. White II. Marion duPont Scott Equine Medical Center, Virginia-Maryland Regional College of Veterinary Medicine, Leesburg, Virginia, and *Department of Large Animal Clinical Sciences, Virginia-Maryland Regional College of Veterinary Medicine, Blacksburg, Virginia.

2:30-3:30 **Student Poster Presentations and Break**

Session III: Graduate Student Presentations
Chair: Dr. Gregory Daniel

3:30 Hsing-Ho Hsu (MS Student), **Abstract 14**

PREVALENCE OF IGG ANTIBODIES TO *T. GONDII* AND *E. CUNICULI* IN CATS WITH AND WITHOUT CHRONIC KIDNEY DISEASE. Hsing-Ho Vasha Hsu[1], David C. Grant[2], and David S. Lindsay[1]. The Departments of Biomedical Sciences and Pathobiology and Small Animal Clinical Sciences [2], Virginia-Maryland Regional College of Veterinary Medicine, Blacksburg, Virginia.

3:45 Jessica Gilbertie (MS Student), **Abstract 15**

IMMUNOLOGICAL MARKERS OF STRESS IN HEIFER BEEF CALVES. Jessica Gilbertie, Michelle Todd, Stewart Harvey, William Swecker. Department of Large Animal Clinical Sciences, Virginia-Maryland Regional College of Veterinary Medicine, Virginia.

4:00 Sarah Davies (MS Student, Small Animal Resident), **Abstract 16**

QUANTITATIVE PERTECHNETATE THYROID SCINTIGRAPHY AND ULTRASONOGRAPHIC APPEARANCE OF THE THYROID GLAND IN EUTHYROID HORSES. S. Davies, G. Daniel, D. Barber, M. Crisman and M. Larson. Virginia-Maryland Regional College of Veterinary Medicine, Virginia, 24061.

4:15 Linda Frellstedt (MS Student, Equine Medical Center Resident), **Abstract 17**

ENDOTOXIN TOLERANCE INDUCED IN VITRO IN EQUINE LYMPHOCYTES. L. Frellstedt and M. Furr. Marion DuPont Scott Equine Medical Center, Virginia-Maryland Regional College of Veterinary Medicine, Leesburg, Virginia.

4:30 Jessica Gentile (MS Student, Small Animal Resident), **Abstract 18**

ECHOCARDIOGRAPHIC ASSESSMENT OF THE CANINE RIGHT HEART: REFERENCE INTERVALS AND REPEATABILITY. J.M. Gentile and J.A. Abbott. Department of Small Animal Clinical Sciences, Virginia-Maryland Regional College of Veterinary Medicine, Blacksburg, Virginia.

4:45 Vrushali Chavan (MS Student), **Abstract 19**

NEWCASTLE DISEASE VIRUS BIO-NANOPARTICLES FOR TUMOR SELECTIVE TARGETING AND ONCOLYSIS. Vrushali Chavan[1], Shobana Raghunath[1], Dan Qiao[1], Nikorn Pothayee[2], Judy Riffle[2], and Subbiah Elankumaran[1]. [1]Center for Molecular Medicine and Infectious Diseases, Virginia-Maryland Regional College of Veterinary Medicine, and [2] Department of Chemistry, Virginia Polytechnic Institute and State University, Blacksburg, VA.

5:00-6:00 **Judges Meeting**

6:30 **Dinner and Award Presentations at Blacksburg Country Club**

Graduate Student Awards
Research Staff Awards
Pfizer Award for Research Excellence

Session I

Graduate Student Presentations

Abstracts (1-7)

Chair: Dr. Ansar Ahmed

Abstract 1 - Anya Hawthorn

A STAT-1 KNOCKOUT MOUSE MODEL FOR THE HEPATITIS E VIRUS INFECTION. A.C. Hawthorn, X.J. Meng, T. LeRoith. Department of Biomedical Science and Pathobiology, Virginia-Maryland Regional College of Veterinary Medicine, Blacksburg, Blacksburg, Virginia.

This is a pilot study to determine the susceptibility of a STAT1 knockout mouse model to Hepatitis E virus (HEV). Previous attempts to infect rodents with HEV have been unsuccessful, or have not been able to be replicated. If this strain of mouse shows clinical signs of disease with HEV infection, it will provide an enormously useful tool in the study of HEV pathogenesis and the role of the innate immune response in viral infection. HEV is an important and understudied pathogen of humans, swine and chickens; the swine strain is zoonotic to humans, but the avian strain is not. Attempts to infect mice with HEV have so far been largely unsuccessful, although wild mice have been found to have antibodies against HEV. The STAT1 transcription factor is important in the innate immune response to viral infection. STAT-1 -/- mice are extremely susceptible to infection by enteric viruses; they become viremic and systemically clinically ill with norovirus infection, a virus related to HEV, while wild type mice are relatively unaffected. We hypothesize that STAT -/- mice are susceptible to HEV infection, and therefore provide a model for HEV infection to elucidate the innate and T cell mediated immune response to the virus. Mice were inoculated via orogastric tube with infectious Hepatitis E virus, and monitored for viremia and virus shedding with weekly PCR of blood and fecal pellets. After 3 weeks post inoculation, mice were sacrificed, and tissues collected for analysis by PCR and histopathology.

Abstract 2 - Burouj Ajlouni

POLYMORPHISMS IN THE FLT1 GENE AND THEIR RELATION TO EXPRESSION OF THE SECRETED FLT1 VARIANT. B. Ajlouni and W.R.Huckle. Department of Pathobiology and Biomedical Sciences, Virginia-Maryland Regional College of Veterinary Medicine, Blacksburg, Virginia.

Vascular endothelial growth factor (VEGF) is a potent angiogenic agent. VEGF activates its biologic responses through two cell-surface receptors, Flt1 and Flk1. In addition to the transmembrane form of Flt1, the Flt1 gene also encodes a secreted, truncated form of the receptor (sFlt1) translated from an mRNA in which a portion of intron 13 is preserved. sFlt1 retains high affinity for VEGF and thereby inhibits its angiogenic activity. Intron 13 contains important *cis* elements involved in sFlt1 mRNA formation. Here, we test the hypothesis that polymorphisms in the human Flt1 gene, particularly SNPs at sites suspected to contain splicing or cleavage-polyadenylation signals, influence Flt1 pre-mRNA processing and rates of Flt1 and sFlt1 expression. The NCBI SNP database contained 23 SNPs in the region of interest, one each in exons 13 and 14. An independent human SNP screen confirmed several of the reported SNPs. The web-based ESEfinder software predicted that the exon 13/14 SNPs had reduced potential for recruitment of splicing components. To test effects of exonic SNPs on Flt1 pre-mRNA processing, wild-type and mutant Flt1 minigene plasmids were transfected into NIH/3T3 cells. Both exonic SNPs were associated with 50-60% decreases in Flt1:sFlt1 mRNA ratios determined by real-time PCR. To facilitate exploration of ESEs in regulated RNA splicing, a PERL computer program, "EXONerator," was written to silence predicted ESEs without altering polypeptide sequence. These results support the notion that small changes in exon composition can have significant effects on splicing activity and underscore the utility of software tools for hypothesis generation.

Abstract 3 - David Goodwin

EFFECTS OF DOPAMINE ON THE DEVELOPMENT OF *TOXOPLASMA GONDII* IN CELL CULTURES. David Goodwin[1], Jeannine Strobl[2], Terry Hrubec[1,2], Brad Klein[1], Anne Zajac[1], and David S. Lindsay[1] [1]Department of Biomedical and Veterinary Science. Virginia Polytechnic Institute. [2]Edward Via Virginia College of Osteopathic Medicine. Blacksburg, Virginia.

Toxoplasma gondii is capable of infecting every warm-blooded animal. Infections with *T. gondii* in the intermediate host has two phases, acute phase of infection where it disseminates throughout the body and chronic phase when it encysts in all tissues but neural tissue of the brain is a frequent location. Traditional thought is there are no adverse side effects of chronic *T. gondii* infection because there is little to no reaction around tissue cysts. Research demonstrates a correlation between prevalence of antibody titers to *T. gondii* and psychological illness in humans. Recent research has shown a correlation between people with psychotic disorders, schizophrenia, bipolar disease and *T. gondii* infection. The hallmark of these psychotic disorders is elevated levels of the neurotransmitter dopamine. Dopamine may play a role in proliferation/chemotraction of *T. gondii*. The link between mental illness and *T. gondii* infection is not 100%, however there is a strong correlation between the two, indicating *T. gondii* infection could be an environmental factor for some mental disorders. Research in rodents with toxoplasmosis has indicated alterations in cognitive learning, fear response, and overall open field activity. Some of these behavior changes can be related to altered neurotransmitter levels, in specific dopamine. In an in vitro cell culture assay dopamine was tested against developing tachyzoites. Dopamine was tested at 2 concentrations, 100 nM and 250 nM. An increase of tachyzoite proliferation and increase destruction in cell monolayer was observed at both concentrations. The highest concentration, 250 nM, yielded the greatest increase in tachyzoites proliferation.

Abstract 4 - Parthiban Rajasekaran

EFFECT OF DIFFERENT GROWTH CONDITIONS ON GENE EXPRESSION OF *BRUCELLA SUIS*. P. Rajasekaran[1], S. Mane[2], S. Halling[3], N. Sriranganathan[1], C. Evans[2], B. Sobral[2], O. Crasta[2], Y. He[4], G. Yu[5], S. M. Boyle[1]. [1]Virginia-Maryland Regional College of Veterinary Medicine, Virginia Tech, Blacksburg, VA, [2]Cyberinfrastructure Group, Virginia Bioinformatics Institute, Virginia Tech, Blacksburg, VA, [3]Bacterial Diseases of Livestock Research Unit, NADC/ARS/USDA, Ames, IA, [4]Unit for Laboratory Animal Medicine, School of Medicine, University of Michigan, Ann Arbour, MI, [5]Boise State University, Boise, ID.

Brucella suis is a gram negative, facultative intracellular bacterium that primarily infects swine but can also infect humans causing severe clinical manifestations. To gain a comprehensive picture of the infection process, genomic analyses of both the pathogen and host is mandatory. Expression profiling of the pathogen's genome at both pre- and post-infection stages would help us in identifying virulence factors or critical steps in the infection. Since *B. suis* is known for its pronounced urease activity compared to other species of *Brucella*, an urease mutant was also included in this experiment to assess the effect of urease mutation on candidate virulence genes**.** As a baseline study, analysis of RNA from wild type *B. suis* strain 1330 and an urease mutant *B, suis* ure1K were grown in enriched (control) and minimal media (mimicking host environment) was performed using a custom designed affymetrics microarray containing 4770 *B. suis* probe sets. A total of 432 genes were differentially regulated ($P<0.05$ and at least 2 fold) between the two treatments with 280 up-regulated and 152 down-regulated. The major group of genes (33 genes) down-regulated is transporters, In case of urease mutant; additional genes were differentially regulated when compared to wild type strain that could play a role in infection. Overall, 375 genes were differentially regulated (at least 2 fold, $p<0.05$) where 236 genes were down-regulated while only 139 were up-regulated. A mutation in urease has pleiotrophic effects on gene expression and precludes direct assessment of whether the urease is directly responsible for virulence.

Abstract 5 - Pergentino Balbuena

NEUROTOXICANTS MALATHION AND LEAD ACETATE INCREASE GENE EXPRESSION OF SCAFFOLD PROTEINS ZO1 AND ZO2, AND CALCIUM CHANNEL PROTEIN TRPC1 IN ENDOTHELIAL CELLS. P. Balbuena, W. Li and M Ehrich, Virginia-Maryland Regional College of Veterinary Medicine, Virginia Tech.

