



UNIVERSITY OF  
**GEORGIA**

College of  
Veterinary Medicine



**Steeve Giguère Science of Veterinary Medicine**

**symposium**

**- VIRTUAL -**

**October 7 - 14, 2020**

University of Georgia

College of Veterinary Medicine

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## About the Symposium

Veterinary medicine and veterinary research have critical roles in both animal and human health. While caring for animals is inherent to the profession, veterinary professionals have a much broader impact. By improving the health and welfare of our companion animals, agricultural animals and wildlife animals, as well as by expanding our understanding of animals in health and disease, veterinarians and veterinarian scientists directly and indirectly impact public health. Moreover, veterinarians and research conducted at veterinary institutions are critical to the One Health concept, which recognizes that human, animal and environmental health are interconnected. The University of Georgia College of Veterinary Medicine is home to a spectrum of research training programs for the next generation of veterinary medicine practitioners and researchers. Each year we highlight the cutting-edge research conducted in these programs at the Science of Veterinary Medicine Symposium. The symposium provides an opportunity for research trainees at all levels to present their work to faculty and students from across the College of Veterinary Medicine community. The symposium also provides first and second year veterinary students the opportunity to explore the scope of veterinary research and the career opportunities available to them while learning about the rigor involved in scientific inquiry. Each year The Symposium also features a nationally recognized Keynote Speaker from industry, academia or government. In 2019, the Symposium was renamed in honor of Dr. Steeve Giguère, former faculty member, world-renowned clinician scientist, and tireless advocate for the training of veterinarians and scientists.

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## About Our Keynote Speaker: Yoshihiro Kawaoka, DVM, PhD



**Dr. Yoshihiro Kawaoka** is a Professor at the School of Veterinary Medicine at the University of Wisconsin-Madison in the Department of Pathobiological Science. He was educated in Japan, receiving his DVM in 1978 and his PhD in 1983 from Hokkaido University. He established the technique of reverse genetics, which allows the generation of ‘designer’ influenza viruses. This technology was used to develop the flu vaccine FluMist® and candidate bird flu vaccines. Dr. Kawaoka discovered what makes bird flu viruses so deadly and how they jump from birds to humans. In addition to his influenza research, Dr. Kawaoka also studies Ebola virus. His group worked in Sierra Leone during the 2014–2016 Ebola outbreak and continues to work with Ebola survivors. He is currently developing an Ebola vaccine, which entered clinical trials in 2019. In February 2020, he initiated research on SARS-CoV-2 including developing viral assays and establishing animal models of infection. In recognition of his achievements, Dr. Kawaoka was awarded the Robert Koch Award in 2006; he received the Medal of Honor (Purple Ribbon) in 2011 and the Japan Academy Award in 2016 from the Emperor of Japan for his influenza research. In 2013, he was elected as an international member of the United States National Academy of Sciences. In 2015, he received the UNESCO Carlos J. Finlay Prize for Microbiology.

# WEDNESDAY, OCTOBER 7

## KEYNOTE PRESENTATION

8:00 - 10:00 AM	<a href="#">ZOOM LINK</a>	Opening Remarks with Dean Lisa Nolan
		Keynote Introduction by Dr. Daniel Perez
		<b>Keynote Presentation - “Emerging viral infections: our recent work”</b> <b>Yoshihiro Kawaoka, DVM, PhD</b>

# THURSDAY, OCTOBER 8

10:00 - 11:15 AM	Oral Presentations - Concurrent Sessions I-V
<b>Session 1</b> Graduate Students  <a href="#">ZOOM LINK</a>	<b>10:00 / Alec Thompson</b> - Development of a molecular genetic key for the identification of <i>Haemaphysalis</i> ticks in North America
	<b>10:15 / Shufan Zhang</b> - The effect of sampling bias in genomic surveillance
	<b>10:30 / Jonathan Wilson</b> - Temporal and spatial patterns in Canine Distemper Virus cases in wildlife diagnosed at the Southeastern Cooperative Wildlife Disease Study (SCWDS), 1975-2019
	<b>10:45 / Ruijie Xu</b> - Reconstructing the evolutionary history and global spread of Leptospirosis using genomic data: preliminary results
	<b>11:00 / Noah Legall</b> - Pangenome analysis of <i>Mycobacterium bovis</i> isolates reveal geographic and host specific genomic signatures
<b>Session 2</b> Graduate Students  <a href="#">ZOOM LINK</a>	<b>10:00 / Sarah Vaughn</b> - Effects of oral RRR- $\alpha$ -tocopherol (vitamin E) on plasma oxidative stress and endocrine markers in healthy horses
	<b>10:15 / Gabriella Sandberg</b> - Review of kinematic analysis in dogs
	<b>10:30 / Raquel Francisco</b> - Experimental susceptibility of striped skunks ( <i>Mephitis mephitis</i> ) and raccoons ( <i>Procyon lotor</i> ) to SARS-CoV-2, causative agent of Coronavirus Disease 2019 (COVID-19)
	<b>10:45 / Ileia Scheibe</b> - Pro-inflammatory and pruritogenic transcriptome of compound 48/80-mediated skin lesions in healthy dogs resembles spontaneous atopic dermatitis
	<b>11:00 / Cheryl Vargo</b> - Characterization of the serum cytokine profile in feline non-flea hypersensitivity dermatitis
<b>Session 3</b> Veterinary & Undergraduate Students  <a href="#">ZOOM LINK</a>	<b>10:00 / Caroline Hawkins</b> - Investigating the role of equine platelet lysate on wound healing in vitro
	<b>10:15 / Alison Blackshire</b> - The effect of platelet lysate on biofilm development
	<b>10:30 / Alisha Muscatwala</b> - Histochemistry, Immunohistochemistry, and Electron Microscopy of Globule Leukocytes in Bovine
	<b>10:45 / Jazz Stephens</b> - Pathologic cardiovascular lesions associated with experimental anti-fibrotic therapies
	<b>11:00 / Suvitha Viswanathan</b> - Detection of <i>Atp6v0d2</i> and <i>Mcoln1</i> mRNAs in the mouse female reproductive system using <i>in situ</i> hybridization
<b>Session 4</b> Veterinary & Undergraduate Students  <a href="#">ZOOM LINK</a>	<b>10:00 / Nic Rinke</b> - A comparison of targeted-Selective and rotational treatment strategies for controlling parasites of horses
	<b>10:15 / Margaret Lemons</b> - Serological detection of <i>Borrelia burgdorferi</i> antibodies in serum and CSF from horses in Georgia
	<b>10:30 / Jessie Cathcart</b> - Vitamin D regulation in foals experimentally infected with <i>Rhodococcus equi</i>
	<b>10:45 / Jacob Crotts</b> - Development of a mobile app for the prevention and control of equine asthma: a survey of 195 horse owners
	<b>11:00 / Ava Mohiuddin</b> - Surgical site infection following ventral midline celiotomy in horses and association with duration of antimicrobial therapy: a retrospective analysis.

<b>Session 5</b> Interns & Residents  <a href="#">ZOOM LINK</a>	<b>10:00 / Chloe Goodwin</b> - Disorders of the central nervous system in new world camelids
	<b>10:15 / Troy Mulder</b> - Something on my mind: neuronal ceroid lipofuscinosis in a cat
	<b>10:30 / Heather Trenholme</b> - Effect of meperidine on equine blood histamine, tryptase, and immunoglobulin-E concentrations
	<b>10:45 / Jessica Elbert</b> - Pyogranulomatous dermatitis in a dog caused by <i>Paralagenidium karlingii</i> : a case report
<b>11:15 - 12:00 PM</b>	<b>Lightning Presentations (pre-recorded)</b> <a href="#">CLICK HERE</a>

## FRIDAY, OCTOBER 9

<b>1:00 - 2:15 PM</b>	<b>Oral Presentations - Concurrent Sessions VI-XI</b>
<b>Session 6</b> Post-Docs  <a href="#">ZOOM LINK</a>	<b>1:00 / Jéssica Assis</b> - Evaluation of CXCR4 and CD26 gene expression correlated with cell differentiation and proliferation biomarkers in human colorectal cancer
	<b>1:15 / C. Joaquin Caceres</b> - A live attenuated Influenza vaccine carrying the IgA-inducing protein (IGIP) is safe and confers protection against lethal challenge in mice
	<b>1:30 / Jennifer Schmiedt</b> - Increased incidence of keratoconjunctivitis sicca in a mixed breed research colony
	<b>1:45 / Blake Shessel</b> - Nephrocystostomy as a novel ureteral bypass procedure in cats
<b>Session 7</b> Interns & Residents  <a href="#">ZOOM LINK</a>	<b>1:00 / Brittany Feldhaeusser</b> - Proteasome inhibition via bortezomib induces apoptosis in canine glioma cells
	<b>1:15 / Ashley Iodence</b> - Retrospective comparison of incisional complications in dogs with mast cell tumor versus soft tissue sarcoma
	<b>1:30 / Heather Trenholme</b> - Pharmacokinetics and pharmacodynamics of meperidine in healthy horses
	<b>1:45 / Andhika Putra</b> - Evaluation of oral mycophenolate mofetil (MMF) steroid-sparing effect as an adjunct immunosuppressant for inducing remission of canine pemphigus foliaceus
<b>Session 8</b> Veterinary & Undergraduate Students  <a href="#">ZOOM LINK</a>	<b>1:00 / Emily Onyekwere</b> - Determining the cost-effectiveness of VacSIM through a swine flu market analysis
	<b>1:15 / Matthew Sharp</b> - Parainfluenza virus 5 (PIV5) expressing 3-cysteine-like proteinase and RNA-dependent RNA polymerase as novel vaccines against SARS-CoV-2
	<b>1:30 / Shreya Kuturu</b> - Genotyping Global Gene Knockout Mice
	<b>1:45 / John Smith</b> - Identifying Genes of the Shikimate, Isoprenoid & Ubiquinone Pathways from <i>T. gondii</i> : Current and Novel Drug Targets
	<b>2:00 / Lauren Thompson</b> - <i>Bordetella pseudohinzii</i> 's Pertussis Toxin-Like Genes Contribute to Persistence in Otitis Media Infections
<b>Session 9</b> Veterinary & Undergraduate Students  <a href="#">ZOOM LINK</a>	<b>1:00 / Abbey Parsons</b> - Analysis of Infectious Bronchitis Virus Survey throughout the Southern United States
	<b>1:15 / Aidan O'Reilly</b> - Bald eagle morbidity and mortality in the southeastern United States: a five-year retrospective study
	<b>1:30 / Amanda Wonn</b> - In vitro modeling of clinical dosing regimens of Adequan® in Chilean flamingoes ( <i>Phoenicopterus chilensis</i> ) for assessment for potential anticoagulant effect
	<b>1:45 / HaeYeun Byun</b> - Effects of chemotherapeutic agent doxorubicin on the corpus luteum
	<b>2:00 / Meenakshi Rajeev</b> - Scar revision in the absence of gross disease for incompletely excised mast cell tumors and soft tissue sarcomas in dogs: 101 surgeries (2010-2019)

<b>Session 10</b> Graduate Students  <a href="#">ZOOM LINK</a>	<b>1:00 / Adrea Mueller</b> - Investigating the role of Infectious Bronchitis Virus variant DMV/1639 in incidence of False Layer Syndrome
	<b>1:15 / Melanie Kunkel</b> - Experimental West Nile virus infection in wild turkeys ( <i>Meleagris gallopavo</i> ) and northern bobwhite quail ( <i>Colinus virginianus</i> )
	<b>1:30 / Brittany Seibert</b> - Effect of antibiotic growth promoter administration on the intestinal microbiota and virus shedding post avian influenza virus infection in chickens
	<b>1:45 / Aline De Oliveira</b> - Type 6 Secretion System plays a role in virulence traits of Avian Pathogenic Escherichia coli (APEC) and Newborn Meningitis Escherichia coli (NMEC) strains
	<b>2:00 / Benjamin Jackwood</b> - Analysis of ITS-1, 18S, and CO1 genes for identification of <i>Eimeria</i> parasites in poultry.
<b>Session 11</b> Graduate Students  <a href="#">ZOOM LINK</a>	<b>1:00 / Krishna Latha</b> - Tumor Progression Locus 2 (Tpl2) prevents immunopathology during Influenza infections
	<b>1:15 / Justin Stilwell</b> - Fish host susceptibility influences myxozoan community composition of Proliferative Gill Disease in catfish aquaculture
	<b>1:30 / Jonathan Hancock</b> - Molecular mechanism of PRAP1 regulation by estrogen receptor alpha (ER $\alpha$ ) in mouse uterus
	<b>1:45 / Yuehuan Li</b> - Lysosomal functions in female reproduction
	<b>2:00 / Christian Andersen</b> - Long-term effects of chemotherapy during adolescence on uterine receptivity in adulthood
<b>2:15 - 3:00 PM</b>	<b>Lightning Presentations (pre-recorded)</b> <a href="#">CLICK HERE</a>

## WEDNESDAY, OCTOBER 14

9:00 - 10:30 AM	<a href="#">ZOOM LINK</a>	Zoom Networking Event
10:30 - 11:00 AM		Closing Remarks & Awards Presentation

# LIGHTNING TALKS - Available October 8 - 14

<p><b>Flipgrid Room 1</b> Graduate Students, Interns, &amp; Residents</p> <p><a href="#">FLIPGRID LINK</a></p>	<p><b>Eliana De Luca</b> - Bisgaard Taxon 40 associated with high mortality in seabirds: characterization and comparative analyses of virulence factors and antimicrobial resistance.</p>
	<p><b>Priscilla Filgueiras</b> - Diagnostic potential of Ag ECO test as a screening method for SARS CoV 2 compared to RT-qPCR in Brazil</p>
	<p><b>Morgan Friedman</b> - Multi-season survey of ixodid ticks on domestic dogs in Chad, Africa</p>
	<p><b>Ryan Grunert</b> - Determining the effects of temephos (Abate™) and spinosad (Natular™) on survival of cyclopoid copepods</p>
	<p><b>Jongsuk Mo</b> - Transmission and Adaptation of an influenza H3N2 virus with human seasonal HA in pigs</p>
	<p><b>Juliet Armstrong</b> - Comparative MRI and CT characteristics of otogenic meningitis in cases of canine otitis media-interna</p>
	<p><b>Kathryn McCullough</b> - Phaeohyphomycosis cases at the University of Georgia from 2010 to 2020</p>
<p><b>Flipgrid Room 2</b> Veterinary &amp; Undergraduate Students</p> <p><a href="#">FLIPGRID LINK</a></p>	<p><b>Rebecca Radisic</b> - Feline leukoencephalomyelopathy in bobcats (<i>Lynx Rufus</i>) in Florida, United States: Pathology and Potential Etiologies</p>
	<p><b>Grace Boothby</b> - Effects of genetic selection on hormonal control of calcium and phosphorus metabolism in chickens</p>
	<p><b>Danielle Creamer</b> - Influence of stimulation, immunosuppressive drugs and chronic kidney disease on endogenous immunomodulatory gene expression from adipose-derived mesenchymal stem cells in cats</p>
	<p><b>Natália Custódio</b> - Definition of the immunomodulatory profile of the Tetraspanin C0029, CXCR4 and CD26 molecular markers of colorectal cancer in peripheral blood mononuclear cells</p>
	<p><b>Molly Goldberg</b> - The use of photostimulation to improve <i>in vitro</i> fertilization</p>
	<p><b>Alexia Johnson</b> - Immunogenicity of Parainfluenza Virus (PIV) Vectored SARS-CoV-2 Vaccines in the Ferret Animal Model</p>
	<p><b>David Lee</b> - Alphacoronavirus 1 spike gene phylogenetics and survey for risk factors of feline infectious peritonitis</p>
	<p><b>Maru Orbay</b> - Skunk adenovirus in three domestic ferrets (<i>Mustela putorius</i>)</p>
	<p><b>Natalia Rivera-Viscal</b> - Defining vector and host factors predisposing to canine heartworm infection in dogs from communities east and west of the Panama Canal</p>
	<p><b>Kate Sarkan</b> - Association of equine abdominal fluid neutrophil counts with colic lesion, duration, and prognosis</p>
	<p><b>Kenzie Schwartz</b> - Abundance and diversity of ticks in different habitat types within parks in Athens-Clarke County, Georgia</p>
	<p><b>Xinyi Xu</b> - Intrinsic traits and extrinsic factors predictive of RNA viral spillover between wildlife and livestock</p>

## Long-term effects of chemotherapy during adolescence on uterine receptivity in adulthood

Christian Andersen<sup>1,2</sup>, Xiaoqin Ye<sup>1,2</sup>

<sup>1</sup>Interdisciplinary Toxicology Program, University of Georgia, Athens, GA

<sup>2</sup>Physiology and Pharmacology, College of Veterinary Medicine, University of Georgia, Athens, GA

The number of childhood (from birth to adolescence) cancer survivors increases because of increased cancer incidence and decreased mortality rate from cancer therapy. A unique side effect of concern on these cancer survivors from cancer therapy (e.g., chemotherapy and radiotherapy) is fertility impairment. Some chemotherapeutic drugs have been shown to target ovarian follicles to impair female fertility. The gonadotoxic effect on female fertility can be circumvented by cryopreservation of ovarian tissues, oocytes, and/or embryos coupled with in vitro fertilization-embryo transfer (IVF-ET). The rate-limiting step for IVF-ET success is embryo implantation, in which the uterus transiently transforms into a receptive state for an embryo to implant. Uterine receptivity is under the control of ovarian hormones estrogen (E2) and progesterone (P4). We showed that a single, human-relevant dose of doxorubicin (10 mg/kg, single dose) in young adult ovariectomized CD-1 mice had a long-term effect on uterine transcriptome to E2 treatment (Andersen CL *et al*, 2018). Since some of the differentially expressed genes are associated with uterine receptivity, we hypothesize that chemotherapy could disrupt uterine receptivity for embryo implantation. To test this hypothesis, C57BL6J mice are ovariectomized on postnatal day (PND) 23 to remove the indirect effect of ovarian toxicity on the uterus, then treated with vehicle (negative control), doxorubicin (2 mg/kg, 5 daily doses), doxorubicin (10 mg/kg, single dose, positive control), cyclophosphamide (20 mg/kg, 5 daily doses), or cisplatin (6 mg/kg, single dose) starting on PND30. Decidualization is a uterine response to an implanting embryo and an indication of uterine receptivity. We use artificial decidualization to determine uterine receptivity as following: Beginning on PND56, mice are treated with ovarian hormones E2 and P4 to establish a receptivity uterus; one PND63, mice are given an intraluminal uterine oil (mimicking embryos) injection to induce artificial decidualization, which is detected using a blue dye reaction on PND64. Preliminary data show that the above four chemotherapeutic regimens have varied adverse effects on uterine receptivity. Uterine transcriptomes will be determined to uncover molecular mechanisms of chemotherapy on uterine receptivity. We are filling in the knowledge gap about long-term effects of chemotherapy during childhood on uterine receptivity in adulthood.



## Comparative MRI and CT characteristics of otogenic meningitis in cases of canine otitis media-interna

Juliet F Armstrong<sup>1</sup>, Simon Platt<sup>1</sup>

<sup>1</sup>Department of Small Animal Medicine and Surgery, College of Veterinary Medicine, University of Georgia, Athens, GA.

In many cases of acute and chronic canine otitis externa, there may be no clinical signs specifically related to the concurrent presence of inflammation in the middle or inner ear. Moreover, the presence of an intact tympanic membrane on physical examination does not exclude the possibility of otitis media, with or without otitis interna. Lack of identifying this inflammation appropriately may contribute to a decreased recognition of intracranial complications, termed otogenic meningitis. Previous studies in humans indicate a significant morbidity and mortality associated with intracranial complications of otitis media-interna without appropriate and early antibiotic therapy. With the use of computed tomography (CT) and magnetic resonance imaging (MRI), earlier identification of intracranial complications has been possible. In veterinary studies comparing imaging modalities for the diagnosis of inner ear disease, CT has been determined to be more sensitive but less specific than radiography, however studies evaluating concurrent MRI results are limited. In order to better identify the utility of MRI and CT for identifying features of canine otogenic meningitis, cases with clinical signs suggestive of central vestibular disease and MRI confirmed otitis media-interna were retrospectively evaluated. Concurrent CT findings, clinical history, and CSF analysis were also reviewed. Ten cases were evaluated. MRI was performed in all cases, CT in 7 (5 with contrast), and CSF analysis in 9. On CT images, 2 cases had evidence of bulla osteolysis without evidence of intracranial extension on MRI; 3 cases showed no evidence of bulla osteolysis despite meningeal enhancement suggestive of intracranial extension on MRI and 2 cases revealed bulla osteolysis with concurrent evidence of intracranial extension on MRI. Only two cases had meningeal changes suggesting intracranial extension identified on both CT and MRI. For cases in which CSF analysis was completed, despite a pleocytosis being identified in those with evidence of intracranial extension on MRI, only 1 demonstrated direct visualization of infective organisms. Emphasizing the benefit of using both modalities as complimentary diagnostics, evaluation of these cases demonstrated that MRI not only identified changes that CT imaging did not detect, but that changes suggestive of otogenic meningitis did not necessarily compare between modalities.

# Evaluation of CXCR4 and CD26 gene expression correlated with cell differentiation and proliferation biomarkers in human colorectal cancer

Jéssica V. Assis<sup>1,2</sup>, Lucélia A. Coutinho<sup>1</sup>, Viviane F. Santos<sup>3</sup>, Wander J. Jeremias<sup>1,4</sup>, Ana S. Mesquita<sup>5</sup>, Matheus M. Viviani<sup>5</sup>, Mônica A. Cabral<sup>5</sup>, Geovanni D. Cassali<sup>6</sup>, Balazs Rada<sup>7</sup>, Rafaella F. Queiroz<sup>1,7</sup>.

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Due to the molecular heterogeneity of colorectal cancer (CRC), the evaluation of tumor gene expression and the understanding of the relationship of biomarkers to tumor progression and/or regression is suggested. Among the markers studied today, CXCR4 and CD26 are known for their role in tumor progression. Like them, the markers of cell differentiation and proliferative profile have their applications constantly discussed in the tumor context, as they lead to the metastatic event. Data on the association of markers with tumor and patient variables are scarce, and their understanding may ensure greater chances of cure and disease-free survival, seeking to make the approach more assertive. Thus, the aim of the study is to determine the expression profile of the CXCR4, CD26, CD133, GAPDH, vimentin, e-cadherin and ki67 genes in colon and rectum tumors by correlating them to the patient and tumor variables. For such analyzes, the evaluation of the gene expression profile of the markers was performed using qPCR using frozen tumor fragments and the data generated were correlated as intended. Intergroup results indicate that CD26 overexpression is closely related to increased GAPDH expression, as well as vimentin, e-cadherin, CD133 and ki67 have their expression regulated positively according to CXCR4. Findings demonstrate that the pathological condition increases the expression of GAPDH, CXCR4 and vimentin, since they are markers related to cell differentiation and proliferation. It was observed that GAPDH has positive expression in peri-tumor samples from the left side of involvement, as well as vimentin, since it is a region in intense inflammatory process. The data generated point to the association of CD26 and GAPDH, as well as CXCR4 and CD133, vimentin and e-cadherin in the development and progression of CRC. Thus, it can be concluded that the present panel of markers has the potential to be the target of further studies regarding its application in determining the diagnosis and prognosis of CRC.