ZO1 and ZO2 are scaffold proteins that support tight junction structures in the blood-brain barrier (BBB). Damage to these proteins affects tight junction structure and therefore compromises BBB function. Transient receptor potential canonical channel protein 1 (TRPC1) forms store-operated calcium channels associated with the calcium signaling that regulates cell permeability in the endothelial cells of the BBB. Since lead is known to mimic calcium in several calcium related pathways, it may affect TRPC1 expression. The present experiment assessed changes in gene expression of ZO1 and ZO2 scaffold proteins, and of TRPC1 protein in rat brain vascular endothelial cells (RBE4) in response to treatments with neurotoxicants malathion and lead acetate alone and in combination. We utilized real time polymerase chain reaction (Q-PCR) analysis to assess changes in patterns of gene expression for these proteins, applying the comparative C_T method for relative quantification. Lead alone did not increase gene expression for any of the genes at any of the concentrations tested. In contrast, when applied alone, malathion increased gene expression for all three genes. Combinations of malathion and lead significantly increased gene expression of ZO1, ZO2, and TRPC1 proteins. Increases of ZO1 and ZO2 expression may be indicative of damage to the scaffold support of the tight junctions; increases in TRPC1 expression may be associated with formation of more store-operated calcium channels, which would increase intracellular accumulation of lead. In both events, the immediate result is the increasing permeability of the BBB.

Abstract 6 - Deena Khan

ESTROGEN UPREGULATES IL-17 SECRETING CELLS AND LEVELS IN C57BL/6 AND LUPUS-PRONE NZB/W MICE. Deena Khan*, Rujuan Dai*, S Ansar Ahmed*. *Department of Biomedical Sciences and Pathobiology, Virginia-Maryland Regional College of Veterinary Medicine, Virginia Polytechnic Institute and State University, Blacksburg, Virginia.

Estrogen has been shown to have profound effects on immune biology and pathological conditions, such as female predominant autoimmune diseases particularly systemic lupus erythematosus (SLE). IL-17, a novel proinflammatory cytokine is now rapidly recognized to play a pivotal role in autoimmune disease progression. We have previously shown that estrogen upregulates IFNγ and Th1-related immune response. However, it is still not known whether estrogen modulates the differentiation of IL-17 and Th17-related immune responses. In the present studies in normal (C57BL/6) and autoimmune lupus (NZB/W) mice, we show (for the first time) that estrogen induces IL-17 from splenocytes that were activated with IL-17 inducing stimuli (IL-6+ TGFβ+ anti CD3 antibodies). The upregulation of IL-17 levels by estrogen was also confirmed by enhanced numbers of IL-17-positive cells by flow cytometry and IL-17-secreting cells by ELISPOT analysis. In addition, we found that cells from estrogen-treated mice have higher numbers of cells expressing IL-17-specific transcription factor, RORγT. Importantly, we also found that IL-17 levels can be inhibited in a sustained fashion by IL-27 but not by IFNg. Together, our studies are the first to show that estrogen promotes IL-17 in both normal and autoimmune mice and document the IL-27-mediated suppression of IL-17. These studies add new knowledge to estrogen regulation of inflammation.

Abstract 7 - Neeraj Singh

MOLECULAR MECHANISMS REGULATING INNATE IMMUNE CELL'S FREE RADICAL GENERATION IN RESPONSE TO LPS CHALLENGE. Neeraj Singh1 and Liwu Li1,2. 1 Department of Biomedical Sciences and Pathobiology, VMRCVM, Blacksburg, VA, 2Laboratory o Innate immunity and inflammation, Department of Biology, Virginia Tech, Balcksburg, VA.

LPS induces free radical production via TLR-4 signaling pathways. Excessive ROS generated following LPS administration are responsible for oxidative stress related tissue injury. IRAK-1, an intracellular signaling kinase, downstream of LPS-mediated signaling pathway, play a vital role in regulating many LPS-induced proinflammatory gene products. The present study was undertaken to evaluate the role of IRAK-1 in regulating ROS/oxidative stress following LPS challenge. BMDM isolated from IRAK-$1^{-/-}$ & WT mice were used for invitro studies while invivo septic shock studies were conducted by injecting 25 mg/kg LPS. Plasma and vital organs were collected 8 hrs following LPS challenge for measuring oxidative stress related parameters. BMDM were treated with LPS and free radicals generated were quantified by using DCFDA. Biochemical, PCR and western blot techniques were used out to determine the role of IRAK-1 on P47 translocation, NOX-1, Rac-1 activity and antioxidative enzymes status following LPS challenge. Our study revelas that IRAK-1 plays an important role in modulating LPS-induced ROS generation. IRAK-$1^{-/-}$ cells exhibited reduced ROS production following LPS challenge. IRAK-1 facilitates ROS generation by regulating p47 membrane translocation, NOX-1 & Rac-1 activity while suppressing the expression of antioxidant enzymes like GPx & Catalase, IRAK-1 deletion protected mice against sepsis-associated mortality by significantly reducing plasma proinflammatory cytokines level, lipid peroxidation products and nitrite levels. Irak-1 deletion maintained level of protective antioxidant like GSH, GPx activity. HE revealed comapartively lesser necrotic lesions in IRAK-$1^{-/-}$ mice liver. In conclusion these findings suggest that irak-1 is critical in the host inflammatory and antioxidative enzymes regulation.

Keynote Presentation

Nammalwar Sriranganathan

Pfizer Award for Research Excellence Recipient

NANOMEDICINE: DRUG DELIVERY AGAINST INTRA-CELLULAR PATHOGENS. N. Sriranganathan. November 20th, 2009. Pfizer Award Presentation

Nanotechnology is a multidisciplinary scientific field dealing with materials whose physical and chemical properties can be controlled at molecular level by incorporating engineering and manufacturing principles. Nanomedicine specifically is defined as the use of such materials in which at least one of the dimensions that affects their function is in the size range of 1-100 nm, for a specific diagnostic or therapeutic purpose. Nanomedicine based site directed drug therapy should promote reduced dosage, frequency and toxicity especially in chronic intracellular infection. For example, the manifestation of nephrotoxicity and ototoxicity on prolonged administration of aminoglycosides or the potential problems of liver damage by tetracyclines.

Intracellular pathogens like *Mycobacterium*, *Brucella,* and *Salmonella* have developed various mechanisms to evade host defenses, and consequently establish chronic infections. They colonize mononuclear phagocytes such as blood-borne monocytes, tissue macrophages, dendritic cells, and microglia as cell reservoirs. These pathogens have also evolved to avoid activation of the immune system, interfere with intracellular trafficking, resist respiratory burst, and adapt to oxygen-limited conditions encountered inside macrophages. Treatment and eradication are difficult because infections are localized within phagocytic cells. Many effective antimicrobials, although highly active *in vitro*, do not actively pass through cellular membranes. Nanomedicine should address targeting of active drugs to the intracellular compartment, where the bacteria hide and replicate, along with prolonged release of the antimicrobials so that the number of doses and associated toxicity can be reduced. I would like to discuss our current efforts in team building and some of the encouraging results from our study.

Session II

Graduate Student Presentations

Abstracts (8-13)

Chair: Dr. David Hodgson

Abstract 8 - Naveen Surendran

ENHANCED ABILITY OF *BRUCELLA ABORTUS* ROUGH VACCINE STRAINS TO INDUCE INNATE IMMUNITY IN A MURINE MODEL *IN VITRO* AND *IN VIVO*. N. Surendran[1]*, B.Hiltbold[3], B. Heid[1], H. Lawler[1], N. Sriranganathan[2], S. Boyle[2], Kurt Zimmerman[2], M. Makris[2], S. Witonsky[1]. [1]Department of Large Animal Clinical Sciences, [2]Department of Biomedical Sciences and Pathobiology, Virginia-Maryland Regional College of Veterinary Medicine, Virginia Tech, Blacksburg, VA. [3]Department of Microbiology and Immunology, Wake Forest University, Winston-Salem, NC.

Brucella spp. are Gram negative facultative intracellular bacterial pathogens. *Brucella* cause infertility, abortion and reduced productivity in a wide range of animals worldwide and cause a chronic debilitating disease in humans. *B. abortus* strain 2308 is one of the pathogenic strains affecting cattle and can induce disease in humans. There are no approved human vaccines available to date. *B. abortus* rough strain RB51, which lacks the O-side chain of lipopolysaccharide, is the live attenuated vaccine strain used for vaccinating cattle in the United States. Another rough strain RB51SOD, which over-expresses Cu-Zn superoxide dismutase, confers better protection than strain RB51 in murine model of brucellosis. Limited information is available regarding how *Brucella* stimulates innate immunity, although subsequent Th1 and Tc1 cell mediated immunity are critical for protection. Furthermore, there is lack of information on how intranasally administered *Brucella* vaccine stimulates the pulmonary innate immunity. Understanding the protective immune response is critical in improving the vaccines for their ultimate use in humans. Dendritic cells (DC) are key mediators of innate and adaptive immunity. Here, we report using murine bone marrow derived dendritic cells, that rough strain RB51 stimulates significant ($p \leq 0.05$) DC activation *in vitro* compared to strain RB51SOD, characterized by MHC class II, costimulatory marker expression and Th1 cytokine production. This result was corroborated *in vivo* as intranasally infected mice with strain RB51 induced higher DC activation, cytokine production and proinflammatory histopathological changes compared to strains RB51SOD and 2308.

Abstract 9 - Ronald Tyler

***MYCOBACTERIUM AVIUM* SUBSPECIES *PARATUBERCULOSIS* DNA EXTRACTION AND DETECTION BY A COMMERCIAL REAL-TIME PCR ASSAY FROM FORMALIN-FIXED PARAFFIN EMBEDDED TISSUES AND CORRELATION WITH HISTOPATHOLOGY.** R.D. Tyler Jr.†, K. Anklam‡, E. Manning‡, M. Collins‡, N. Sriranganathan†. † Department of Biomedical Science and Pathobiology, Virginia-Marland Regional College of Veterinary Medicine, Blacksburg, VA. ‡ Department of Biological and Pathological Sciences, University of Wisconsin-Madison School of Veterinary Medicine, Madison, WI.

Mycobacterium avium subspecies *paratuberculosis* (***Map***) causes Johne's disease, a chronic granulomatous enteric disease of ruminant animals. Animals typically present with prolonged weight loss, diarrhea, and generalized edema. In this study a newly described method of extracting ***Map*** DNA from formalin-fixed paraffin-embedded (FFPE) tissues collected from animals diagnosed with Johne's disease at necropsy was used to evaluate the detection of the ***Map*** DNA by a new commercial real-time PCR assay. In addition, results of the real-time PCR assay are correlated with histopathology and numbers of acid fast bacteria within tissue sections. Of 35 case definition FFPE tissues, 25 tested positive with the commercial quantitative real-time PCR yielding a sensitivity of **71.4**%. Of 21 FFPE tissues from the negative controls (small intestine and lymph nodes from 8 animals with no gastrointestinal disease), none tested positive giving a specificity of **100**%. Last, there was a distinct **inverse correlation** of the cycle threshold time results compared to the acid fast tissue score. These results indicate that FFPE tissues can be used to detect ***Map*** genetic material with results comparable to culture methods but with a much shorter turnaround time. Additionally these results show that there is an inverse correlation of the quantitative real-time PCR cycle threshold time results and the Zeihl-Neelsen's staining intensity in tissue sections.

Abstract 10 - Alicia Feagins

CONSTRUCTION AND CHARACTERIZATION OF A HEV CHIMERIC VIRUS USING THE GENOMIC BACKBONE OF A GENOTYPE 1 HEV WITH A LIMITED HOST RANGE AND THE CAPSID GENE OF A GENOTYPE 4 HEV WITH AN EXPANDED HOST RANGE. A. R. Feagins[1], T. Opriessnig[2], Y. W. Huang[1], S. U. Emerson[3], and X. J. Meng[1]. [1]Center for Molecular Medicine and Infectious Diseases, Department of Biomedical Sciences and Pathobiology, College of Veterinary Medicine, Virginia Polytechnic Institute and State University, Blacksburg, VA, [2]Department of Veterinary Diagnostic and Production Animal Medicine, College of Veterinary Medicine, Iowa State University, Ames, IA, [3]Laboratory of Infectious Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD.