## The effect of platelet lysate on biofilm development.

Alison Blackshire, Julie Gordon<sup>1</sup>, John Peroni<sup>1</sup>

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Infections that occur in tissues containing a biomedical device are a major medical challenge and are complicated by the development of bacterial biofilm rendering the infection difficult to eradicate. Bacteria within a biofilm are protected by an extracellular matrix that inhibits the host's immune response and antibiotic penetration. Because of these concerns there is a pressing need to identify antibiotic treatment alternatives especially for biofilm-mediated infections. An acellular platelet product known as platelet lysate (PL), manufactured in our lab from donor horses, has shown promising antimicrobial activity by directly inhibiting bacterial growth. *We hypothesize that PL will sustain its antimicrobial effects in the presence of biofilm.* In a preliminary study we have shown that PL effectively reduces *Pseudomonas aeruginosa* (PA) dependent biofilm formation and enhances its breakdown. Using 24-well culture plates, PA biofilm was formed in wells containing stainless steel (SS) disks or orthopedic screws and treated with 50% PL or regular bacterial growth media. After a 48-hour treatment period, the SS pieces were stained to quantify biofilm mass after treatment, and bacteria were recovered to determine viability by counting colony forming units (CFUs). PL inhibited biofilm formation and promoted biofilm breakdown on both SS disks and screws. PL decreased bacteria viability during biofilm inhibition and breakdown on SS disks. Regarding SS screws, CFUs were equivalent between both control and treatment groups for biofilm inhibition and breakdown. Replicates are needed to determine statistical significance, but the initial results are promising and will provide valuable information for future studies focused on developing PL as a biological antimicrobial treatment against biofilm. Furthermore, we plan to determine the mechanisms by which PL affects the structural polysaccharides and proteins in biofilm.

# Effects of genetic selection on hormonal control of calcium and phosphorus metabolism in chickens

Grace L. Boothby<sup>1</sup>, Jason A. Payne<sup>1</sup>, Brett Marshall<sup>1</sup>, and Laura E. Ellestad<sup>1</sup>

<sup>1</sup>Department of Poultry Science, University of Georgia, Athens, Ga.

Modern commercial broilers have undergone decades of genetic selection; as a result, they have more efficient growth performance, but this is accompanied by negative consequences including decreased skeletal health. Three hormones, active vitamin D (1,25(OH)<sub>2</sub>D<sub>3</sub>), parathyroid hormone (PTH), and calcitonin, work to control the flux of calcium and phosphorus in order to maintain skeletal homeostasis. Little research has been done to investigate how genetic selection has impacted these systems. The purpose of this study was to compare gene expression of enzymes, receptors, and transporters involved in the regulation of calcium and phosphorus metabolism between a modern broiler strain (Cobb 500) and a strain of birds representative of a mid-1950s broiler (Athens Canadian Random Bred; ACRB). Liver, kidney, and jejunal tissue samples were collected from D21 birds (n=8 per strain), and total RNA was extracted from these tissues. Reverse transcription reactions were performed to yield cDNA, and expression of target genes was quantified using real-time PCR. Levels of target mRNA were normalized to levels of beta-actin. Gene expression was analyzed for vitamin D conversion enzymes; vitamin D, calcitonin, and PTH receptors (PTHrRs); calcium transporters; calcium sensing receptor; and phosphorus transporters. Statistical analysis was done using a two-tailed Student's t-test. Results indicate that the modern broiler may have a diminished ability to activate vitamin D due to decreased expression of the activating enzyme 1 $\alpha$ ,25-dihydroxylase in the liver ( $P \leq 0.05$ ) and an enhanced ability to deactivate vitamin D due to increased expression of deactivating enzyme 24-hydroxylase in the kidney ( $P \leq 0.05$ ). Decreased expression of PTHrRs in the liver and jejunum of the modern broiler ( $P \leq 0.05$ ) indicate a decreased sensitivity to PTH. Furthermore, differing expression of calcium and phosphorus transporters in the jejunum and the kidney ( $P \leq 0.05$ ) suggests that genetic selection has resulted in an improved ability to absorb dietary calcium and reabsorb renal calcium. Overall, the difference between strains indicates that genetic selection for rapid and efficient growth has also had an impact on physiological systems regulating skeletal health, potentially resulting in the problems seen in the industry today and providing targets for strategies that could be developed to ameliorate these problems.

## Effects of chemotherapeutic agent doxorubicin on the corpus luteum

HaeYeun (Rachel) Byun<sup>1</sup>, Christian Lee Andersen<sup>1,2</sup>, and Xiaoqin Ye<sup>1,2</sup>

<sup>1</sup>Department of Physiology and Pharmacology, College of Veterinary Medicine; <sup>2</sup>Interdisciplinary Toxicology Program, University of Georgia, Athens, GA, USA.

For many premenopausal female cancer patients, fertility impairment is an inevitable outcome due to gonadotoxic chemotherapeutics such as doxorubicin (DOX). It's already known that doxorubicin interferes with mechanisms essential for pregnancy; however, there is no literature on the impact that DOX has on the corpus luteum. The corpus luteum is a transient endocrine gland that produces progesterone. And progesterone(P4) facilitates embryo development, embryo transport, and embryo implantation -- all of which are absolutely necessary for a successful pregnancy to occur. Previous studies have shown toxicity to the main cell types of the corpus luteum – luteal cells and endothelial cells -- therefore, we hypothesized that DOX could be potentially toxic to the corpus luteum to impair progesterone steroidogenesis. To test our hypothesis, we mated C57BL/6 mice and randomly assigned them to the control group (1xPBS) or the DOX group (a single dose of DOX at 10 mg/kg, on post-coitus day 0.5 (D0.5)). We then sacrificed the mice on D3.5. The data collected so far indicate altered serum hormone signaling in 50% (5/10) of the DOX-treated group but 0% (0/11) in the control group. These DOX mice with altered serum hormones showed a low progesterone and high estrogen environment. Our results indicate no difference in the number of corpora lutea across the different treatment groups -- ultimately indicating that the progesterone deficiency is due to an impairment of progesterone synthesis. Furthermore, our results indicate a disruption of the vasculature of DOX treated mice which can potentially affect progesterone transport. We also found an increase in the size of lipid droplets which contains the substrate of P4. Our results demonstrate the potential effect that DOX has on the corpus luteum to impair progesterone synthesis. We anticipate our study to be a starting point for the further analysis of the interaction that DOX has with the progesterone synthesis pathway. For instance, we plan to increase our sample size and investigate the expression of key players of the progesterone synthesis pathway: StAR, CYP11A1, and 3 $\beta$ -HSD -- ultimately closing the knowledge gap regarding DOX's interaction with the corpus luteum.

## A live attenuated Influenza vaccine carrying the IgA-inducing protein (IGIP) is safe and confers protection against lethal challenge in mice.

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The World Health Organization (WHO) estimates that seasonal influenza virus (IAV) infections result in about 3-5 million cases of severe diseases around the world every year. Vaccination is considered the first line of defense against IAV, but the ever-changing nature of these viruses make vaccines ineffective after a single season or against pandemic strains. Hence, the appeal for more broadly protective influenza virus vaccines that result in protection against all or several influenza viruses and long-lasting immunity. IgA responses are considered of great significance to prevent and/or control intestinal and respiratory infections, including IAV. IgA is typically more broadly-neutralizing than IgG. Therefore, IAV vaccines designed to stimulate IgA can provide a more broadly protection. In this study, we used the IgA-inducing protein (IGIP) to stimulate the IgA response against IAV. A live attenuated Influenza vaccine (LAIV) with temperature sensitive mutations carrying the IGIP sequence in the hemagglutinin segment (IGIP-HA) was obtained through reverse genetics. IGIP viruses of the H1N1 or H3N2 subtype exhibited similar growth profiles in-vitro in comparison with the isogenic controls. We tested the safety and immunogenicity of the IGIP-H1-LAIV in mice. The results showed that the mice vaccinated with IGIP-H1-LAIV do not show signs of disease demonstrating that the vaccine is safe. Additionally, neutralizing antibodies against the homologous virus were detected after vaccination. Furthermore, upon challenge mice do not show weight loss or clinical signs demonstrating that the IGIP-H1-LAIV confers protection against lethal challenge. Finally, tissues collected after challenge showed no IAV replication and milder lesions in the vaccinated mice. In conclusion, the results propose IGIP as a natural immunomodulator for LAIV to generate a safer and broader immune response.

## Vitamin D regulation in foals experimentally infected with *Rhodococcus equi*

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1,25 (OH)<sub>2</sub> vitamin D<sub>3</sub>, the active metabolite of vitamin D, helps regulate skeletal homeostasis through calcium and phosphorus metabolism. In people, Vitamin D also modulates the immune response. Immune cells express the vitamin D receptor and contain enzymes that convert inactive vitamin D to the active form. Active vitamin D then regulates production of antibacterial protein synthesis to support clearance of intracellular bacteria like *Mycobacterium tuberculosis*, which causes severe chronic pneumonia in people. *Rhodococcus equi* is an important cause of pneumonia in young foals, and exhibits a similar pathogenesis to *M. tuberculosis* centered on replication within alveolar macrophages. The role of vitamin D in the equine immune system and in *R. equi* infection has not been described. We hypothesized that *in vivo* and *ex vivo* *R. equi* infection will induce the synthesis of the active metabolite of vitamin D, suggesting vitamin D may have a similar immunomodulatory role in foal pneumonia as in other species. Active and inactive vitamin D concentrations were quantified via ELISA in serum samples from 7 foals with experimental *R. equi* infection from days 0, 3, 7, & 14 post-infection and from 10 age-matched healthy foals. In addition, pulmonary macrophages from 6 healthy foals obtained via bronchoalveolar lavage were cultured with and without a low concentration of virulent *R. equi*. Culture supernatant vitamin D concentrations were quantified before and 2 and 7 days post-infection as above. *In vivo* *R. equi* infection induced the synthesis of the active metabolite of vitamin D by day 14, suggesting vitamin D may have a similar immunomodulatory role in foal pneumonia as *M. tuberculosis* in people. *Ex vivo* *R. equi* infection did not result in a significant change in either metabolite by day 7, but longer culture duration may be needed to better mimic chronic natural exposure. This is the first study to examine vitamin D metabolism during *R. equi* infection in foals. Our results will determine if further investigation of vitamin D as an immunomodulator in *R. equi* prophylaxis or treatment is warranted.

# **Influence of stimulation, immunosuppressive drugs and chronic kidney disease on endogenous immunomodulatory gene expression from adipose-derived mesenchymal stem cells in cats**

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To date, most work to enhance understanding of the function of feline MSCs has been performed on cells derived from healthy cats. It is possible that the immunomodulatory efficacy of autologous, adipose-derived MSCs from cats with systemic diseases may be different. To make progress towards using autologous MSCs as part of the therapeutic regimen in cats receiving renal transplantation, one must understand endogenous gene expression of key immunomodulators in healthy cats and cats with chronic kidney disease (CKD) as any differences may reveal limits to their *in vivo* application. We assessed the phenotype and gene expression of six MSC lines from healthy cats and six MSC lines from cats with CKD. One CKD cat had ischemia induced chronic kidney insufficiency. RT-qPCR was used to measure the expression of immune modulatory genes (IL-6, IL-10, IL-12p40, IL-18, TGF- $\beta$ ). Furthermore, the MSCs were exposed to immunosuppressive drugs (dexamethasone, methotrexate and cyclosporine) and pro-inflammatory stimuli (peptidoglycan, poly I:C). Our null hypothesis was- there is no difference in gene expression between MSCs from healthy and CKD cats under the conditions we tested. Results showed that there was no significant difference in expression of the immune modulatory genes between MSCs from healthy cats and from cats with CKD in this trial. However, statistically significant differences between untreated MSCs and MSCs treated to induce immunosuppression or stimulated with proinflammatory ligands were observed. IL-18 expression by MSC from both CKD and healthy cats was upregulated after treatment with methotrexate. Expression of IL-6 was increased following treatment with both Poly I:C and methotrexate. Expression of IL-10 was increased following treatment with cyclosporine and methotrexate, and expression of IL-12p40 was higher in cells treated with cyclosporine, dexamethasone and methotrexate. Downregulation of TGF $\beta$  expression was evident in MSCs treated with dexamethasone or Poly I:C. If proven valid, the upregulation of anti-inflammatory cytokines, i.e. IL-12p40 and IL-10, in response to immunosuppressive treatment is interesting. Even with a limited sample size, this data helps to shape the future assessment of protocols for clinical use of adipose-derived MSCs in the post-renal transplantation protocol in cats. This study suggests that further research is warranted.

## **Development of a mobile app for the prevention and control of equine asthma: a survey of 195 horse owners**

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Equine asthma, like asthma in humans, is characterized by lower airway inflammation and bronchoconstriction. The disease has worldwide distribution and affects up to 20% of some equine populations. Exposure to inhaled dust/allergens is a risk factor for development and exacerbation of the disease, making environmental control a vital component of managing affected horses. However, equine practitioners perceive that horse owners struggle with adherence to management recommendations. In human medicine, multiple cell phone apps have been developed to help people manage asthma by tracking symptoms, providing daily medication reminders, and providing educational materials. Despite similar prevention and control measure recommendations for human and equine asthma, no such app exists to help horse owners manage equine asthma. The overall goal of this project is to design a cell phone app, intended to increase adherence to asthma control measures, for horse owners with horses affected by equine asthma. To better understand the treatments and environmental measures horse owners currently employ, a questionnaire was developed. 86% of respondents with horses in work said their horse's performance was affected by a respiratory condition. Owners reported difficulty controlling asthma symptoms in their horses, and recurrence of symptoms after treatment was common. 73% of owners reported interest in using an app to help monitor and control their horses' symptoms. Results of this survey confirm that equine asthma represents a significant and recurrent problem for horse owners, many of whom would be interested in using an app. Survey results directly support ongoing efforts to develop an equine asthma app.



## **Definition of the immunomodulatory profile of the Tetraspanin CO029, CXCR4 and CD26 molecular markers of colorectal cancer in peripheral blood mononuclear cells**

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Colorectal cancer (CRC) is the fourth cancer with the highest mortality in the world, with almost 900 thousand deaths annually. The complex etiology and the heterogeneity of this tumors have made molecular markers important targets for studies to understand their possible interaction with the progression of tumor lesions, metastases, resistance to treatment and recurrences, which may lead to a more assertive prognosis, diagnosis and treatments. The chemokine receptor CXCR4, responsible for enabling metastasis in CCR, peptidase CD26, an important regulator of tumor progression and Tetraspanin CO029 (TSP029), mainly related to cell motility, are the focus of this study. Thus, the main objective is to determine the immunomodulatory profile of these recombinant proteins in culture of peripheral blood mononuclear cells (PBMCs) from healthy individuals (UGA # 2012-10769-06), added in isolation and associated. Therefore, after the production of recombinant proteins, PBMCs from donors by Histopaque 1077 were isolated and maintained in culture with RPMI and, subsequently, incubated in three different ways: with lipopolysaccharide (LPS) as control; with the isolated recombinant proteins; and with the associated proteins, in two concentrations (10 and 25µg / mL) to obtain supernatant in four times (6, 24, 48 and 72 hours ) and subsequent evaluation of the immune response by ELISA for cytokines IL-10, IL-6 and IFN-γ. The results showed that the proteins stimulated IL-10 and IL-6 comparatively with the non-stimulated medium at all times analyzed. The treatments TSP029, CXCR4, CD26, TSP029 + CXCR4, TSP029 + CD26, CXCR4 + CD26 demonstrated peak stimulation of IL-10 in 24 hours that remained until 72 hours. TSP029 was able to stimulate IL-10 in 6 hours and, when associated with CXCR4 or CD26 or both, this stimulus was regulated. The next steps will be the determination of the TNFα curve and the production of antibodies from the supernatants obtained in the cultures already carried out. It is also intended to outline the immunomodulation curve with PBMCs of patients with colorectal cancer and further comparison with the data obtained so far. This work aims to elucidate the immunogenic profile of these tumor markers for targeting studies on immunotherapeutic targets.

## **Bisgaard Taxon 40 associated with high mortality in seabirds: characterization and comparative analyses of virulence factors and antimicrobial resistance.**

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The *Pasteurellaceae* family has been associated with fatal infectious diseases in numerous avian species. Several new species within this family have been recently described, but their pathogenic roles are not well understood. Here, we describe the phenotypic and whole genome characterization of Bisgaard taxon 40 strain A25201, which was isolated from a mass mortality event affecting terns (*Thalasseus sandvicensis* and *Sterna hirundo*) in Florida, USA. The comparative genomics was performed by phylogenetic analysis targeting gene comparisons of virulence factors implicated in disease associated with *Pasteurellaceae*. This analysis included the present isolate as well as four publicly available Bisgaard taxon 40 genomes and twenty-seven *Pasteurellaceae* reference strains. In addition, *in vitro* antimicrobial susceptibility patterns and the detection of antimicrobial resistance genes were also investigated.  $\beta$ -hemolytic, shiny, circular, and whitish-cream colonies were observed on blood agar. The length of the whole genome sequence obtained was 1,887,008 bp and contained 1,693 predicted protein coding DNA sequences. Bisgaard taxon 40 strains shared high similarity values (98.3-100.0% of nucleotide identity) and phylogenetic analyses grouped them into a distinct branch within the *Pasteurellaceae* family, alongside members of the *Gallibacterium* genus. Major virulence factors were identified, including capsule biosynthesis protein, outer membrane protein (*ompA*, *ompH*), cytolethal distending toxin (*cdt*), iron metabolism (*fur*, *exbD*), superoxide dismutases (*sodA*) and lipooligosaccharide (LOS) (*galU*, *galE*, *lpxA*, *lpxC*, *kdsA*). Representative members of the *Gallibacterium* genus were most closely related to Bisgaard taxon 40, sharing nucleotide identities ranging from 63.1 to 82.8% for the virulence genes described above. Antimicrobial resistance was detected for amoxicillin-clavulanate and tetracycline, whereas susceptibility patterns were detected for ampicillin, doxycycline, enrofloxacin and trimethoprim-sulfamethoxazole. No resistance genes matched the corresponding categories of phenotypic resistances. This is the first study reporting the whole genome sequence characterization and comparative genomics for this microorganism. The role of this bacterium in the mortality event as a primary or opportunistic pathogen remained unclear, but importantly, the findings presented in this study reveal novel genomic characteristics, which should provide insightful information on genome evolution and pathogenic potential of Bisgaard taxon 40.

## Type 6 Secretion System plays a role in virulence traits of Avian Pathogenic *Escherichia coli* (APEC) and Newborn Meningitis *Escherichia coli* (NMEC) strains

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Extraintestinal pathogenic *Escherichia coli* (ExPEC) are a pathotype of *E. coli* responsible for extraintestinal infections in humans and animals and include the subpathotypes Neonatal meningitis-causing *E. coli* (NMEC) and Avian Pathogenic *E. coli* (APEC). NMEC is the leading cause of neonatal meningitis caused by Gram-negative bacteria with mortality rates up to 50%. APEC strains cause extraintestinal infections in poultry resulting in economic losses for the industry and present zoonotic potential as they serve as a reservoir of virulence factors that can lead to the emergence of human pathogens.

Despite the identification of several virulence determinants in NMEC and APEC, the involvement of secretion systems in their virulence remains poorly understood. The Type 6 Secretion System (T6SS) functions as a molecular syringe that secretes proteins into the external milieu or directly into the host cells. Although the T6SS is associated to virulence mechanisms used by several bacterial pathogens, current knowledge on the role of T6SS in NMEC and APEC pathogenesis is limited. Here, we aim to investigate the role of T6SS in ExPEC pathogenesis. We have screened a collection of 92 NMEC, 454 APEC, and 102 human fecal *E. coli* isolates for the presence of 5 T6SS1 genes including effector and uncharacterized structural components. Results show a significantly higher prevalence of these genes in NMEC than in human fecal isolates, and in APEC when compared to avian fecal isolates. We then created deletion mutants for three T6SS genes in two ExPEC strains: NMEC15 and APEC018. These mutants were assessed regarding biofilm formation, adhesion and invasion to cell lines and resistance to predation by the social amoeba *Dictyostelium discoideum*. We found that the deletion of *hcp*, which encodes an effector secreted by T6SS, affects the ability of NMEC15 to form biofilm and to resist to predation by *D. discoideum*. Furthermore, deletion of *hcp* in APEC380 affected the ability of this strain to adhere to and invade chicken fibroblasts DF-1. Our findings provide evidence that T6SS acts as a virulence factor in NMEC and APEC strains.

## Pyogranulomatous dermatitis in a dog caused by *Paralagenidium karlingii*: a case report

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An 8-year-old, neutered male, mixed breed dog presented to the University of Georgia Veterinary Teaching Hospital's Dermatology service in October 2019 for evaluation of multifocal to coalescing, erythematous plaques and nodules with mild crusting over the right thorax of four months duration. Four punch biopsies of the masses were taken and submitted to the Department of Pathology's Biopsy Service for histological evaluation.

Affecting approximately 90% of examined sections, the dermis and panniculus were multifocally infiltrated and expanded by high numbers of degenerate and viable neutrophils, epithelioid macrophages, and reactive fibroblasts, as well as scattered lymphocytes, plasma cells, eosinophils, and multinucleated giant cells (pyogranulomatous dermatitis), frequently admixed with a finely granular, lightly basophilic, myxomatous matrix. Multifocally throughout the tissue and centrally located within dense inflammatory aggregates were numerous, transverse and cross-sections of 8-10 micron in diameter, myceloid, septate, presumptive hyphae, with nonparallel walls and nondichotomous acute and right angle branching, which were positive using both a standard GMS stain and a modified GMS stain optimized to detect oomycetes. The overlying epidermis was mildly acanthotic with minimal orthokeratotic hyperkeratosis and a locally extensive aggregate of eosinophilic, amorphous, homogenous material admixed with pyknotic and karyorrhectic cellular debris, inflammatory cells, hemorrhage, keratin, and low numbers of coccobacilli (serocellular crust).

Tissue fungal culture was negative. Conventional pan-oomycete PCR and *Lagenidium* PCR was performed on formalin-fixed, paraffin-embedded tissue targeting the PCR amplicon of the internal transcribed spacer (ITS) region. The pan-oomycete and *Lagenidium* PCR yielded a positive and negative result, respectively. The product from the pan-oomycete PCR was purified using the QIAquick PCR purification kit and sequenced by Sanger method at the AVDL. This yielded a sequence 100% homologous to *Paralagenidium karlingii* available in the BLAST database.

*Paralagenidium karlingii* is an exceedingly rare oomycete pathogen, previously recognized as a cause of cutaneous lesions in four dogs from the southeastern USA as well as from a single human patient from the midwestern United States. While opportunistic fungal organisms and *Pythium* spp. are more frequently recognized and diagnosed in dogs, paralagenidiosis due to *Paralagenidium karlingii* is an important differential diagnosis for canine mycotic/oomycotic dermatitis and cellulitis.