Genotype 3 swine HEV can cross species barriers and infect humans and the genotype 3 US2 strain of human HEV can infect pigs. The genotype 4 TW6196E strain of human HEV can also infect SPF pigs. However, a genotype 1 strain of human HEV (Sar-55) and a genotype 2 strain of human HEV (Mex-14) were unable to infect pigs. This data suggests that both genotype 1 and genotype 2 strains of human HEV may have a limited host range and infect only humans. The capsid protein, encoded by open reading frame 2 (ORF2), is the main, perhaps only, structural protein responsible for host cell interaction, which suggests a possible role in determining the host tropism and susceptibility. To determine if the capsid gene can influence HEV host range, a chimeric virus using the genomic backbone of limited host range genotype 1 HEV and the capsid gene of an expanded host range genotype 4 HEV was constructed. The capsid gene of genotype 1 human HEV (Sar-55 strain) was replaced by a genotype 4 human HEV (Taiwan TW6196E) capsid gene in the genomic backbone of a genotype 1 infectious cDNA clone of human HEV Sar-55 to generate a chimeric virus. The viability of the chimeric virus is being tested *in vitro*. An animal experiment in pigs will follow to definitively determine the infectivity of the chimeric virus. The results of this study will enhance our understanding of the mechanism of cross-species infection.

Abstract 11 - Jamie Wearn

THE PHARMACOKINETICS AND PHARMACODYNAMICS OF PIOGLITAZONE IN HORSES AND ITS POTENTIAL ROLE AS AN INSULIN SENSITIZING AGENT FOR THE MANAGEMENT OF EQUINE METABOLIC SYNDROME. J.M.G. Wearn[1], L. J. McCutcheon[1], J.L. Davis[2], D.R. Hodgson[1], M. Ashraf-Khorassani[3], R. J. Geor[4], R.S. Pleasant[1], J. K. Suagee[4], M.V Crisman[1], 1. Department of Large Animal Clinical Sciences, Virginia-Maryland Regional College of Veterinary Medicine, Virginia Polytechnic Institute and State University, Blacksburg, Virginia. 2. Department of Clinical Sciences, College of Veterinary Medicine, North Carolina State University, Raleigh, North Carolina. 3. Department of Chemistry, Virginia Polytechnic Institute and State University, Blacksburg. 4. Department of Animal and Poultry Sciences, Virginia Polytechnic Institute and State University, Blacksburg, Virginia.

Equine Metabolic Syndrome (EMS) is a unique condition of horses characterized by adiposity, insulin resistance, and an increased risk of laminitis. Although the underlying mechanism of EMS is unknown, compensatory hyperinsulinemia resulting from insulin resistance, obesity, and a pro-inflammatory state, are considered integral to the heightened risk of laminitis. Reducing insulin resistance may decrease the incidence of laminitis in horses with EMS. Pioglitazone increases insulin sensitivity in humans with type 2 diabetes, a syndrome of insulin resistance sharing some similarities with EMS. Our hypothesis was that PIO would preserve insulin sensitivity in a model of induced insulin resistance. The specific aims were to investigate the pharmacokinetics and pharmacodynamics of pioglitazone in an endotoxin infusion model of insulin resistance. 16 normal horses were enrolled. Pioglitazone was administered to 8 horses (1 mg/kg, PO, q24h) for 14 days. Liquid chromatography-ion spray tandem mass spectroscopy was used to quantitate plasma concentration of pioglitazone. A frequently sampled intravenous glucose tolerance test with minimum model analysis was used to compare indices of glucose and insulin dynamics prior to, and following, endotoxin infusion in horses treated with pioglitazone and their controls. Pioglitazone administration resulted in plasma concentrations similar to those considered therapeutic in humans. No significant effect of drug treatment was detected on indices of insulin dynamics following endotoxin challenge. Although no significant effects on insulin dynamics were identified in this model, the authors suggest further investigations of the effects of pioglitazone on inflammation and in horses with EMS are warranted.

Abstract 12 - Kelly Johnson

IN VITRO 3-DIMENSIONAL KINEMATIC EVALUATION OF THE TIBIAL PLATEAU LEVELING OSTEOTOMY THROUGHOUT THE STANCE PHASE OF GAIT IN THE DOG. KA Johnson, OI Lanz, S Elder, RM McLaughlin, TA Harper. Department of Small Animal Clinical Sciences, Virginia-Maryland Regional College of Veterinary Medicine, Blacksburg, Virginia.

To determine the ability of the Tibial Plateau Leveling Osteotomy (TPLO) to restore normal joint kinematics in a cranial cruciate ligament (CrCL) deficient stifle throughout the weight bearing range of motion. Paired pelvic limbs from 12 dogs, weighing 20-40kg, were compared in an in vitro biomechanical study. Each limb was placed in a custom designed jig at 120° of stifle extension under an axial load of 20% body weight. Electromagnetic motion tracking sensors were placed on the lateral aspect of the distal femur and proximal tibia. A force was applied to mimic the action of the quadriceps muscle. Force application allowed the limb to move from 120° to maximal extension. Positional data was acquired at 60 points/second. Each limb was tested under normal, CrCL deficient, and TPLO-treated conditions. Transection of the CrCL resulted in a median cranial tibial translation of 5.92mm and a median of 0.77° of internal rotation throughout the weight bearing range of motion. TPLO failed to normalize cranial tibial translation within the CrCL deficient stifle; however, values trended towards intact values throughout the range of motion. No significant differences were noted in internal rotation in any of the three conditions from 120° – 140°. Hyperextension did not differ significantly between conditions. Data from this in vitro biomechanical model suggests that the TPLO fails to neutralize cranial tibial translation throughout the weight bearing range of motion. The effectiveness of the TPLO in restoring normal biomechanics is more significant at greater angles of flexion.

Abstract 13 - James Brown

DOES GROWTH ON COLLAGEN-COATED PLATES ENHANCE MESENCHYMAL CELL DIFFERENTIATION TOWARD TENDON? J.A. Brown, J.G. Barrett, L.A. Dahlgren* and N.A. White II. Marion duPont Scott Equine Medical Center, Virginia-Maryland Regional College of Veterinary Medicine, Leesburg, Virginia, and *Department of Large Animal Clinical Sciences, Virginia-Maryland Regional College of Veterinary Medicine, Blacksburg, Virginia.

Compare cell growth kinetics, matrix synthesis (collagen and glycosaminoglycans), matrix metalloproteinase expression and relative expression of selected genes characteristic of tendon fibroblast phenotype, between bone marrow mesenchymal stromal cells (BMMSCs) and tendon progenitor cells (TPCs) cultured on porcine, bovine and rat-tail collagen coated tissue culture surfaces. BMMSCs and TPCs were harvested and cultured from 6 adult horses. Tissue culture dishes were pre-coated with porcine, bovine and rat-tail collagen and cells were seeded at uniform density in triplicate. Standard plastic culture plates were used as the negative control. Cell number and sulfated glycosaminoglycan production were determined on days 4 & 7 by measuring mitochondrial enzyme activity and dimethylmethylene blue dye binding colometric conversion assays, respectively. Twenty-four hour production of collagen and sulfated glycosaminoglycan was determined on day 7 by measurement of incorporation of tritiated hydrogen and ^{35}Sulfur, respectively. Relative expression of collagen types I & III, COMP, decorin, and degradative enzymes (MMPs) was determined on day 7 using real time PCR and the comparative threshold cycle method ($\Delta\Delta C_t$) with internal GAPDH RNA control. Pair-wise comparisons and repeated measures ANOVA were used to determine statistical significance ($p<0.05$). Preliminary results suggest superior cell growth on all types of collagen compared with no collagen coating without significant difference among the different collagen sources. Further results are pending. Improved cell growth kinetics and differentiation of mesenchymal cells towards the tenocyte cell lineage with production of tendon-specific matrix is highly desirable for application of stem cell based therapies in equine tendonitis.

Session III

Graduate Student Presentations

Abstracts (14-19)

Chair: Dr. Gregory Daniel

Abstract 14 - Hsing-Ho Hsu

PREVALENCE OF IGG ANTIBODIES TO *T. GONDII* AND *E. CUNICULI* IN CATS WITH AND WITHOUT CHRONIC KIDNEY DISEASE. Hsing-Ho Vasha Hsu[1], David C. Grant[2], and David S. Lindsay[1]. The Departments of Biomedical Sciences and Pathobiology and Small Animal Clinical Sciences [2], Virginia-Maryland Regional College of Veterinary Medicine, Blacksburg, Virginia.

Chronic kidney disease (CKD) is common in cats. Two zoonotic parasites *Toxoplasma gondii* and *Encephalitozoon cuniculi* can infect the kidneys of cats and cause disease. We are interested in examining the prevalence of *T. gondii* and *E. cuniculi* antibodies in cats with and without renal disease for two reasons; CKD is a major health concern in cats and renal transplants in cats are increasing. Plasma and sera were obtained from 192 feline patients at the VMRCVM teaching hospital. With the investigators blinded, samples were screened via indirect immunofluorescent antibody assays at a dilution of 1:25 for antibodies to *T. gondii* RH strain tachyzoites and a dilution of 1:10 for antibodies to *E. cuniculi* type III spores. Of the 192 samples, 27 (14%) were from cats diagnosed with chronic kidney disease. Antibodies to *T. gondii* were found in 46 (24%) of the 192 samples, and 5 (18%) of the 27 cat with renal disease were positive for *T. gondii*. Antibodies to *E. cuniculi* were found in 20 (10%) of the 192 samples, and 4 (14%) of the 27 cats with renal disease were positive for *E. cuniculi*. The 24% prevalence of *T. gondii* antibodies is higher than in previous reports on owned cats from the US. There are no previous studies on the prevalence of antibodies to *E. cuniculi* in cats from the US. Further investigation of the health reports to look for the influence of age, sex, stage of CKD and breed on the prevalence of antibodies is in progress.

Abstract 15 - Jessica Gilbertie

IMMUNOLOGICAL MARKERS OF STRESS IN HEIFER BEEF CALVES. Jessica Gilbertie, Michelle Todd, Stewart Harvey, William Swecker. Department of Large Animal Clinical Sciences, Virginia-Maryland Regional College of Veterinary Medicine, Virginia.

Weaning is known to cause stress in beef calves, which increases glucocorticoid production. T helper lymphocytes can be divided into two subsets, T helper 1 (Th1) and T helper 2 (Th2). T helper 1 is characterized by IFNγ production; IFNγ increases IgG_2 concentrations. T helper 2 is characterized by IL-4 production; IL-4 increases in IgG_1. Glucocorticoids cause a Th2 bias; therefore cytokines involved in Th1/Th2 responses can be used to measure the stress response. Twelve Angus heifer calves were allotted into a fence line or abrupt weaning group as fence line weaning is less stressful, based on behavior. Calves were vaccinated the day after weaning (Day 0) with RB51 vaccine, which leads to a Th1 response in mice. Blood was collected on days -7, 0, 7, 14, 21, and 42. Fecal samples were taken at day -7, day 0, and day 3. Serum was analyzed for RB51-specific IgG_1 and IgG_2 antibodies, IFNγ and IL-4 using an ELISA. Fecal samples were analyzed for cortisol metabolites. At day 0, fecal cortisol was higher in abrupt versus fence line weaned calves ($P<0.001$). IL-4 was undetectable. No difference was detected in IgG_1 or IgG_2 antibodies to RB51 between treatments, but both IgG_1 and IgG_2 increased from day 0 to day 14 ($P<0.05$). Fence line calves had higher IFNγ concentrations than abrupt at day -7 and day 0 ($P<0.04$). Results suggest that RB51 did not initiate a strong Th1 response in either treatment group, because no differences were found between IgG_1 and IgG_2 antibodies.

Abstract 16 - Sarah Davies

QUANTITATIVE PERTECHNETATE THYROID SCINTIGRAPHY AND ULTRASONOGRAPHIC APPEARANCE OF THE THYROID GLAND IN EUTHYROID HORSES. S. Davies, G. Daniel, D. Barber, M. Crisman and M. Larson. Virginia-Maryland Regional College of Veterinary Medicine, Virginia, 24061.