## Proteasome inhibition via bortezomib induces apoptosis in canine glioma cells

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Gliomas are the second most common canine primary brain tumor. These tumors are most commonly treated with radiation therapy and generally have a guarded prognosis. The role of systemic therapy in treating canine gliomas is largely unresolved; However, recent human glioma data suggest that bortezomib (BORT) (Velcade®), a proteasome inhibitor, in combination with radiation therapy may lead to an improved clinical outcome. We hypothesized that canine glioma cell lines will be sensitive to BORT and treatment will lead to decreased cell viability and increased radiosensitivity *in vitro* when compared to untreated cells. We identified the IC<sub>50</sub> of BORT in two canine glioma cell lines, SDT3G and J3TBG, using an ATP-based cell viability assay. We then treated cells with two doses of BORT (50 nM and 75 nM) and assessed the effects of this treatment on apoptosis via flow cytometry. Treatment of canine glioma cells resulted in a dose-dependent decrease in cell-viability with IC<sub>50</sub> values calculated as 45 nM (J3TBG) and 67 nM (SDT3G). Treatment of the J3TBG cell line with BORT resulted in a decreased percentage of viable cells and an increased percentage of apoptotic cells at both 48 and 72 hr. Both of these effects were statistically significant in comparison to the vehicle only (DMSO) treated cells ( $p < 0.01$ ). Furthermore, there was a statistically significant increase in apoptosis between the 50 nM and 75 nM doses, indicating a dose-dependent response. Similar responses were seen in the SDT3G cell line. Analysis of the cytoplasmic and nuclear proteins harvested from cells treated concurrently with the apoptosis experiments demonstrated accumulation of total ubiquitinated proteins in cells treated with bortezomib, consistent with inhibition of the proteasome. Experiments to assess the effect of radiation plus BORT on the clonogenic potential of canine glioma cells are ongoing, but preliminary data in the J3TBG cell line suggests an added benefit when combining both treatments in low doses. In conclusion, BORT significantly reduces the viability of canine glioma cells via induction of apoptosis and may result in increased cytotoxicity when used in combination with radiation.

## Diagnostic potential of Ag ECO test as a screening method for SARS CoV 2 compared to RT-qPCR in Brazil

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Molecular identification (RT-qPCR) of SARS CoV-2 from nasopharyngeal swab is regarded as the conclusive diagnostic method. With more than four million confirmed cases, and more than one hundred and twenty-eight thousand deaths in Brazil, it becomes apparently impossible to undertake mass diagnostic tests in the country. The molecular analysis is time-consuming and the goal of early detection and isolation of COVID-19 patients in order to prevent community transmission may be compromised. The development of an efficient and reliable rapid diagnostic test as an alternative or complementary test to molecular assay is therefore necessary. The aim of this study was to evaluate the SARS CoV-2 diagnostic potential of Ag ECO; a new diagnostic tool. The study was conducted among 135 patients suspected to have contracted SARS CoV-2. Two nasopharyngeal swabs were collected from the patients during their first visit at Baleia Hospital, Brazil. SARS CoV-2 was diagnosed in nasopharyngeal samples in the patients using the newly developed Ag ECO test and the diagnostic performance was compared with RT-PCR (a reference diagnostic standard). Thirty nine percent (39.0%) of the patients were positive to SARS CoV-2 by molecular detection while 28.0% of the patients were diagnosed positive by the Ag ECO test. Three (3) of the 135 patients examined presented false positive results to Ag ECO, while 18 showed false negative diagnostic outcomes. The sensitivity and specificity of diagnosis of SARS CoV-2 by Ag ECO test using RT-PCR as the reference diagnostic standard were 66.0 (CI 51.7 – 78.5%) and 96.3 (89.7 – 99.2%) respectively. Our study showed that Ag ECO diagnostic test showed substantial diagnostic performance. This diagnostic method can be recommended as the first line diagnostic tool in resource-limited areas due to the few false positive results observed. However, the use of RT-PCR is recommended for the confirmation of the negative results obtained from this new diagnostic approach.

## Experimental susceptibility of striped skunks (*Mephitis mephitis*) and raccoons (*Procyon lotor*) to SARS-CoV-2, causative agent of Coronavirus Disease 2019 (COVID-19)

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Anthropogenic factors and socio-economic systems (e.g., habitat destruction, animal trade, international movement of people and animals, healthcare infrastructure, etc.) have drastically influenced host-pathogen dynamics, creating new opportunities for pathogens to colonize novel hosts. SARS-CoV-2, a recently emerged zoonotic pathogen, has further demonstrated this relationship by not only causing a global pandemic resulting in mass human mortalities, but also worldwide economic instability. Unlike previous coronavirus outbreaks, such as SARS-CoV and MERS, SARS-CoV-2 has established community spread within the North American continent. A One Health approach to the current COVID-19 pandemic in North America provides a new ecological context to host-microbe interactions and is needed to identify sources of emerging pathogens and potential new routes of exposure and transmission. We conducted a study to identify if two North American native wildlife species that represent a high likelihood of susceptibility and ecological opportunity—striped skunks (*Mephitis mephitis*) and raccoons (*Procyon lotor*)—are susceptible to infection with SARS-CoV-2. Groups of 8 young, captive-bred animals housed in a BSL-3 containment facility were intranasally inoculated with one of two doses of SARS-CoV-2 (2 pairs for each dose). To test for direct transmission, on 2 days post inoculation (DPI), we added 1 contact animal to the same cage of each pair of inoculated animals. From DPI 1, we collected nasal and rectal swabs for qrtPCR and virus isolation (VI) at regular intervals from both inoculated and transmission animals. On DPI 5, 9, and 15/17 blood samples were collected for VI and serology. Animals from each group were then euthanized and necropsied at staggered intervals until DPI 15/17. Four control animals housed in a separate room were also sampled as described above. Preliminary results suggest skunks are susceptible to infection with and shed SARS-CoV-2, and raccoon results are still pending. We expect this data to improve our understanding of the role of striped skunks and raccoons as hosts for SARS-CoV-2. Evaluating these findings within a One Health context will inform the risk of spillback from people to animals, the management and surveillance of these species, and could, in turn, prevent or reduce the threat of future outbreaks.

## Multi-season survey of ixodid ticks on domestic dogs in Chad, Africa

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Ticks are important vectors for pathogens of great concern for human, wildlife, and domestic animal populations. Different tick-borne pathogens can be transmitted by select tick species, which means that an understanding of tick distributions and seasonality is critical for disease prevention efforts. Limited studies on ticks have been performed in Chad, Africa, highlighting a significant knowledge gap in vector data for the region. For this study, ticks were collected from domestic dogs in Chad by a team of University of Georgia researchers, Afrique One/IRED personnel, and Carter Center Staff. Tick collections occurred in May 2019, November 2019, and May 2020, representing both rainy and dry seasons. Ticks were preserved in 70% ethanol and are currently being identified to species level using a combination of morphologic characteristics and sequence analysis of 16S rRNA gene region. This is an ongoing study, species identification processes have not concluded. Tick species diversity will be analyzed in association with data on host dog sex, age, and condition, as well as region and season. My group is interested in cataloguing the ranges of all ixodid tick species across Chad; however, we are particularly keen to understand the range of the invasive tick, *Rhipicephalus (Boophilus) microplus*, which has been linked to economic losses in cattle due to the pathogen, *Babesia bovis*, which causes bovine babesiosis. This tick has been aggressively spreading across the African continent, though it has yet to be identified in Chad. We have identified an unusual detection of the endemic species *Rhipicephalus (Boophilus) decloratus* in our preliminary identification processes. This species is a common parasite of cattle, but less pathogenic than the invasive species *Rhipicephalus (Boophilus) microplus*. We have also identified large numbers of *Rhipicephalus sanguineus* and *Amblyomma variegatum*, both common ticks of dogs and vectors of several important pathogens of people and animals. Additional research on the changing ranges and biodiversity of African ixodid ticks, as well as pathogens they may transmit, is needed to better understand the potential risk of tick-borne diseases in this understudied region.

## The use of photostimulation to improve *in vitro* fertilization

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Photostimulation involves the exposure of cells and tissues to light energy in the red to near-infrared range. Such energy is absorbed by the mitochondria leading to enhanced activity with positive effects on cellular energy metabolism, cell division and other cellular functions. The objective of this study was to evaluate the effect of photostimulation on early bovine embryos both for potential enhancement to bovine assisted reproduction and as a model for intervention in human clinical reproduction. Immature bovine oocytes were collected and matured according to standard *in vitro* maturation protocols. Mature oocytes were separated into 25 oocytes/well and subjected to *in vitro* fertilization at 38.5°C in 5% CO<sub>2</sub> and high humidity, in the absence (control) or presence of photostimulation (treatment: 660-665 nm LED array, 10 min duration, 1h after the onset of fertilization). Zygotes were cultured for 7 days; cleavage and blastocyst rates were recorded as a percentage of the number of oocytes subjected to each treatment. There were 6 replicates and the Mixed procedure (SAS 9.4, SAS Institute Inc.) was used for statistical analysis. There was no difference ( $p > 0.05$ ) between treatments; cleavage ( $71.5 \pm 4.5$  vs.  $71.1 \pm 4.5$  mean  $\pm$  s.e.) and blastocyst ( $16.4 \pm 4.5$  vs.  $18.7 \pm 4.5$ ) rates for control and treatment respectively. This result indicates no stimulatory or toxic effects from the photostimulation protocol. Further research is needed to optimize a protocol, with possible changes to the wavelengths of light used, to enhance metabolic activity during sperm-oocyte interaction, improving the success of *in vitro* fertilization treatments for bovine and human patients. This abstract has been previously accepted to the internal Prisma Health Showcase.



## Disorders of the central nervous system in new world camelids

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New World camelids (NWC; llamas and alpacas) are popular in the United States for fiber production and companionship. Although neurologic disease is commonly diagnosed in NWC residing in the southeastern United States, etiology is not well-documented. To characterize patterns of inflammation, distribution, and identify potential etiologic agents, the autopsy records from 2008 to 2020 from the Athens Veterinary Diagnostic Laboratory were reviewed for cases of neurologic disease in llamas and alpacas. A total of 318 NWC were submitted for autopsy, of which 67 cases (50 alpacas and 17 llamas) were diagnosed with neurologic disease. Females (38/67) and males (29/67) with ages ranging between 4 days to 15 years (mean age = 6 years) were affected. Inflammatory diseases occurred in 33/67 cases; infectious organisms were identified in 14/33 cases, including *Parelaphostrongylus tenuis* (5/14), *Listeria monocytogenes* (2/14), *Fusobacterium necrophorum*, unidentified mixed bacteria, *Cladophilophora bantianum*, rabies virus, Eastern equine encephalitis virus, suspect *Blastomyces* spp., and protozoal cysts (1/14 each). Lesions characterized by necrosis, scattered axonal degeneration, lymphocytic infiltration, hemosiderin-laden macrophages, and glial scars were observed in 27/67 cases. While these lesions were attributed to *P. tenuis* infection, no intralesional nematodes were detected and the diagnosis could not be confirmed. Necrosis and/or axonal degeneration with no apparent cause was found in 7/67 cases. Axonal degeneration due to intervertebral disc disease was diagnosed in 2/67 cases, and hepatic encephalopathy due to severe liver dysfunction was found in 1/67 cases. As highlighted by our study, *P. tenuis* is an important cause of neurologic disease in NWC, but other infectious diseases, including rabies, should also be considered.

## Determining the effects of temephos (Abate™) and spinosad (Natular™) on survival of cyclopoid copepods

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Guinea worm (*Dracunculus medinensis*) is a parasitic nematode that infects humans and a few other mammalian species in Africa. Dracunculiasis (Guinea Worm disease) is a painful debilitating disease and is the focus of the Guinea Worm Eradication Program (GWEP) led by The Carter Center and other public health organizations. Historically, the primary transmission route of dracunculiasis is ingestion of cyclopoid copepods (the intermediate hosts) from untreated water sources. Currently, the primary chemical control method for cyclopoid copepods is the application of the organophosphate larvicide Abate™ (temephos). However, it can be difficult to apply Abate™ at the appropriate concentration in some water bodies. Therefore, there is a need for an alternative chemical compound to manage copepods. Natular™ (spinosad) is a mosquito larvicide that is currently used to control common mosquito species such as *Aedes*, *Anopheles*, and *Culex* spp. We hypothesized that Natular™ would be as effective as Abate™ in causing copepod immobilization due to the similar mode of action and its effectiveness as a larvicide. The results indicate that Natular™ is less effective at immobilizing copepods compared with Abate™. Although increasing the concentration of Natular™ can result in higher copepod immobilization rates, these increased doses (double or triple) may not be safe for field application. Additional research on the synergistic effects of the two compounds and the toxicity rates of Natular™ on other organisms is needed in order to conclude if Natular™ would be a good alternative control intervention for the Guinea Worm Eradication Program.