The purpose of this study was to document features of pertechnetate scintigraphy and B mode ultrasonography of the thyroid gland in a group of normal horses. Thyroid to salivary (TS) ratio and percent dose uptake of pertechnetate by the thyroid gland (% uptake) were calculated and compared between horses in two age groups. Group A consisted of 8 horses ≤ 10 years of age. Group B included 7 horses > 11 years of age. Inclusion criterion included normal physical exam, hematology and serum biochemistry, and total thyroxine (Tt_4). Ultrasound was used to determine gland volume and document the presence of nodules. Scintigraphic images were acquired following injection of 30mCi of $^{99m}NaTcO_4$. Regions of interest were drawn around the thyroid and salivary glands to calculate TS ratio and % uptake. T-tests were used to determine if there was a significant difference in mean Tt_4 concentration, thyroid gland volume, TS ratio or % dose uptake between groups A and B. Group B had significantly lower mean Tt_4 concentrations than group A (2.34 μd/dl versus 2.97 μg/dl) with $p<0.05$. There was no significant difference in thyroid gland volume, number of nodules or TS ratio between groups. There was a trend for increased % uptake in group B. There was greater variation in Tt_4 concentrations, thyroid size and % uptake in older horses. Further study is needed to determine if there is an age relationship with % dose uptake and if increased uptake in older horses is a normal finding or represents early disease.

Abstract 17 - Linda Frellstedt

ENDOTOXIN TOLERANCE INDUCED IN VITRO IN EQUINE LYMPHOCYTES. L. Frellstedt and M. Furr. Marion DuPont Scott Equine Medical Center, Virginia-Maryland Regional College of Veterinary Medicine, Leesburg, Virginia.

Endotoxin tolerance (ET) has been induced in horses in vivo, however the cytokine responses associated with this have not been investigated. The purpose of this study was to describe a method for inducing ET in equine cells in vitro, and to describe the cytokine profile which is associated with the tolerance. Blood was collected from 6 healthy horses and peripheral blood mononuclear cells were isolated. ET was induced by culturing cells with a variety of concentrations of endotoxin (0.1, 1.0 and 10 ng/ml), followed by a second endotoxin challenge (1.0, 10 and 100 ng/ml). The state of tolerance was identified by determining TNF-α concentrations (ELISA). ET was confirmed when TNF-α production was reduced by >75% compared to non-tolerized control cells. Wide individual variation in the response of equine cells to endotoxin was identified. Non-tolerized cells produced TNF-α concentrations of 108 to 4713 pg/ml. ET was induced in all cells exposed to the 2-step endotoxin challenge. The most consistent results were achieved when cells were exposed to 1.0 ng/ml of endotoxin initially and then challenged with 10 ng/ml of endotoxin. The TNF-α production was reduced by >85% in all cells exposed to described protocol. For the second aim of the study ET was induced using this protocol. The cytokine profiles associated with ET are currently being determined using RT-PCR. This experiment proved that ET can be induced in vitro in equine lymphocytes. The determination of the associated cytokine profile will provide important information regarding ET in equine cells.

Abstract 18 - Jessica Gentile

ECHOCARDIOGRAPHIC ASSESSMENT OF THE CANINE RIGHT HEART: REFERENCE INTERVALS AND REPEATABILITY. J.M. Gentile and J.A. Abbott. Department of Small Animal Clinical Sciences, Virginia-Maryland Regional College of Veterinary Medicine, Blacksburg, Virginia.

Historically, echocardiographic evaluation of left heart function has been emphasized but there has been recent interest in diseases characterized by changes in right ventricular structure and function including pulmonary hypertension (PH) and arrhythmogenic right ventricular dysplasia (ARVD). Currently, neither reference intervals for canine right heart dimensions, nor repeatability of echocardiographic indices of right heart structure and function, have been reported. Measurement of tricuspid regurgitation (TR) velocity is the principal echocardiographic method for estimation of pulmonary artery pressure but because measureable TR is not always present, alternative echocardiographic indices of PH have been identified. However, repeatability of these indices is unpublished. We obtained echocardiographic data from 43 healthy dogs. Images from standard and modified planes that accentuate the right atrium and right ventricle were digitally recorded. Six of the 43 healthy dogs were randomly selected and subject to repeated echocardiographic examinations by two operators in order to describe measurement repeatability. To evaluate repeatability of echocardiographic indices used in assessment of PH, four asymptomatic dogs with clinically stable disease and measurable TR velocities were subject to repeated echocardiographic examination. Currently, measurements are being obtained from the stored echocardiographic images. Numerical data from the 43 healthy dogs will be used to define reference intervals for echocardiographic dimensions of the right atrium and ventricle. In order to assess measurement repeatability, a mixed model will be developed to evaluate the effect of time and operator on numerical data. Variance component analysis and coefficients of variation will be used to evaluate measurement repeatability.

Abstract 19 - Vrushali Chavan

NEWCASTLE DISEASE VIRUS BIO-NANOPARTICLES FOR TUMOR SELECTIVE TARGETING AND ONCOLYSIS. Vrushali Chavan[1], Shobana Raghunath[1], Dan Qiao[1], Nikorn Pothayee[2], Judy Riffle[2], and Subbiah Elankumaran[1]. [1]Center for Molecular Medicine and Infectious Diseases, Virginia-Maryland Regional College of Veterinary Medicine, and [2] Department of Chemistry, Virginia Polytechnic Institute and State University, Blacksburg, VA.

Oncolytic viruses such as Newcastle disease virus (NDV) afford rapid and rational design through recombinant DNA technology to facilitate the targeting of a broad spectrum of malignancies. Ligand directed, receptor mediated targeting is the most common approach for targeting oncolytic viruses to specific cell types. However, the use of replication competent NDV for cancer therapy is limited by the widespread availability of the sialic acid receptors on normal and cancer cell surface and neutralization of virus infectivity after repeated administration. Here, we have developed a method for obtaining virus-like bio-nanoparticles (BNPs) of NDV that are engineered to carry therapeutic molecules or transgenes on the surface or the core. The BNPs were made by co-expression of the NDV matrix (M), hemagglutinin (HN) and fusion (F) proteins in DF-1 chicken embryo fibroblast cells. The recombinant BNPs are non-replicating and selectively targeted to folate receptors on cancer cells. Most cancer cells bear folate receptors but normal human cells lack them. The dynamics of budding and release of BNPs in transfected human cells examined by transmission electron microscopy were reminiscent of infectious NDV. The BNPs were also evaluated *in vitro* in human cancer cell lines for cytoplasmic diffusion and cytotoxicity. Our results show that NDV BNPs are selectively infective to folate receptor bearing cancer cells and cytotoxic. This personalized approach based on receptor distribution would help in delivering small molecules, cytotoxins, reporter genes or antibodies to selective tumor cell types and also aid in generating tumor specific immunity by custom design.

Graduate Student Poster Presentations

Abstracts (20-40)

Abstract 20 – Suzanne Hirst (MS Student)

ANTI-INFLAMMATORY PROPERTIES OF CERIUM OXIDE NANOPARTICLES. S.M. Hirst, A. Karakoti, R. Tyler, N. Sriranganathan, S. Seal and C.M. Reilly. Department of Biomedical Sciences and Pathobiology, Virginia-Maryland Regional College of Veterinary Medicine, Blacksburg, Virginia.

Cerium oxide nanoparticles have oxygen defects in their lattice structure that enable them to scavenge oxygen radicals. Recent reports have shown nanoceria to act a regenerative free radical scavenger in a physiological environment. The goal of our study was to perform a comprehensive analysis of nanoceria administered through three routes: peroral (PO), intravenous (IV), and intraperitoneal (IP). We examined bio-accumulation, organ deposition, clearance, histology and WBC differentials to determine target organs and immunologic response of nanoceria. The most extensive and cumulative nano-deposition was found in IV and IP administered mice, while PO mice excreted greater than 95% of their doses. As determined by ICP-MS, nanoceria organ deposition for IV and IP mice was greatest in the spleen followed by the liver, lungs, and kidneys. Elimination for all administration routes was through feces. Nanoceria distribution was confirmed with IVIS in vivo imaging, and deposition was confirmed with TEM. There was no statistical difference in histology grades between control and nanoceria dosed mice, however, nanoceria dosed mice generally showed elevated WBC counts. In vitro, we demonstrate that ROS, nitrates, and iNOS protein and mRNA levels decrease in a dose dependent manner with nanoceria administration in a J774A.1 murine macrophages. Our in vitro studies show that nanoceria reduces ROS and inflammatory mediators, and our in vivo studies show that the spleen, the largest lymphoid organ, has the greatest deposition than any other organ in the body. Our studies therefore suggest that cerium oxide may decrease inflammatory mediators and oxygen radicals in biological systems.

Abstract 21 – Amitesh Mandal (MS Student)

IMMUNE RESPONSE OF HYBRID STRIPED BASS FOLLOWING ORAL DELIVERY OF A CHITOSAN-ENCAPSULATED DNA VACCINE FOR MYCOBACTERIOSIS. Amitesh Mandal and Stephen A.Smith. Department of Biomedical Sciences and Pathobiology, Virginia-Maryland Regional College of Veterinary Medicne , Virginia Tech, Blacksburg , VA 24061-0442.

Utilization of DNA vaccines in aquaculture has been gaining interest and recently efforts have been focused on methods of delivering DNA vaccines to fish. The immune response of hybrid striped bass *(Morone saxatilis x Morone chrysops)* following oral delivery of a DNA vaccine containing Mycobacterium marinum Ag85a plasmid in a chitosan nanoparticle encapsulation was determined. Hybrid striped bass (20-40g) were acquired from a local hatchery and maintained in the laboratory in recirculation systems at 1-2 ppt salinity and 23-26 C. Fish were individually tagged for identification and fed a commercial pelleted diet (Zeigler Bros. Inc.). Fish were arbitrarily divided into four experimental groups of twenty fish each: an IM immunization of the DNA vaccine as a positive control, an oral delivery of uncomplexed DNA vaccine, an oral delivery of chitosan alone as a negative control, and an oral delivery of complexed chitosan-DNA vaccine. The vaccine dose for both the intramuscular injection and oral delivery was fixed at 50µg/fish. Fish were bled non-lethally every two weeks for 10 weeks post immunization. Blood was allowed to clot overnight, serum obtained the following day by centrifugation, and the serum frozen in individual tubes. An ELISA, using a whole-cell lysate of Mycobacterium marinum as the substrate , an affinity purified rabbit anti-hybrid bass antibody and a peroxidase-labeled goat anti-rabbit antibody, was used to evaluate antibody levels in individual fish. ELISA reactions were read at 450 nanometers and analyzed using Molecular Device's Softmax Pro ELISA Plate Reader Software program.

Abstract 22 – Robert Solberg (MS Student)

INDUCTION OF BLOOD TRAUMA BY COMMONLY USED EXTRACORPOREAL BLOOD PUMPS. R.G Solberg and J.L. Robertson, VMD/PhD. Department of Biomedical Sciences and Pathology, Virginia-Maryland Regional College of Veterinary Medicine, Blacksburg, VA.

Blood circulated outside of the body reacts with artificial surfaces and pumping mechanisms, causing complications that are potentially dangerous to patients. These reactions include thrombogenesis, complement activation, inflammatory reactions and hemolysis. We believe these reactions to be dependent on the conditions of extracorporeal circulation: namely time, temperature, and method of pumping. Most studies of extracorporeal circulation limit the circulation time to 6 hours, as cardiopulmonary bypass and hemodialysis (the most common extracorporeal circulation procedures) are limited to 6 hours and usually take much less time. However, other applications such as isolated organ require longer periods of artificial circulation. Our research is aimed at exploring the changes in blood artificially circulated for longer periods of time, up to 48 hours. Our hypothesis is that blood components will exhibit different rates of degradation based on the conditions of circulation. Data collected to date indicates that blood components do exhibit time-based changes, however no data has been collected to determine whether changes are dependent on temperature or pumping condition.

Abstract 23 – Marianne Werner (MS Student)

DEVELOPING A METHOD FOR EQUINE LYMPHOCYTE RESPONSE TO BETA 2 AGONIST. Werner MP, Buechner-Maxwell VA, Ehrich MF, Prater MR, Eyre P. Department of Large Animal Clinical Sciences and Department of Biomedical Sciences, Virginia-Maryland regional College of Veterinary Medicine, Blacksburg, Virginia.

Beta-2 agonists promote bronchodilation in RAO horses and humans with asthma. Stimulation of the beta-2 adrenoreceptor on lymphocytes also serves to modulate inflammatory cytokine production in a dose dependent fashion. This response is partly achieved through activation of protein kinase A (PKA) by adenylyl cyclase using magnesium as a cofactor. *The aim of this study is to develop a method for evaluating PKA activity as a measure of beta-2 agonist stimulation in equine peripheral blood lymphocytes (PBLs), and to determine if adding magnesium to the cell environment has a positive effect on this activity.* **Methods:** Lymphocyte-enriched populations were obtained from equine PB using density gradient centrifugation. Cells were placed in RPMI complete media and incubated at 37°C, 5%CO_2. Phosphorylation of the 157th serine residue of vasodilator stimulated phosphoprotein (VASP) was detected by flow cytometry. The effect of magnesium pre-incubation time and concentration, albuterol concentration, and antibody staining techniques were compared as was intra and inter-experiment repeatability. **Results:** Pre-incubation of lymphocytes with magnesium did not alter intracellular concentration or response to albuterol stimulation. Optimal range of concentrations were identified for magnesium (2.5 -5 mM) and albuterol (4-8 uM) and the addition of magnesium amplified the response to albuterol, but not in a consistent fashion. The use of FITC-labeled secondary antibody was determined to be the best method of staining. Repeatability was most consistent within populations of cells from the same horse. **Conclusions:** Further experimentation and development of alternative techniques (Western blot analysis) are essential to validate this methodology in horse tissues.

Abstract 24 – Kristin Cameron (MS Student, Small Animal Resident)

THE EFFECT OF ILLNESS ON URINE CONCENTRATIONS OF CATECHOLAMINES AND THEIR METABOLITES IN DOGS. K.N. Cameron, W.E. Monroe, D.L. Panciera, and J.B. Meldrum, Departments of Small Animal Clinical Sciences and Biomedical Sciences and Pathobiology, Virginia-Maryland Regional College of Veterinary Medicine, Blacksburg, VA 24061.

Pheochromocytomas are uncommon tumors in dogs that cause non-specific and episodic clinical signs associated with excessive secretion of catecholamines. Currently, the only method of definitive diagnosis is with a biopsy, but physical manipulation of the tumors can have serious complications. In humans, urine concentrations of catecholamines and their metabolites, metanephrine and normetanephrine, have proven to be highly sensitive and specific methods of testing for pheochromocytomas. The application of these tests in veterinary medicine to date has been limited and test results can be difficult to interpret as the effects of illness and the stress of hospitalization have an unknown impact on catecholamine and metanephrine concentrations. The purpose of this study is to evaluate the effects of illness on urine concentrations of catecholamines and metanephrines in dogs. Twenty-five critically ill dogs from the VMRCVM ICU population with non-adrenal illness will have urine samples collected to measure concentrations of catecholamines and metanephrines. Twenty-five age and gender matched pet dogs will act as clinically normal controls. Epinephrine, norepinephrine, metanephrine, and normetanephrine concentrations in urine will be measured and expressed as a ratio of the metabolite concentration to that of urine creatinine. The results will be compared between the two groups to determine the magnitude of the effect of illness on these parameters. Knowledge of these values in ill and healthy dogs will help determine the utility of these tests in the diagnosis of canine pheochromocytomas.

Abstract 25 – Erik Noschka (MS Student, Large Animal Resident)

CAN MEASUREMENT OF URINE ISOPROSTANES PREDICT SURVIVAL IN HORSES WITH COLIC? E. Noschka, M.V. Crisman, C.D. Thatcher[†], G.L. Milne[‡], L.A. Dahlgren. Department of Large Animal Clinical Sciences, Virginia-Maryland Regional College of Veterinary Medicine, Blacksburg, Virginia. [‡]Departments of Medicine and Pharmacology, Vanderbilt University School of Medicine, Nashville, Tennessee, [†]School of Applied Arts and Sciences at the Polytechnic Campus, Arizona State University.

Approximately 4% of horses suffer from one colic episode per year. The outcome is fatal in 11% of cases. The long term goal of this work is to reduce the number of deaths due to colic by developing a test to identify horses needing surgery and expediting their timely referral. F2-isoprostanes are the "gold standard" for assessment of oxidative stress in vivo, and have been used extensively to quantify lipid peroxidation in association with risk factors in various diseases. Horses with colic may have intestinal ischemia or inflammation characterized by oxidative stress and increased production of isoprostanes. The purpose of this study is twofold: (1) Determine urine concentrations of the isoprostane $PGF_{2\alpha}$ (iso-$PGF_{2\alpha}$) in normal horses, and a matched cohort of horses with colic, and (2) compare concentrations of iso-$PGF_{2\alpha}$ to estimate the prognostic value of urine iso-$PGF_{2\alpha}$. We hypothesize that urine concentrations of iso-$PGF_{2\alpha}$ will be significantly higher in horse with colic compared to normal horses, and horses requiring surgery compared to those treated medically. Urine samples were collected from 40 normal horses and 40 horses with colic (20 medical and 20 surgical). Urine iso-$PGF_{2\alpha}$ levels were measured by mass spectrometry and normalized to urine creatinine levels. Significance was set at $p \leq 0.05$. The measurement of urinary concentrations of isoprostanes may be an important prognostic indicator in equine colic. The results will be the basis for future studies designed to reduce the generation of isoprostanes or prevent their deleterious effects and development of a "stall-side" assay.

Abstract 26 – Clifton Cassidy (MS Student)

OUTER MEMBRANE VESICLE BASED BRUCELLOSIS VACCINE. Clifton Cassidy, Isis Mullarky, Nammalwar Sriranganathan, Stephen Boyle. Virginia-Maryland Regional College of Veterinary Medicine, Blacksburg VA 2406, Dairy Science Department, Virginia Tech, Blacksburg VA 24060.

Brucella abortus is a zoonotic agent that primarily infects cattle and causes brucellosis. ***B. abortus*** strain RB51 is a live, attenuated vaccine licensed for cattle. However, there is no available vaccine to prevent human brucellosis. Outer membrane vesicles (OMV) are constantly released from Gram-negative bacteria. They are comprised of the outer membrane and periplasmic proteins from the bacteria. OMV have been used in other bacterial species as vaccines (i.e. ***N. meningitides***). In this study, ***B. abortus*** strain RB51 and strain RB51 overproducing Cu/Zn super oxide dismutase (SOD) will be used to produce OMV. These OMV will be used as vaccines against brucellosis in the mouse model. Immune response towards the OMV will be determined and protection assays will follow. This study is currently in progress and all data may not be complete by the time of the symposium.

Abstract 27 – Hedie Bustamante (PhD Student)

THE POTASSIUM-SENSITIVE ATP CHANNEL IN PARKINSON'S DISEASE: DOPAMINE AND DOPAMINE METABOLITE (DOPAC) CONCENTRATION A PD MOUSE MODEL EXPOSED TO THE TYPE 2 DIABETES DRUG GLIBENCLAMIDE. H.A. Bustamante[1], T. Rogers-Cotrone[1], C.A. Dodd[1], J.R. Bloomquist[2], B.G. Klein[1]. [1]Department of Biomedical Sciences and Pathobiology, Virginia-Maryland Regional College of Veterinary Medicine, [2]Department of Entomology. Blacksburg, Virginia.

Mitochondrial dysfunction has been observed in Parkinson's disease (PD) and compounds that induce experimental parkinsonism interfere with the ATP-producing mitochondrial electron transport chain. Dopaminergic nigrostriatal neurons have a high concentration of ATP-sensitive potassium channels (KATP). The type II diabetes drug glibenclamide (GB) is a KATP blocker. There is some evidence that GB may facilitate toxicity to the nigrostriatal pathway in the MPTP mouse model of PD. This supports the potential involvement of KATP in the nigrostriatal degeneration of PD and suggests diabetics using GB could be at greater risk for PD. This study examined striatal dopamine and DOPAC concentrations in C57BL/6 retired breeder mice treated with GB or DMSO vehicle after administration of MPTP. Six treatment groups of 12 mice were used. GB (30 and 60 mg/kg) in DMSO, or DMSO alone were injected daily for 14 days. On day 2 mice received a single dose of MPTP (15 mg/kg) or vehicle. Mice were sacrificed 24 hours after the last GB dose. HPLC-UV revealed a significant main effect decrease in dopamine produced by MPTP but no main effect of GB upon dopamine. Moreover, there was no interaction between MPTP and GB in effects upon dopamine. Similar findings for DOPAC. From these results it can be concluded that GB does not affect MPTP-induced dopaminergic toxicity in the mouse model of PD.

Abstract 28 – Anna Champion (PhD Student)

FURTHER COMPOSITIONAL CHARACTERIZATION OF THE CAPSULE-LIKE MATERIAL OF FRANCISELLA TULARENSIS. A.E. Champion and T. Inzana. Department of Biomedical Sciences and Pathobiology, Virginia-Maryland Regional College of Veterinary Medicine, Blacksburg, Virginia.

Francisella tularensis is a category A select agent and the etiologic agent of tularemia. Two carbohydrate-containing surface antigens have been identified on this bacterium: lipopolysaccharide (LPS) and a capsule-like material (CLM). **Aims:** The CLM was thought to consist predominately of neutral sugars, but upon further study multiple components including protein, amino acids, and lipid were also identified. Our goal is to determine the complete composition of the CLM. **Methods:** Extraction of the cells with 0.5% phenol was used to isolate the CLM. Fractions were collected at specific points throughout the extraction, and analyzed for protein, carbohydrate, LPS, and lipid using gas-chromatography mass-spectrometry, SDS-PAGE with Coomassie and silver staining, and Western blotting using hyperimmune serum against whole cells of *F. tularensis* or purified CLM. **Results:** The most CLM was expressed when the bacteria were subcultured in defined medium and grown for 5 days. The CLM appeared as a faint, high molecular weight smear or bands by SDS-PAGE and Western blotting. Twenty-percent of the purified material was carbohydrate. The remaining 80% included protein, nucleic acids, and minimal LPS contamination. Testing for lipid content was inconclusive. **Discussion:** We hypothesize the carbohydrate component acts as a scaffold to maintain, as an aggregated structure, the mix of components. Current work has strengthened this theory. Mutagenesis of glycosyltransferases in a putative "capsule" locus has shown the collapse of this electron dense layer around the bacterial cell. Knowledge of the components of the CLM will aid in identifying other possible genes involved in regulation of the CLM.

Abstract 29 – Tila Khan (PhD Student)

MEMBRANE-BOUND IMMUNOMODULATORY INFLUENZA VACCINES PROVIDE PROTECTION AGAINST LETHAL HOMOTYPIC VIRUS CHALLENGE. Tila Khan, Lynn Heffron and Paul Christopher Roberts, Department of Biomedical Sciences and Pathobiology, Virginia-Maryland Regional College of Veterinary Medicine, Virginia Polytechnic Institute and State University, Blacksburg, Virginia.