## Molecular mechanism of PRAP1 regulation by estrogen receptor alpha (ER $\alpha$ ) in mouse uterus

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Proline rich acidic protein 1 (PRAP1) is a secretory protein highly expressed in the uterine epithelium. While its function and mechanism of regulation in the uterus remain largely unknown, limited studies in the literature have proposed a role of PRAP1 as a negative regulator of cell growth in epithelial tissue homeostasis as well as a protector for irradiation-induced apoptosis in the gastrointestinal epithelium. Our lab demonstrated dynamic expression of *Prap1* mRNA in the uterine epithelium during early pregnancy, with high expression on day 0.5 post-coitus (D0.5), low expression on pre-implantation D3.5 and again high expression on D4.5 upon embryo implantation initiation. In ovariectomized mouse uterus, *Prap1* mRNA is downregulated by progesterone but upregulated by estrogen. Estrogen mainly exerts its function in the uterus via estrogen receptor alpha (ER $\alpha$ ), which is expressed in different uterine compartments, including uterine epithelium, stroma, and myometrium. Female mice with ER $\alpha$ -deficiency in the whole uterus (gER $\alpha$ KO) or in the uterine epithelium (epiER $\alpha$ KO) are both infertile. Public microarray data from ovariectomized (OVX) uteri revealed upregulation of *Prap1* in the gER $\alpha$ KO OVX uteri but downregulation of *Prap1* in the epiER $\alpha$ KO OVX uteri compared to the *Prap1* expression level in wild type OVX uteri. With collaborations, our lab has established epiER $\alpha$ KO mouse colony and newly obtained anti-PRAP1 antibody. I am working to determine the spatiotemporal expression of PRAP1 in the wild type and epiER $\alpha$ KO mouse uteri during early pregnancy. Meanwhile, I am using bioinformatics to analyze the regulatory elements of *Prap1* in order to provide instructional information for determining the molecular mechanisms of *Prap1* regulation. In the future, I will study PRAP1 function in the uterus using a PRAP1-deficiency mouse model. Our study will provide novel information on PRAP1 regulation and function(s) in the uterus.

## Investigating the role of equine platelet lysate on wound healing in vitro

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Aberrant wound healing is a significant issue in equine medicine and we hypothesize equine platelet lysate (ePL) will promote timely and organized healing. The aim of this study was to determine if using ePL in cell culture could enhance wound healing as determined by an in vitro scratch assay on equine primary dermal fibroblasts. Fibroblasts were isolated from skin biopsies obtained from the cannon bone region of 6 healthy horses. Fibroblasts were passaged to P2 before seeding at a density of 100,000 cells per well onto glass coverslips within 24-well plates. After incubating and reaching 80% confluence, a scratch assay was performed and media was replaced with treatment groups containing: 10%FBS, 10%ePL, 20% ePL, 10% DHS, and 20% DHS with and without 2ng/mL TNF- $\alpha$ . Stimulation of cells via TNF- $\alpha$  was utilized to represent the presence of inflammatory mediators naturally found at the site of injury. Cell supernatant was obtained before the scratch assay as well as 24, 48, and 72h afterwards. Supernatants were frozen at -80°C until further analysis via ELISA for TGF- $\beta$ 1 and IL-6. At the same time points, coverslips were formalin fixed and immunohistochemically stained for the presence of  $\alpha$ -SMA, a marker for myofibroblast differentiation. Once  $\alpha$ -SMA was evaluated from a subset of samples, all samples were stained via the Diff-Quik method before being digitally scanned. Images generated from the digital scanner were used to measure the rate of scratch closure expressed as the area of the scratch over time using the software program Image J. This study is ongoing and results are anticipated within the coming weeks.

## Retrospective comparison of incisional complications in dogs with mast cell tumor versus soft tissue sarcoma

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Mast cell tumors (MCT) contain bioactive compounds, which have been implicated in delayed wound healing and may predispose dogs to post-operative incisional complications (IC) compared to other tumors that do not have marked peritumoral inflammation. The objective was to compare the incidence of IC in dogs undergoing excision of MCT and soft tissue sarcomas (STS). The hypothesis was dogs undergoing MCT resection would experience greater post-operative IC than dogs undergoing STS resection. Retrospective medical records search from January 2014 to July 2019 was performed. Data were compared for incidence and descriptive features of IC between MCT and STS groups. Logistic regression analysis was used to evaluate risk factors for IC, including: tumor grade, surgical and histologic margins, pre- and/or post-operative chemotherapy (excluding steroids), steroid/antihistamine use, anesthesia and surgical time, and propofol use. Tumors (n=293) were analyzed from 218 dogs. IC were not significantly different between MCT (28/209; 13%) and STS excision (13/84; 15%; p=0.64). MCT with incomplete margins were more likely to have IC than MCT with complete margins (OR=2.8 (95%CI 1.2-6.7) p=0.04). Post-operative chemotherapy increased the odds of IC in MCT (OR=2.7 (95%CI 1.2-6.3) p=0.02). The risk of IC with either tumor increased with body weight (p=0.03). Steroid use did not increase IC risk in MCT. Anesthesia (p<0.0001) and surgical (p<0.0002) times were higher for MCT than STS. The main study limitation is the retrospective nature. Incomplete MCT excisions are at greater risk for IC. Post-operative chemotherapy should be used with caution for MCT.

## Analysis of ITS-1, 18S, and CO1 genes for identification of *Eimeria* parasites in poultry.

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Coccidiosis is a costly enteric disease of commercial poultry worldwide caused by a single-celled, parasitic protozoa of the *Eimeria* genus. The parasite reproduces rapidly both sexually and asexually causing substantial damage to intestinal linings, and often mortality. The most common method for the identification and differentiation of *Eimeria* of species is classic microscopy, using morphometric characteristics (size, shape) and region of the gut parasitized. While a simple technique, microscopy can be subjective, and the sensitivity is not ideal. As molecular tools have advanced, using PCR may afford the opportunity to further characterize *Eimeria* samples past simple species differentiation. Therefore, the overall goal of this research is to produce high quality profiles from sequence data to promptly identify *Eimeria* strains. Three different genome regions have previously been used to molecularly differentiate species of coccidia; Internal Transcribed Spacer-1 (ITS1), Ribosomal 18s DNA (18S), and Cytochrome Oxidase C-1 (CO1). When evaluated together, inter- and intra-species variation in these regions allows for a molecular fingerprint of current multispecies samples. Additionally, using a combination of PCR and next generation sequencing (NGS) makes the sample and sequencing processing rapid, allowing for mixed samples to be sequenced at one time. To date, our results show that individual vaccines carry an identifiable sequence combination of these three genome regions, allowing us to differentiate them. With this data, we hope to build a profile of field coccidia versus vaccine coccidia, which will be an extremely useful diagnostic tool in the future.

# Immunogenicity of Parainfluenza Virus (PIV) Vectored SARS-CoV-2 Vaccines in the Ferret Animal Model

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SARS-CoV-2 emerged in late 2019, quickly spread across the globe and has infected more than eleven million individuals resulting in more than 500 thousand deaths. There is limited information on this zoonotic coronavirus that causes COVID-19 (Coronavirus Disease 2019), but rapid strides have been made in the first half of 2020 including development of diagnostic tests, experimental treatments, and experimental vaccines to prevent SARS-CoV-2 infection or COVID-19. There is a critical need to develop a safe and effective vaccine to decrease the number of people affected by SARS-CoV-2.

In collaboration with the laboratory of Dr. Biao He, we assessed the antibody response to ferrets vaccinated with novel parainfluenza virus 5 (PIV5) vectored vaccine expressing the spike protein and/or N protein of SARS-CoV-2 (PIV5-S and PIV5-N). Ferrets were selected as an appropriate animal model because they have been widely used in influenza vaccine and infection studies, they are naturally susceptible to SARS-CoV-2 infection and can transmit virus to naïve ferrets. Groups of ferrets were vaccinated by intranasal administration of vaccine or mock-vaccinated and blood samples were collected every 7 days (0, 7, 14, 21, and 28) to assess serum antibody responses to the SARS-CoV-2 S protein. About 40 days post-vaccination, ferrets were infected with SARS-CoV-2, monitored for clinical disease and sampled for infection. The ferrets were humanely euthanized on day 7 post-challenge, necropsied, and blood collected for post-challenge serology. All blood samples were processed for serum to run enzyme-linked immunosorbent assays (ELISAs). The objective of this study was to determine the immunogenicity of the PIV5 vaccines by assessment of serum antibody responses and whether serum antibody responses confer protection from infection.

Ferrets had a robust antibody response to vaccines. While the PIV5-S had low antibody responses post-vaccination, the day seven post-challenge responses suggest the vaccine primed S-specific immune responses as mock-vaccinated ferrets had undetectable antibody responses seven days after challenge. While analysis of infection is pending, these data suggest that the PIV5-based COVID-19 vaccine elicited immune responses against SARS-CoV-2 and likely protected against infection and disease.

## Experimental West Nile virus infection in wild turkeys (*Meleagris gallopavo*) and northern bobwhite quail (*Colinus virginianus*)

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The potential impacts of West Nile virus (WNV; Family *Flaviviridae*, Genus *Flavivirus*) on many North American upland game bird species remain largely understudied. Although regional declines in northern bobwhite quail (*Colinus virginianus*) and wild turkey (*Meleagris gallopavo*) populations have recently been documented without a known cause, data on the susceptibility of these species to WNV-associated disease are lacking. To address this knowledge gap, we subcutaneously inoculated two age cohorts of wild turkey poults (n=25) and northern bobwhite chicks (n=20) with WNV and monitored virologic, clinical, pathologic, and serologic responses. Viremia titers were low in both species compared to more susceptible avian species, such as some corvids and raptors. However, while less than half of inoculated bobwhites (8/20; 40%) developed detectable viremia titers, all turkey poults had transient viremia titers from 1-2 days post-inoculation (DPI) to 3-5 DPI. The mean peak viremia titer among bobwhites was  $10^{2.0}$  plaque-forming units (PFU)/ml serum (range  $<10^{1.7}$ - $10^{3.0}$  PFU/ml serum; detection threshold:  $10^{1.7}$  PFU/ml), which was slightly lower than the mean peak viremia titer among turkey poults ( $10^{3.8}$  PFU/ml serum; range  $10^{1.7}$ - $10^{4.8}$  PFU/ml serum). Most poults (20/24; 83%) shed WNV either orally or cloacally, while oral or cloacal shedding occurred rarely in bobwhites (4/20; 5%). One of 25 (4%) WNV-inoculated turkey poults and no bobwhites developed clinical signs potentially attributable to WNV infection. All inoculated turkeys and bobwhites, as well as one contact-control turkey, seroconverted by 14-15 DPI. West Nile virus rarely was isolated from tissues of inoculated poults (1/24; 4%) and was not isolated from bobwhite tissues. No WNV-associated macroscopic lesions were detected in either species at 14-15 DPI. Histopathological examination of the tissues of the young cohorts indicated that most turkey poults (10/12; 83%) and half of the bobwhites (5/10; 50%) had mild myocarditis and 11/12 (92%) poults and 0/10 (0%) bobwhites had mild encephalitis. These data suggest wild turkeys and bobwhites have low susceptibility to WNV-associated morbidity and mortality and neither species likely serves as a competent reservoir host for WNV. Serologic surveys of wild counterparts would complement these data and are in progress.