Influenza virus continues to be responsible for widespread respiratory disease, resulting in deaths and economic loss worldwide, despite massive vaccination efforts and eradication programs. Vaccination remains most effective way to protect against influenza. Novel strategies are required to provide rapid vaccine coverage in the event of epidemic. **AIMS: 1.**The goal of this study is to construct Cytokine-bearing Influenza Vaccines (CYT-IVACs) by incorporating bioactive, membrane-bound immunomodulatory proteins into influenza A virus to serve as immune stimulators. **2**. Evaluation of CYT-IVACs ability to confer protection against lethal challenge and induce humoral and cellular immunity. **METHODS.** Influenza permissive MDCK cell lines expressing immunomodulators at the cell surface were used to propagate influenza virus A/PR/8/34 (H1N1) and released virus was concentrated, purified and inactivated with ß-propiolactone. Immunomodulator incorporation and associated bioactivity was verified by western blot analysis and in vitro cytokine specific bioassays, respectively. The protective efficacy of these vaccines were evaluated using a mouse model of influenza virus infection. Vaccine was administered either intranasally or subcutaneously and mice were challenged with a lethal mouse adapted A/PR8/34 virus infection. Animals were sacrificed at day 4 post-challenge and viral loads determined. **RESULTS.** We found that CYT-IVAC is efficient at preventing weight loss and reducing viral lung titres compared to the conventional wild-type vaccine without membrane-bound cytokine**. DISCUSSION** Together the study shows membrane bound immunomodulatory proteins augment antiviral host defenses.

Abstract 30 – YanYan Ni (PhD Student)

CONSTRUCTION OF A FULL-LENGTH cDNA CLONE OF PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME VIRUS (PRRSV) AND ASSESSMENT OF VIRUS RESCUE FROM THE CLONE. Y.Y. Ni , Y.W. Huang, and X.J. Meng. Department of Biological Science and Pathology, Virginia-Maryland Regional College of Veterinary Medicine, Blacksburg, Virginia.

Porcine reproductive and respiratory syndrome virus (PRRSV) is an enveloped, positive-stranded RNA virus, which causes highly contagious disease in swine characterized by severe respiratory syndrome in nursery pigs and reproductive failure in sows. Reverse genetics of RNA virus is a powerful tool for studying the structural and and functional relationship of virus genes. The present work describes the construction of a full-length cDNA clone of Type II PRRSV isolate VR-2385. Seven overlapping genomic fragments covering the entire viral genome of PRRSV flanked by unique restriction enzyme sites were amplified by RT-PCR from PRRSV-infected MARC-145 cells. A stuffer fragment containing the corresponding unique restriction sites was inserted between BamHI and BglI sites on a low-copy-number pACYC177 vector. Each of the RT-PCR fragments was ligated stepwise into the modified vector, resulting in the assembly of a full-length cDNA clone. The rescue of cloned PRRSV from in vitro transcripts in culture cells is in progress. This project will provide a valuable tool for further study of pathogenic mechanisms of PRRSV and for development of chimeric PRRSV as vaccine candidate that offers cross-protection to various genetically diversified PRRSV strains.

Abstract 31 – Shobana Raghunath (PhD Student)

TARGETED ONCOLYTIC VIROTHERAPY FOR PROSTATE CANCER. R. Shobana and S. Elankumaran, Department of Biomedical Sciences and Pathobiology, Virginia-Maryland Regional College of Veterinary Medicine, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061.

Oncolytic virus (OV) therapies are based on the use of replication competent, tumor selective viruses with limited toxicity. OVs destroy tumor cells but spare normal cells owing to defects in the type I interferon antiviral system in the tumor cells. Newcastle Disease Virus (NDV), an avian paramyxovirus, is a promising OV used in many clinical trials. Despite encouraging pre-clinical, and clinical studies with NDV, further refinements for increased tumor penetration and overcoming tumor resistance are needed in order to become an established OV. The aim of this study is to enhance the oncolytic potential of a recombinant NDV (rNDV) to treat prostate cancer (CaP). NDV fusion (F) protein activation is a prerequisite for viral entry into the target cell and for effective spread by cell fusion. We engineered the F protein of NDV to be specifically cleaved by prostate specific antigen (PSA), abundant only in the CaP microenvironment. The NDV F protein with a PSA-cleavable site (F-PSA) is not activated by common cell surface proteases in plasmid based assays. Formation of syncytia and nuclear fusion were seen only when exogenous PSA was overlaid in F-PSA plasmid transfected cells. Surface immunofluorescence and western blots confirmed that the F-PSA protein is efficiently processed and transported to the cell surface and is cleaved by PSA. Additional PSA mutants are being tested for effective cleavage. Different strategies are being employed to recover the PSA-specific rNDV and its oncolytic efficacy will be tested in prostate cancer cell lines and animal models.

Abstract 32 – Eva Restis (PhD Student)

NANOMEDICINE AND DRUG DELIVERY: RECEPTOR MEDIATED DRUG TARGETING OF ANTIMICROBIAL LOADED NANOPARTICLES AGAINST *MYCOBACTERIUM AVIUM* AS A MODEL FOR *M. TUBERCULOSIS* INFECTIONS. Eva Restis[1], Mohamed Seleem[2], Judy Riffle[3], Nikorn Pothayee[3], Joseph Falkinham III[4], and Nammalwar Sriranganathan[1], [1]Department of Biomedical Sciences and Pathobiology, [2]Institute of Critical Technology and Applied Science, [3]Department of Chemistry, [4]Department of Biology at Virginia Polytechnic Institute, Blacksburg, VA.

Currently, about one third of the world's population is latently infected with *M. tuberculosis* (Mtb) and about 3 million people die from the disease annually worldwide. In the US, a resurgence of Mtb cases has occurred in recent years. It is attributable to emigration from countries with high Mtb prevalence, the HIV epidemic, outdated control strategies and poor patient compliance. Failure to complete the full treatment course can increase the risk of disease re-emergence, spread of infection and development of drug resistant Mtb. An improved treatment approach is urgently needed. Developing a safe and effective colloidal drug delivery system that targets the macrophage directly may translate into reduced dosage as well as frequency, which leads to better patient compliance and management of tuberculosis. The goal of this project is to explore if different nanoparticles loaded with anti-tuberculosis drugs will effectively enter and eliminate *M. avium* in the macrophage as well as treat *M. avium* infection in a mouse model without toxic side effects. *In-vitro* cell culture studies using J774A.1 murine macrophage cell line were done with Rifampicin loaded silica and polyacrylic acid (PAA) nanoparticles (NP) with equivocal clearance results. Results of MTS and Trypan blue toxicity assays revealed Rifampicin-PAA-NP's were non-toxic in J774A.1 cells, but several constructs of Rifampicin-silica-NP's were toxic. Future studies include development of PAA and silica-NP's without toxicity, determination of zeta potential, drug loading/release profiles, intracellular trafficking, and *in vivo* efficacy using a mouse model.

Abstract 33 – Cheryl Ryder (PhD Student)

PROETECTION AGAINST TULAREMIA IN MICE USING AN ATTENUATED TYPE A MUTANT. Cheryl E. Ryder, Abey Bandara, Tom Inzana. Dept. of Biomedical Science and Pathobiolgy. VMRCVM at Virginia Tech. Blacksburg, VA 24061.

Aim: To characterize a potential vaccine candidate against Tularemia by studying the surface antigens of F. tularensis and their impact on the host's response. *Methods:* O-antigen, chemical mutants of *F. tularensis* type A were found to be highly attenuated, but inadequately protective against challenge with the parent strain. Genome sequencing of the O-antigen locus identified a single amino acid change in gene *wbtK*. Complementation of the mutated gene resulted in twenty-two isolates, which were examined for O-antigen expression. Balb/c mice were challenged to determine if virulence was restored, and the tissues examined for clearance. The immune response was assessed by collecting sera post-vaccination and post-challenge for antibody and cytokine ELISAs. *Results:* O-antigen was restored in all complemented recombinant isolates. Challenge studies showed that some, but not all, of the recombinant isolates remained avirulent with an $LD_{50} > 5 \times 10^7$ cells. Vaccination with recombinant strains showed two were protective against challenge with $> 3.8 \times 10^7$ cells of a highly virulent type A strain. Clearance data revealed all the bacteria were cleared from the tissues by day 10, similar to the avirulent, O-antigen mutant. *Discussion:* Avirulent mutants expressing O-antigen by complementation *in trans* provide optimal protection against type A challenge. Although O-antigen was restored, virulence was not, possibly due to expression of the gene transform an extrachromosomal element. Current work with a site-directed *wbtK* mutant may discern whether this lack of virulence is due to expression of the gene *in trans* or if another mutation exists in the chemical mutant.

Abstract 34 – Brent Sanford (PhD Student)

CROSS-PROTECTION OF HEPATITIS E VIRUS INFECTION IN PIGS INFECTED WITH A GENOTYPE 3 SWINE HEV AND SUBSEQUENTLY CHALLENGED WITH HOMOLOGOUS AND HETEROLOGOUS GENOTYPES OF SWINE AND HUMAN HEV. B. Sanford, B.A. Dryman, Y.W. Huang, A.R. Feagins, T. LeRoith and X.J. Meng. Department of Biomedical Sciences and Pathobiology, Virginia Polytechnic Institute and State University, Blacksburg, VA.

Hepatitis E virus (HEV) is an important cause of acute viral hepatitis worldwide. The virus has adapted into a number of host animals including swine, chickens and humans. Genotypes 3 and 4 HEV strains have been identified from pigs, and have been proven capable of causing zoonotic human infections whereas genotypes 1 and 2 HEV are restricted in humans. The objective of this study is to evaluate the protective and cross-protective nature of prior HEV infection against subsequent challenges with different genotypes of HEV in a pig model. Three treatment groups of 6 weaned pigs per group were each inoculated with a genotype 3 strain of swine HEV, and 12 weeks later, challenged with a genotype 3 swine HEV, a genotype 3 human HEV, and a genotype 4 human HEV, respectively. The control group was initially inoculated with PBS buffer and challenged with PBS buffer. Weekly serum samples were collected to detect viral RNA and anti-HEV antibody levels. Weekly fecal samples were tested for HEV RNA. Consistent with infection, all treatment group pigs shed viral RNA after initial inoculation with genotype 3 swine HEV, and most seroconverted to IgG anti-HEV antibody. After subsequent homologous or heterologous challenge, the vast majority of animals did not have detectable HEV RNA in feces or sera. This data indicates that prior infection of pigs with a genotype 3 swine HEV confers protection against subsequent challenges with homologous and heterologous genotypes of HEV, although the protection appears not to be 100%.

Abstract 35 – Lori Settle (PhD Student)

EXPRESSION AND PURIFICATION OF THE BACTERIOPHAGE FELIX O1 ENDOLYSIN. L. L. Settle[1*], M. Seleem[2], N. Sriranganathan[1], F. W. Pierson[1]. [1]Center for Molecular Medicine and Infectious Diseases, Virginia Maryland Regional College of Veterinary Medicine, Virginia Tech, Blacksburg, VA. [2]Institute for Critical Technology and Applied Science, Virginia Tech, Blacksburg, VA.

Bacteriophage Felix O1 is a bacteria-infecting virus specific for and lethal to most members of the genus *Salmonella*, a common cause of foodborne illness. Previous studies conducted by our lab have demonstrated that whole Felix O1 is a successful control measure when applied to artificially contaminated chicken frankfurter samples. The aim of this study is to determine the efficacy of the purified Felix O1 endolysin as an application for control of *Salmonella* in raw and ready-to-eat poultry products. The lysin gene *lys*, identified in a previous study, was amplified by PCR and cloned into the expression vector pRSET A to create pRSETA/Lys1. The recombinant plasmid was transformed into chemically competent Mach 1 *E. coli* for propagation. The ideal expression conditions for Lys, using both BL21 and BL21 Codon Plus *E. coli* hosts, are currently being determined. The protein will be purified by Ni-NTA affinity chromatography, characterized, and tested for anti-*Salmonella* activity. Results of recent expression efforts will be discussed.