## Genotyping Global Gene Knockout Mice

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A genotype is a particular gene or set of genes carried by an individual that codes for a specific trait. The process of determining the genetic variants each individual possesses is called genotyping. In any lab that involves experimentation on organisms, genotyping is a basic yet crucial process that is used to identify an individual before it is tested. In our lab, the testing individuals are mice. We use global gene knockout mouse models and cell-type specific conditional gene knockout mouse models to study molecular mechanisms in regulating female reproduction. Genotyping a single gene global knockout mouse involves three primers in a single round of Polymerase Chain Reaction (PCR). There are three expected genotypes of both alleles: wildtype (+/+), heterozygous (+/-), and knockout (-/-). Genotyping allows us to assign wild type mice, in the control group, heterozygous mice as breeders and knockout mice for studying their fertility. There are four main steps in genotyping global gene knockout mice: tailing, DNA extraction, PCR, and gel electrophoresis. Tailing is to snip the tip of a mouse tail. The tail piece is then submerged in a lysis buffer to be extracted for genomic DNA (gDNA). The gDNA is used as a template for PCR. PCR is a commonly used technique that quickly makes millions of copies of DNA segments. The PCR product from each mouse tail is then applied to a gel, which is made of agarose and a small amount of ethidium bromide (EtBr). EtBr intercalates into DNA and makes DNA “visible” under UV light. Gel electrophoresis separates DNA segments based on their sizes. An image of the gel with DNA segments can be captured with a UV transilluminator. A wild type allele will yield a larger DNA band, a knockout will produce a smaller band. The three genotypes will be determined as following: +/+, a single larger band; +/-, one larger and one smaller band; -/-, a single smaller band. With the genotype information, we will continue our studies on female fertility in the gene knockout mouse models.

## Tumor Progression Locus 2 (Tpl2) prevents immunopathology during Influenza infections

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Tumor progression locus 2 (Tpl2) is a serine-threonine kinase known to promote inflammation in response to various infectious agents. We have previously shown that Tpl2<sup>-/-</sup> mice succumb to infection with a normally low-pathogenicity strain of influenza (x31, H3N2), however the root cause of morbidity has yet to be identified, which is the goal of the current study. Histological examination of the lungs of influenza-infected Tpl2<sup>-/-</sup> mice showed increased alveolar septal necrosis, pleuritis, and more widespread lesions than seen in WT mice, all signs of epithelial-endothelial barrier damage and inflammation. Although Tpl2<sup>-/-</sup> mice display modestly delayed viral control throughout the infection, no virus was observed in the lungs of Tpl2<sup>-/-</sup> mice on the day of peak morbidity and mortality, suggesting that pathology is not due to uncontrolled virus replication or virus cytopathic effects but rather to an overactive immune response to infection. Indeed, an increase in lung levels of interferon- $\beta$  (IFN- $\beta$ ) and the IFN-inducible monocyte chemoattractant protein MCP-1 (CCL2) was observed in influenza-infected Tpl2<sup>-/-</sup> mice at 7 days post infection (dpi). This increase in MCP-1 expression was accompanied by excess influx of inflammatory monocytes, which have the potential to damage the epithelium and thereby mediate the observed pathology. Thus, ongoing studies are examining whether Tpl2 paradoxically limits inflammation during influenza infection by constraining the production of type I IFNs. This information will enhance our understanding of Tpl2 signaling and interferon regulation, which could provide insights into host-targeted therapies for treating highly pathogenic influenza which is typically associated with excess type I IFNs and robust inflammatory monocyte recruitment.

## Alphacoronavirus 1 spike gene phylogenetics and survey for risk factors of feline infectious peritonitis

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Feline coronavirus (FCoV) is a common, fecal-orally transmitted, enteric virus in cats, and is the cause of feline infectious peritonitis (FIP), which is a frequently fatal, immune-mediated disease. Although FCoV transmission can be due to transiently infected cats, a major source of FCoV is persistently infected cats without FIP. Previous data in FCoV and other viruses suggest that shedding phenotypes and/or virulence may be associated with specific viral genotypes; thus, identification of genetic markers for FCoV is needed. The spike (S) protein of coronaviruses is responsible for receptor binding and viral entry. As such, the spike is exposed to diversifying immunological pressure and variation may result in immune evasion allowing for persistent infection and shedding. Currently, there are no accepted phylogenetic divisions of FCoV that correctly identify the relationship between strains. Using Geneious and the sequences available on GenBank, a meta-analysis of all available FCoV spike gene sequences was performed to evaluate current phylogenetics.

Secondly, several risk factors for the development of FIP in FCoV-infected cats have been suggested, including vaccination. We hypothesized that cats that developed FIP will be more likely to have been vaccinated recently than age-matched cats that do not develop FIP. We will investigate this hypothesis by means of an online survey.

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## Pangenome analysis of *Mycobacterium bovis* isolates reveal geographic and host specific genomic signatures

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*Mycobacterium bovis*, the bacterial cause of bovine tuberculosis (bTB), is a damaging disease for the veterinary and public health sectors. Genotyping of *M. bovis* isolates extracted from host species have been a fundamental tool to understand the transmission dynamics of the pathogen, and modern genomic studies have more recently used the pangenome to understand the evolutionary underpinnings of *M. bovis* spread. Pangenome analysis of microbial pathogens is seen as an emerging tool to investigate adaptive processes in pathogens such as antimicrobial resistance, host range, and disease surveillance. *M. bovis* affects a multitude of host species, however, it is still not clear why some become reservoirs of infection, while others are mere spillover hosts. In this study, we pose the research question, does the *M. bovis* population have specific genomic signatures depending on their environment? If yes, how are they characterized? To answer these questions, I utilize a pangenomic framework of *M. bovis* isolates from the United states of America, the United Kingdom, and New Zealand to determine accessory gene presence/absence and homologous recombination patterns across regions and host-species. The pangenome inferred based on the current *M. bovis* genome annotations (accession no. LT708304) consisted of 3,592 core genes and 12,263 accessory genes. Principal Coordinate Analysis based on the similarity of isolate accessory genes revealed distinct clusters of isolates on both the country of origin scale. Varying frequency distributions of homologous recombination events amongst the isolates based on country and host metadata were observed. These preliminary results reveal that *M. bovis* has geographic and host specific genomic signatures, which suggest that these mycobacteria might be evolving differently to adapt to specific environments. Further work will involve analysis of positive selected sites in the core genome and further phylogenetic analysis of core genes.

## Serological detection of *Borrelia burgdorferi* antibodies in serum and CSF from horses in Georgia

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Lyme disease, a tick-transmitted disease caused by the Gram-negative spirochete *Borrelia burgdorferi*, occurs in dogs, cats, humans, and horses and can have devastating effects on many organs. Infection in the nervous system is particularly hard to diagnose in all species. Serological testing is used to detect current and previous *B. burgdorferi* infections in humans and animals. However, in endemic areas, seropositivity frequently reflects exposure to the organism rather than confirms clinical disease. Approximately 50% seropositivity in healthy horses is described in Lyme-endemic areas in the United States, but the seroprevalence of antibodies against *B. burgdorferi* has not been described in horses in Georgia. The objectives of this study were thus twofold: (1) to determine the seroprevalence of antibodies against *B. burgdorferi* in healthy horses in Georgia; and (2) to compare antibody titers in serum and CSF samples in a subset of these animals. We hypothesized that the seroprevalence of *B. burgdorferi* in non-endemic Georgia is less than 50%, and that seropositivity in serum and CSF would be consistent within animals. 304 samples from healthy horses presented for Coggins testing in Georgia and 27 paired serum and CSF samples collected from neurologically normal horses were analyzed. Antibody titers against *B. burgdorferi* outer surface proteins (OspA, OspC, and OspF) were quantified using the validated Equine Lyme Multiplex Assay. Data were analyzed with descriptive statistics and repeated measures ANOVA ( $P < 0.05$ ). 7.3% of healthy horses in Georgia had detectable serum antibodies against *B. burgdorferi*, with the majority of horses positive for OspF (5.9%) alone. Seroprevalence did not differ between samples from northeastern and southern Georgia ( $P > 0.9999$ ). 2/27 healthy horses with concurrent serum and CSF samples had detectable antibodies in serum and 5/27 had detectable antibodies in CSF. As expected, the seroprevalence of antibodies against *B. burgdorferi* is lower in Georgia than in Lyme-endemic areas. However, some horses in Georgia do demonstrate serum and/or CSF antibody responses reflective of acute or chronic exposure to the organism, which could represent local infection. Thus, Lyme disease should be considered a differential diagnosis in horses with consistent clinical signs in Georgia.



## Lysosomal functions in female reproduction

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Our previous microarray of mouse peri-implantation uterine luminal epithelium (LE) revealed a dramatic upregulation of *Atp6v0d2* (ATPase H<sup>+</sup> transporting V0 subunit D2) in the LE upon embryo implantation initiation. *Atp6v0d2* encodes a tissue-specific V0D2 subunit for V-ATPase (Vacuolar-type H<sup>+</sup>-ATPase), regulates the pH of extracellular space and lumen of intracellular organelles, especially lysosomes. It led us to the novel finding of uterine epithelial lysosomal acidification upon embryo implantation. Lysosomal acidity, which is critical for lysosomal functions, is mainly maintained by V-ATPase to pump H<sup>+</sup> into lysosomal lumen and counter ion channels to dissipate the transmembrane voltage built up by V-ATPase. Transient receptor potential cation channel, mucolipin subfamily, member 1 (TRPML1, encoded by *Mcoln1*) is a highly expressed counter ion channel in the mouse peri-implantation LE. We have been using *Atp6v0d2*<sup>-/-</sup> and *Mcoln1*<sup>-/-</sup> mouse models to study their *in vivo* functions in female reproduction. The *Atp6v0d2*<sup>-/-</sup> female mice have overall normal fertility. However, *Mcoln1*<sup>-/-</sup> female mice have reduced fertility at 2 months old (2M) and quickly become infertile at 5M due to progesterone deficiency caused by defective corpus luteum (CL), which is the main site for progesterone synthesis. Since ATP6V0D2 and TRPML1 regulates lysosomal pH in opposite directions, we hypothesize that deletion of both *Atp6v0d2* and *Mcoln1* could alleviate the adverse effect of *Mcoln1*-deficiency in cells with their co-expression. To test this hypothesis, we generated *Atp6v0d2*<sup>-/-</sup> *Mcoln1*<sup>-/-</sup> double knockout (DKO) mice. Fertility test collected so far shows 27% of DKO females remain fertile at 5M. Correspondingly, 27% of DKO females have restored serum progesterone levels as well as restored CL morphology. These preliminary data reveal partially recovered fertility (27%) of DKO compared to *Mcoln1*<sup>-/-</sup> females (0%) at 5M. To further understand how V-ATPase and TRPML1 coordinately regulate lysosomal functions in the CL to support pregnancy, we will determine the expression *Atp6v0d2* and *Mcoln1* in the CL, and employ TEM coupled with pharmacological and genetic approaches to decipher mechanisms of lysosomes in regulating CL functions. Our study will fill in the knowledge gaps about functions of the lysosome, especially the importance of electrochemical balance of the lysosome, in female reproduction.

## Phaeohyphomycosis cases at the University of Georgia from 2010 to 2020

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The objective was to analyze phaeohyphomycosis cases received by Athens Veterinary Diagnostic Lab and UGA Department of Pathology between 2010 and 2020. Phaeohyphomycosis can be caused by numerous saprophytic, dematiaceous (pigmented) fungi. Infection is considered opportunistic. While the route of infection is poorly understood, respiratory, ocular, aural, and cutaneous wound routes have been suggested. Infection is rare in veterinary species and humans, and such patients are often immunosuppressed or immunocompromised. The University of Georgia, College of Veterinary Medicine laboratory information management system was reviewed from 2010 to 2020 for mammalian biopsy and necropsy cases with histologic diagnoses of pigmented fungi. Fifty-two cases were included, with 46 biopsies and 6 necropsies. Biopsies were equine (46%), canine (33%), and feline (22%) origin, primarily of skin (89%), often originating from the head (24%) or limb (33%). Necropsies were feline (3), equine, canine, and camelid (1 each) origin, with encephalitis in 5 (83%). Three necropsies grossly described skin wounds, with 2 histologically confirmed as fungal dermatitis. Juvenile animals accounted for 80% of encephalitis cases. Fungal culture in biopsies (9%) and necropsies (83%) identified agents in 50% and 100% of attempts, respectively. While rare, accumulated phaeohyphomycosis cases in Georgia demonstrate a predominance of dermatitis in biopsies and encephalitis in necropsies. Relatively abrasion-vulnerable face and limb locations of skin lesions supports that pre-existing wounds likely predispose to infection. Encephalitis with concurrent skin wounds in 2 cases may support that systemic spread follows cutaneous infection. Encephalitis in predominantly young animals may indicate age or an immature immune status as predisposing factors.