Abstract 36 - R. S. Pudupakam (PhD Student)

DELETION ANALYSIS OF HYPERVARIABLE REGION (HVR) OF HEPATITIS E VIRUS: EFFECTS ON VIRAL REPLICATION *IN VITRO*. R. S. Pudupakam[1], Y. W. Huang[1], and X. J. Meng[1]. Department of Biomedical Sciences and Pathobiology, Virginia-Maryland Regional College of Veterinary Medicine, Blacksburg, Virginia

Hepatitis E virus (HEV) is a major cause of acute viral hepatitis in many developing countries. The open reading frame 1 (ORF1) of HEV genome has domains essential for HEV replication and a hypervariable region (HVR), which is highly heterogeneous . In our previous study, we showed that the deletions in HVR of HEV do not affect its replication. We hypothesize that, although deletions in HVR do not affect HEV infectivity, it is possible that the mutant viruses with deletions in HVR may interact with host alleles to influence virus replication efficiency. A replicon expressing luciferase as a reporter was constructed using genotype 1 human HEV (Sar55 strain) in order to quantify viral RNA synthesis *in vitro* and to assess the effect of HVR on RNA replication. Different HVR-deletion mutants were constructed using fusion PCR. Mutants hHVR5a, hHVR5b, and hHVR5c were constructed with the deletion of amino acids (aa) 711-725, 711-740, and 711-750 at the 5' end of the HVR; mutants hHVR3a, hHVR3b, and hHVR3c were constructed with the amino acid deletions aaΔ761-775, aaΔ746-775, and aaΔ736-775 at the 3' end of the HVR; and mutants hHVRma, hHVRmb, and hHVRmc were constructed with the amino acid deletions aaΔ729-759, aaΔ721-766, and aaΔ716-771 in the middle region of HVR. Capped RNA transcripts synthesized *in vitro* from the mutants were transfected into Huh-7 cells, and intracellular luceferase activity which is a measure of viral replication is being monitored. The results of replication kinetics of HVR-deletion mutants will be discussed.

Abstract 37 – Ke Wen (PhD Student)

TOLL-LIKE RECEPTOR (TLR) EXPRESSING ΓΔ T CELL SUBSETS IN GNOTOBIOTIC PIGS INFECTED WITH VIRULENT HUMAN ROTAVIRUS. Wen K, Li G, Liu F, Bui T, and Yuan L. Department of Biomedical Sciences and Pathobiology, VMRCVM, Virginia Tech.

To identify the role of γδ T cells in innate immune responses against rotavirus infection. Gnotobiotic pigs were inoculated at 5 days of age with virulent human rotavirus (VirHRV) or mock inoculated. Frequencies of TLR-expressing γδ T cells (pro-inflammatory CD2-CD8- and CD2+CD8- subsets, and anti-inflammatory CD2+CD8+ subset) in ileum, spleen and blood of the pigs were determined by flow cytometry at post-inoculation day [PID] 0, 3 or 5. Low frequencies of TLRs were expressed constitutively by γδ T cells in spleen whereas almost none in ileum at PID 0. In ileum, the site of virus replication, VirHRV infection stimulated significant increases in TLR2 and 3 expression in all three γδ T subsets and TLR9 in CD2+CD8- subset. In spleen, TLR2 and 3 expression by CD8- subsets increased significantly at PID5. In blood, TLR9 expression increased significantly in all three γδ T subsets at PID5. The upregulation of TLR expression indicates that γδ T cells play a role in innate immune recognition of rotavirus infection. VirHRV enhanced the expression of both pro- and anti-inflammatory γδ T cell subsets in ileum whereas only pro-inflammatory subsets in spleen. Our findings suggest that different subsets of γδ T cells act as innate effectors and/or regulators in mucosal immune responses to rotavirus infection.

Abstract 38 – Bonnie Brenseke (PhD Student, Pathology Resident)

THE FORMATIVE AND CUMULATIVE EFFECTS OF CADMIUM. B.M. Brenseke, T. LeRoith, and M.R. Prater. Department of Biomedical Sciences and Pathobiology, Virginia-Maryland Regional College of Veterinary Medicine, Blacksburg, Virginia.

Cadmium (Cd) is a ubiquitously encountered heavy metal, ingested through food/water, and inhaled from cigarette smoke. Cd is carcinogenic, teratogenic, and an endocrine disruptor that exerts multisystemic effects in genitourinary, metabolic, and musculoskeletal systems, where it is associated with bone loss and susceptibility to fragility fracture. Consequences of gestational Cd may include perinatal morbidity and elevated risk of adult-onset disease. Two putative mechanisms to explain the contribution of gestational Cd exposure to impaired fetal skeletal formation are explored in the present proposal. Firstly, Cd is believed to elevate placental reactive oxygen species (ROS), and indirectly alter fetal programming and development. Secondly, Cd has estrogenic activity and alters placental glucocorticoid (GC), perhaps through dysregulation of the enzyme 11β-hydroxysteroid dehydrogenase 2 (11β-HSD-2). Therefore, our central hypothesis is that perinatal Cd dysregulates placental development, alters fetal skeletal programming, and impairs adult bone health. Aims: Evaluate the role of perinatal exposure to Cd in fetal and placental formation, and in adult offspring. Methods: The following parameters will be evaluated following gestational oral administration of Cd to Wistar rats: 1) quantify Cd accumulation in maternal, fetal, and weanling tissues; 2) measure placental ROS and 11β-HSD-2; 3) evaluate expression of specific osteoblastic, osteoclastic, and estrogen receptor markers in fetal and adult bones; 4) visualize bony phenotype of fetal and adult bones using computed tomographic image analysis. Results: Based on pilot data and previous studies, we expect that maternal Cd will disrupt fetal osteogenic programming and permanently alter skeletal health. Final results are pending.

Abstract 39 – Jessica Walters (PhD Student)

UNUSUAL PHENOTYPIC CHARACTERISTICS OF *ORNITHOBACTERIUM RHINOTRACHEALE* IN VIRGINIA. J. Walters[1], M. Mainous[1], L. Craig[2], R. Evans[3], F.W. Pierson[1]. [1]Virginia-Maryland Regional College of Veterinary Medicine, Department of Large Animal Clinical Sciences, Blacksburg, VA 24061. [2]Virginia Department of Agriculture and Consumer Services Harrisonburg Regional Animal Health Lab, Harrisonburg, VA 22802. [3]Cargill Turkey Production, LLC. Harrisonburg, VA 22802.

Irregular characteristics of *Ornithobacterium rhinotracheale* (ORT) isolates showing increased virulence have been observed in Virginia. Eighteen isolates from turkeys of varying ages showing clinical respiratory symptoms, initially typed as ORT, were obtained. Isolates were plated on blood agar and incubated at 37°C in a standard candle jar for 48 hours. Atypical characteristics of hemolysis and adherence to agar plates were observed in some isolates. All isolates were catalase-negative and oxidase-positive. Each isolate was inoculated on an API 20NE strip and an API ZYM identification system was used to determine isolates as *O. rhinotracheale.* Using all identification methods previously described, eight samples typed out as ORT. Of those eight isolates, seven showed some degree of hemolysis after a 48-hour incubation period. One isolate, 725-09, appeared non-hemolytic when originally observed, but beta-hemolysis was observed after an additional 48 hours incubation, and became more pronounced after 72 hours. Available literature indicates incomplete hemolysis by the bacteria has occasionally been seen only after 96 hours of incubation. Hemolysis may be an indicator of pathogenicity, offering a possible explanation for increased virulence observed. Protein profiles produced by sodium-dodecyl sulfate-polyacrylamide gel electrophoresis revealed similar banding patterns between the hemolytic and non-hemolytic samples. Slight differences may be present around 15 kiloDaltons (kD), 17 kD, and 75 kD. Those possible differences will be examined more closely, and results will be discussed.

Virginia-Maryland Regional College of Veterinary Medicine

Faculty Research Interest Listing

Jonathan Abbott, DVM, DACVIM, (2000) Associate Professor. Cardiology. Echocardiographic assessment of cardiac disease, feline myocardial disease, pharmacotherapy of heart failure.

S. Ansar Ahmed, BSc, BVSc, PhD, (1989) Professor and Head, Department of Biomedical Sciences & Pathobiology. Immunology. Cellular and molecular immunology, immunoendocrinology, autoimmunity, immunology of infectious diseases.

Wendy Archipow, BVSc, MS, (2009) Assistant Professor. Surgery. Soft tissue surgery.

Roger Avery, PhD (1999) Professor and Senior Associate Dean for Research & Graduate Studies. Virology.

Don L. Barber, BS, DVM, MS, Diplomate ACVR, (1984) Professor. Radiology. Diagnostic radiography, nuclear imaging.

Dennis J. Blodgett, BS, DVM, PhD, Diplomate ABVT, (1983) Associate Professor. Toxicology.

Stephen M. Boyle, BA, PhD, (1984) Professor. Microbiology. Brucella vaccine, immuno-contraception vaccines.

Virginia Buechner-Maxwell, BA, DVM, MS, Diplomate ACVIM, (1995) Professor. Evaluation of equine lymphocyte function, regulation of lymphocyte mediated pulmonary inflammation, nutritional support of critically ill neonatal foals and adult horses.

Thomas Caceci, AB, PhD, (1987) Associate Professor. Histology. Aquatic animal structure & function, antifreeze peptides, web-based teaching and learning development.

David L. Caudell, DVM, PhD, (2009) Assistant Professor. Mouse models of hematopoietic malignancies, mouse models of immunodeficiency, immunopathology, bone marrow biology.

Mark V. Crisman, BS, DVM, MS, Diplomate ACVIM, (1987) Professor. Immunopharmacology with an emphasis in inflammation.

Linda A. Dahlgren, AB**,** DVM, MS, PhD, Diplomate ACVS. (2004) Assistant Professor. Tendon biology and healing, wound healing, tissue engineering, mesenchymal stem cells.

Gregory B. Daniel, BS, DVM, MS (2007), Professor and Head, Department of Small Animal Clinical Sciences. Quantitative nuclear medicine, positron emission tomography.

John J. Dascanio, BS, VMD, Diplomate ACT & ABVP, (1993) Associate Professor. Reproduction involving all domestic animals with an emphasis in the horse, use of computers and information technology to enhance veterinary education for students and practitioners.

Sandra Diaz, BVS, DVM, MS, (2007) Assistant Professor. Dermatology. Disorders of hair and hair growth, canine and feline allergic disorders, feline and canine otitis.

C. Kathleen Dorey, M.A**.,** Ph.D. (2009) Affiliate Professor with VirginiaTech Carilion Research Institute. Ocular neovascularization, inflammation, age-related macular degeneration.

Marion F. Ehrich, BS, MS, PhD, RPh, Diplomate ABT, (1980) Professor. Pharmacology, Toxicology. Biochemical neurotoxicology, especially neurotoxicity of organephosphorus components.

François C. Elvinger, Dr.Med.Vet., PhD, Diplomate, ACVPM & ECVPH, (1997) Professor. Epidemiology. Epidemiology of infectious diseases, diagnostic test validation, animal health surveillance, study design.

Ludeman A. Eng, BS, MA, PhD, (1981) Associate Professor and Assistant Dean for Strategic Innovations. Reproductive cell biology, in vitro fertilization and early embryo development, reproductive toxicology.

Willard H. Eyestone, BS, MS, PhD, (2000) Research Assistant Professor. Developmental biology of laboratory and domestic animals, animal cloning by somatic cell nuclear transfer, genetic modification of animals for resistance to prion diseases, assisted reproductive technology.

Larry E. Freeman, BS, DVM, MS, (1978) Associate Professor. Anatomy. Veterinary gross, developmental, and applied anatomy, comparative and functional morphology of mammals, marine mammal morphology. Reproductive system vasculature, developmental malformations in domestic animals.

Martin O. Furr, DVM, PhD, Diplomate ACVIM, (1989) Professor. Equine medicine. (Leesburg Campus).

David C. Grant, BA, DVM, MS, Diplomate ACVIM, (2000) Assistant Professor. Urology, laser Lithotripsy.

Piedad N. Henao Guerrero, DVM, MS, Diplomate ACVA. (2007) Assistant Professor. Anesthesiology. Bispectral index, monitoring equipment, pain management.

Tisha A. Harper, DVM, MS, (2006) Assistant Professor. Stifle surgery, nosocomial infections.

Cindy Hatfield, BS, MS, DVM, (2002) Assistant Professor. Anesthesiology. Pain management, local anesthetic techniques, transdermal drug delivery systems, teaching/learning methods, exotic/marine animal anesthesia.

Ian P. Herring, BVS, DVM, MS, Diplomate ACVO, (1994) Associate Professor. Ophthalmology. Ocular pharmacology, ocular trauma.

David R. Hodgson, BVSc, PhD, Diplomate ACVIM, FACSM, (2007) Professor and Head of Department of Large Animal Clinical Sciences. Horses: physiology of exercise, respiratory disease, internal medicine.

Jennifer L. Hodgson, BVSc, PhD, MRCVS, Diplomate ACVM, (2007) Associate Professor and Associate Dean for Professional Programs. Equine respiratory disease, infectious diseases, anthelminthic resistance of cyathostomins of horses, methicillin resistant staphylococcus aureus in horses.

Theresa Hrubec, DVM, PhD, (2002) Research Assistant Professor. (VCOM) Environmental toxicants change cellular events during gestation and lead to development birth defects.

William R. Huckle, BA, MS, PhD, (1999) Associate Professor. Intracellular signaling mechanisms, regulation of growth factor receptor expression, maintenance and growth of blood vessels in cardiovascular disease and cancer, transgenic models of human diseases.

Karen Inzana, BS, DVM., MS, PhD, Diplomate ACVIM, (1989) Professor. Neurology. Peripheral nerve research, neurotoxicology, brain tumor research.

Thomas J. Inzana, BS, MS, PhD, Diplomate ABMM, (1987) Professor. Molecular bacterial pathogenesis of Haemophilus somnus, Actinobacillus pleuropneumoniae, and Francisella tularensis, development of improved vaccines for bacterial pathogens and biowarefare agents, host immune response to bacterial pathogens.

Jeryl C. Jones, BS, DVM, PhD, Diplomate ACVR, (1995) Associate Professor. Radiology. Computed tomography, canine spinal disease.

Bernard S. Jortner, VMD, MS, Diplomate ACVP, (1980) Professor. Pathology. Neuropathological effects of toxins, effects of heavy metals, solvents and in particular the delayed neurotoxicity of organophosphates, pathological effects of toxins on tissues.

Taranjit Kaur, BS, VMD, MPH, Diplomate, ACLAM, (2001) Assistant Professor. Public health and conservation medicine, wild and captive chimpanzee health and well-being, information technology and GIS, medicinal plants.

Bradley G. Klein, BA, PhD, (1988) Associate Professor. Neurobiology. Role of pesticides as possible environmental triggers for neurodegenerative diseases, role of the environmental contaminants in the etiology of Parkinson's disease.

Andrea C. Lantis, DVM, Diplomate ACVIM, (2009) Assistant Professor. Cardiology. Assessment of the renin-angiotensin-aldosterone system in the setting of congestive heart failure, management of congestive heart failure, advanced imaging techniques in the assessment of cardiac disease.

Otto I. Lanz, DVM, Diplomate ACVS, (1998) Associate Professor. Neurosurgery, reconstructive surgery**.**

Martha M. Larson, DVM, MS, Diplomate ACVR, (1986) Professor. Radiology. Pancreatitis, imaging of pulmonary disease, ultrasound imaging.

John C. Lee, PhD, (1981) Professor. Physiology. Neural and hormonal control of the cardiovascular and respiratory function.

Yong W. Lee, BS, MS, PhD, (2004) Assistant Professor. Cellular and molecular mechanisms of inflammatory vascular disease, nanomedicine: biomedical applications of nanotechnology.

Michael S. Leib, BS, DVM, MS, Diplomate ACVIM, (1983) Professor. Small animal GI diseases, helicobacter, large bowel disease, GI endoscopy, NSAIDs, chronic vomiting, chronic diarrhea.

Tanya LeRoith, BS, DVM, PhD, (2005) Assitant Professor. Anatomic Pathology. Animal models of infectious disease, viral immunology, comparative pathology.

David S. Lindsay, BS, PhD, (1997) Professor. Parasitology , protozoology, apicomplexan biology, immunology, vaccine development.

Harold C. McKenzie III, DVM, MS, Diplomate ACVIM, (2003) Associate Professor. Equine Medicine (Leesburg Campus).

J. Blair Meldrum, DVM, PhD, (1980) Professor. Treatment of metals and minerals toxicoses, mechanisms of antiviral drugs.

Xiang-Jin Meng, MD, MS, PhD, (1999) Professor. Molecular mechanism of viral replication and pathogenesis, developing vaccines against viral diseases, human, swine and avian hepatitis E viruses, porcine reproductive and respiratory syndrome virus, porcine circovirus.

W. Edward Monroe, BS, DVM, MS, Diplomate ACVIM, (1985) Professor. Internal Medicine. Endocrine diseases, diabetes mellitus.

David M. Moore, BS, MS, DVM, Diplomate ACLAM, (1985) Adjunct Associate Professor and Assistant Vice Provost for Research Compliance. Laboratory animal medicine.

Matthew E. Nicholson, DVM, Diplomate ACVS, (2009) Associate Professor. Surgery. Minimally invasive surgery, Soft tissue surgery.

Laurie G. O'Rourke, DVM, PhD, Diplomate ACVP, ECVCP. Comparative Pathology.

David L. Panciera, BS, DVM, M.S., Diplomate ACVIM, (1998) Professor. Internal Medicine. Small animal endocrinology, canine hypothyroidism, thyroid function tests.

Kevin D. Pelzer, BS, DVM, MPVM, Diplomate ACVPM, (1987) Associate Professor. Salmonellosis, bovine leukemia virus, sheep and goats.

Daniel R. Perez, BSc, MS, PhD, Associate Professor. Interspecies transmission, pathogenesis, evolution of avian influenza viruses and the role of cross-protective immunity in the spread of highly pathogenic avian influenza viruses to other birds and mammals. (Maryland Campus)

J. Phillip Pickett, DVM, Diplomate ACVO, (1988) Professor. Ophthalmaology. Glaucoma, equine corneal disease, equine uveitis.

Bess J. Pierce, BS, MZS, DVM, (2007) Associate Professor. Community Practice. Canine sports medicine and rehabilitation.

F. William Pierson, BS, MS, DVM, PhD, Diplomate ACPV, (1993) Associate Professor and Director, Veterinary Teaching Hospital. Biosecurity/Agroterrorism, hospital acquired infections, hemorrhagic enteritis virus of turkeys (Siadenovirus), mutifactorial production diseases of poultry.

R. Scott Pleasant, BS, DVM, MS, Diplomate ACVS, (1991) Associate Professor. Large animal surgery, equine lameness.

M. Renee Prater, DVM, PhD, (2002) Research Assistant Professor. Immune protection against MNU-induced digital defects in mice. (VCOM)

Beverly J. Purswell, DVM, MS, PhD, Diplomate ACT, (1985) Professor. Theriogenology, clinical services.

Christopher Reilly, PhD, Research Assistant Professor. Etiology of systemic lupus erythematosus. (VCOM)

Carolina Ricco, DVM, MS, Diplomate ACVA, (2008) Assistant Professor. Loco-regional anesthesia/analgesia, arterial blood pressure monitoring, radiotelemetry, innovative teaching methods and strategies.

P. Christopher Roberts, BS, MS, PhD, (2007) Associate Professor. Viral vaccine development, host pathogen interactions and viral: bacterial synergy in disease exacerbation , immunotherapy and virotherapy targeting ovarian cancer.

John L. Robertson, BS, MS, VMD, PhD, (1989) Professor. Comparative oncology of malignant melanoma and malignant lymphoma, chronic renal disease, implantable biomaterials.

John H. Rossmeisl, BA, DVM, MS, Diplomate ACVIM, (2003) Associate Professor. Neurology and Neurosurgery. Vascular biology and tumor angiogenesis, primary brain neoplasms, traumatic brain injury, endocrinology.

Siba K. Samal, BVSc, MS, PhD, Diplomate ACVM, (1999) Professor, Chair, and Associate Dean Maryland Campus. Molecular Biology/Virology. (Maryland Campus)

Geoffrey K. Saunders, BS, DVM, MS, Diplomate ACVP, (1982) Associate Professor. Diagnostic pathology.

W. Kent Scarratt, BSc, DVM, Diplomate ACVIM, (1982) Associate Professor. Disorders of the central nervous system in large animals, evaluation of failure of passive transfer of immunity in large animals.

Gerhardt G. Schurig, DVM, MS, PhD, (1978) Professor and Dean, College of Veterinary Medicine. Immunology, vaccine development, brucellosis/tuberculosis.

Bonnie J. Smith, BS, MS, DVM, PhD, (1991) Associate Professor. Classical morphology, functional morphology, teratology.

Stephen A. Smith, BS, MS, DVM, PhD, (1991) Professor. Diseases of finfish, mycobacteriosis, immunology and immunotoxicology, clinical medicine and readiology, pharmacokenetics, horseshoe crab diseases, diseases of Alaskan fish, normal histology of fishes.

D. Phillip Sponenberg, BS, DVM, PhD, (1981) Professor. Genetics of domesticated animals, coat color genetics, conservation of rare breeds of livestock, diagnostic pathology, reproductive pathology.

Nammalwar Sriranganathan, BVSc, MVSc, PhD, Diplomate ACVM, (1984) Professor. Targeted drug delivery for intracellular pathogens, development of vaccines against bioterrorism agents, bacteriophage based remediation of food borne Salmonella in poultry, entertoxigenic E. coli, molecular immunology.

Elankumaran Subbiah, BVSc, MVSc, PhD, Diplomate ACVM (2006) Assistant Professor. Negative strand RNA viruses, their structure and function, pathogenesis, and control of diseases produced by them, development of novel, non-invasive immunization strategies for control of viral diseases, emerging viral diseases.

Kenneth E. Sullins, BS, DVM, MS, Diplomate ACVS, (1984) Professor. Upper airway, laser surgery and subchondral bone cysts. (Leesburg Campus)

William S. Swecker, Jr., BS, DVM, PhD, Diplomate ACVN, (1990) Associate Department Head and Professor. Trace elements and immune function in cattle.

Gregory C. Troy, DVM, MS, Diplomate ACVIM, (1987) Professor. Infectious diseases, urinary tract disorders, oncology.

Reid Tyson, BS, DVM, Diplomate ACVR, (2008) Assistant Professor. Radiology. Functional imaging, e-Learning, informatics.

Don R. Waldron, BS, DVM, Diplomate ACVS, ABVP, (1988) Professor. Surgery. Urology, urinary tract.

Nathaniel A. White, II, BS, DVM, MS, Diplomate ACVS, (1985) Professor and Director, Marion duPont Scott Equine Medical Center. Large animal surgery, colic, orthopedic surgery, equine lameness, reperfusion injury. (Leesburg Campus)

W. Dee Whittier, BS, DVM, MS, (1980) Professor. Applied bovine internal parasitology, applied bovine reproduction, beef cattle marketing and disease.

Nicole M. Weinstein, BS, DVM, Diplomate ACVP. Assistant Professor. Clinical Pathology. Cytologic preparation methodology, immunohematology/transfusion medicine.

Jeff R. Wilcke, DVM, MS, Diplomate ACVCP, (1982) Professor. Veterinary medical informatics, clinical pharmacokinetics, comparative medical nomenclature, medical records systems.

Sharon G. Witonsky, BA, DVM, PhD, (2000) Associate Professor. Equine protozoal myeloencephalitis, immunology and infectious disease: mouse models, equine immunology, brucella.

Lijuan Yuan, MS, PhD, (2007) Assistant Professor. Gnotobiotic pig model of human rotavirus infection and disease, generation of helper-virus free, plasmid-based reverse genetic systems of porcine and human rotavirus to study the determinants of pathogenicity and attenuation markers of rotaviruses.

Anne M. Zajac, BS, DVM, MS, PhD, (1986) Associate Professor. Parasitology.

Kurt Zimmerman, DVM, PhD, Diplomate ACVP, (2004) Assistant Professor. Medical expert systems, medical knowledge representation, knowledge discovery and machine learning algorithms, medical decision making, diagnostic pathology.