## Transmission and Adaptation of an influenza H3N2 virus with human seasonal HA in pigs.

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Influenza A virus (IAV) is known to cause respiratory disease in pigs and humans. IAV circulates worldwide in pigs, with various strains and subtypes being prevalent among the swine population. The viral surface proteins Hemagglutinin (HA) and Neuraminidase (NA) are responsible for the invasion process of host cells and for determining host specificity, and are key factors in driving the evolutionary process of IAVs. Interspecies transmission is common between humans and pigs, with increased prevalence of human origin strains being detected in pigs after the introduction of the triple-reassortment internal gene constellation or TRIG cassette. The TRIG cassette is capable of stabilizing swine-origin strains and can incorporate various HA and NA, contributing to the diversity of IAVs. While it is known that human origin strains can adapt to pigs, the exact molecular process that enables the adaptation is still not fully understood. For the purpose of this study, we generated an H3N2 strain via reverse genetics with the HA and NA from human seasonal H3N2 (A/Victoria/361/2011) and the internal genes from TRIG cassette and the matrix gene (M) from the pandemic 2009 H1N1 strain to evaluate the adaptation of human seasonal surface genes in pigs. To test the transmission capabilities and evolution of the strain in pigs, we conducted a transmission study and swab samples were collected at specified timepoints from the infected and respiratory contact groups. Viral RNA was extracted from the samples and were submitted to Illumina MiSeq platform (NGS) for high-throughput sequencing. Bioinformatic analysis revealed four potential positions in the HA1 region (A154S, V202G, F209Y and synonymous 89D) with one fixed variant (A154S), which was present in all pigs in the contact group. Our data shows that mutations in the HA gene are selected quickly after replication and transmission of human HA in pigs, which may be instrumental in the adaptation process of the human origin H3N2 viruses in pigs. Further studies are needed to test if the fixed mutation results in increased fitness for the human HA in pigs.

## **Surgical site infection following ventral midline celiotomy in horses and association with duration of antimicrobial therapy: a retrospective analysis.**

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Surgical site infection (SSI) is one of the most commonly reported complications following ventral midline celiotomy, resulting in increased morbidity, other incisional complications, and cost. The use of antimicrobial therapy in prevention of SSI remains poorly characterized. This study was designed to evaluate the association of post-celiotomy SSI and duration of antimicrobial therapy, with the hypothesis that SSI rates will not differ between horses receiving different durations of antimicrobial therapy. Horses undergoing ventral midline celiotomy for acute colic (08/13-01/17) that survived and did not have a repeat celiotomy for at least 10 days or until discharge were included. Horses were grouped according to duration of antimicrobial therapy: 1=perioperative; 2=three days; 3=>three days. Signalment, surgical findings and procedures, post-operative complications, and survival to discharge were compared between groups (Chi-square test for categorical data and Kruskal-Wallis test for continuous data). 182 horses met the inclusion criteria. There was no difference between groups with respect to signalment ( $p=0.070-0.484$ ), season of presentation ( $p=0.177$ ), pre-operative temperature ( $p=0.660$ ), and surgeon ( $p=0.310$ ). There was no difference between groups with respect to lesion location ( $p = 0.298$ ), however a significant difference was found with respect to lesion type (strangulating vs. non) ( $p = 0.007$ ) and procedure performed (enterotomies and resections) ( $p < 0.001$ ). The overall SSI rate was 11% and this did not differ between groups ( $p=0.610$ ). A significant difference was found between groups with respect to post-operative fever ( $p<0.041$ ) and other post-operative complications, including other infections ( $p=0.011$ ), gastrointestinal ( $<0.001$ ), and inflammatory complications ( $p=0.003$ ). There was no difference in survival to discharge between groups ( $p=0.257$ ), although groups differed with respect to duration and cost of hospitalization ( $p<0.001$ ). The rate of SSI was not associated with duration of antimicrobial administration. Differences in lesion type, procedures performed, and post-operative complications were identified, although between-groups comparisons are required to further delineate these relationships.

## **Investigating the role of Infectious Bronchitis Virus variant DMV/1639 in incidence of False Layer Syndrome**

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Infectious Bronchitis virus (IBV) is an avian coronavirus that primarily causes respiratory disease but can also affect the reproductive tract of laying type chickens. If infection occurs in pullets, False Layer Syndrome can later develop. False Layer Syndrome is characterized by systemic changes in the oviduct and the development of large, fluid-filled cysts. IBV strain DMV/1639 in Canada and the U.S. has recently been reported in areas experiencing False Layer Syndrome. Our study investigates the role and timing of IBV infection on development of False Layer Syndrome using IBV variants DMV/1639 and Mass strain M41. We hypothesize that IBV variant DMV/1639 causes cystic oviducts and that the age of infection plays a role in the development of False Layer Syndrome. Eight groups of 120 SPF chickens and a control group of 50 were placed into colony houses and challenged at separate timepoints using either DMV/1639 or M41 IBV. Post challenge, we collected choanal swabs and performed necropsies. Based on real-time PCR, the M41 and DMV groups were adequately challenged with average CT values of 26.14 and 24.18, respectively. Tracheas and reproductive organs were collected for histopathology and in-situ hybridization. Oviduct cysts were found in all groups for both challenge viruses. The M41 group challenged at 7 DOA had the highest numbers of cysts and the DMV group challenged at 3 DOA had the second highest numbers of cysts. Our data indicates that IBV variant DMV/1639 causes oviduct cyst formation and that the age of infection with IBV is important in development of False Layer Syndrome. Further analysis will provide valuable information in understanding the pathogenesis of False Layer Syndrome.

## **Something on my mind: neuronal ceroid lipofuscinosis in a cat**

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A two year old, female spayed Siamese cat presented to the rdvm with a four month history of progressive, abnormal behavior, including urinating outside of the litter box, becoming anti-social, hyperexcitable, and exhibiting mild ataxia. Gross necropsy was unremarkable. Histopathology reveals the majority of neuronal and astrocytic cytoplasm expanded by amphophilic to eosinophilic globules which often peripheralized the nuclei. The cerebral white matter and lamina of the gray matter is severely spongiotic, and the associated neurons are hypereosinophilic and angular (neuronal necrosis). Electron microscopy was performed on cerebral tissue. Within the cytoplasmic matrix of neurons are one or multiple bundles of small, electron dense C-shaped to S-shaped lines. This pattern is indicative of the curvilinear profile (CLP), or Type 2 of neuronal ceroid lipofuscinosis (NCL). NCL is a lysosomal storage disease that has been described in several domestic species and humans, and clinical signs involve progressive cognitive decline, motor dysfunction, epileptic seizures, and eventually death or euthanasia. Although therapeutic advances have delayed onset of clinical signs, there is no current cure for this disease.

## **Histochemistry, Immunohistochemistry, and Electron Microscopy of Globule Leukocytes in Bovine**

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Globule leukocytes (GL) are intraepithelial cells found in the mucosa of the respiratory and digestive tract that increase in response to certain hypersensitivities, such as helminth infections and asthma. Although thought to be a subtype of mast cell, in most species these cells are poorly characterized. In this study, histochemical and immunohistochemical staining as well as electron microscopy were used to characterize GL from the bovine gastrointestinal tract. Tissues evaluated were identified from paraffin embedded archival specimens and stained with PTAH, Luna, and Giemsa. Stains were used to highlight morphological differences or similarities between GL and other cells. In bovine, GL were not metachromatic on Giemsa, unlike tissue mast cells. GL stained red like eosinophils on Luna stain and were PTAH positive like reported for large granular lymphocytes (LGL). Bovine GL were immunonegative for CD-3 (T-cell marker), CD-21 (B-cell marker), CD-79a (B cell marker), and carboxypeptidase. However, GL were immunoreactive for CD-117, a mast cell marker, and tryptase and  $\beta$  chymase, which are found in mast cell granules, suggesting a close relationship between GL and mast cells in this species. Electron microscopy revealed GL and mast cells had similar ultrastructure. Though these results suggest that GL and mast cells are closely related cell types, they do not conclusively prove that GLs are derived from mast cells or vice versa.

## Bald eagle morbidity and mortality in the southeastern United States: a five-year retrospective study

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The bald eagle (*Haliaeetus leucocephalus*) is an important North American conservation icon that has historically been a species in peril due to various human-associated activities. Further, as both apex predators and facultative scavengers, bald eagles frequently suffer from toxicoses, notably lead, anticoagulant rodenticides, and insecticides. However, published reports on causes of mortality in bald eagles in the southeastern U.S. are scarce. We retrospectively evaluated diagnostic findings of bald eagle cases submitted to the Southeastern Cooperative Wildlife Disease Study at the University of Georgia from January 2015 to June 2020. Eagle carcasses were submitted for postmortem examination from 21 states (primarily in the southeastern U.S.). Data included date and location found, age, sex, and clinical history, when known. Diagnostic findings included gross pathology, histopathology, and case-specific ancillary test results. Final diagnoses were categorized by primary and contributing causes of mortality. Among the 267 bald eagles examined, non-infectious causes of mortality (209; 78.3%) were more common than infectious (21; 7.9%), with 37 (13.8%) that died of unknown causes. The majority (127; 47.6%) of noninfectious causes were attributed to trauma. The most common attributable sources of trauma were vehicular collision (21), electrocution (19), and gunshot (10). Seventy-three (27.3%) eagles died of toxicoses, including lead (44), anticoagulant rodenticides (18), avian vacuolar myelinopathy (i.e., cyanobacterium toxin; 5), and organophosphate insecticide (1). Additionally, several toxicants were detected at low levels in tissues of 131 eagles, including various anticoagulant rodenticides in 110 (41.2%), lead in 20 (7.5%) and pentobarbital in 1 (0.4%). Infectious causes of mortality were most often viral (11; predominantly West Nile virus, followed by avian poxvirus). This study demonstrates numerous challenges faced by bald eagles in the southeastern U.S. including potential, population-level impacts of human-associated activities. Although trauma was the most common source of mortality, frequent detection of a variety of toxins is concerning. Specifically, the potential health impacts of low-level and possibly chronic toxicant exposure in bald eagle populations warrants further investigation.

## Determining the cost-effectiveness of VacSIM through a swine flu market analysis

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Influenza A virus of swine, known as swine flu, is a significant respiratory pathogen due to being globally ubiquitous, having the potential for transmission from pigs to humans, and causing substantial economic losses to the swine industry. Current swine flu vaccines elicit low levels of efficacy and require multiple doses to induce high antibody titers. Vaccines on the market for this disease include FluSure XP by Zoetis, Sequivity by Merck, and Ingelvac Provenza by Boehringer Ingelheim. VacSIM® is an immunization delivery method that enhances vaccine efficacy and host protection due to the slow release of antigens. The goal of this study is to determine if the addition of VacSIM delivery to swine flu vaccines can improve their immunogenicity, providing a cost effective approach to mitigating industry losses. Data for the market analysis was collected from vaccine manufacturers and professionals in the swine industry. This information is currently being evaluated to determine how the costs of VacSIM, about \$0.50/dose, will affect vaccine manufacturing revenue and the swine production industry. Experimentally, over 6000 doses of VacSIM have been administered to water buffalo and cattle in vaccine trials with no adverse events, showing evidence that VacSIM is safe for use in livestock. The VacSIM delivered *Schistosoma japonicum* vaccine elicits 56% protection against challenge in water buffalo and reduces fecal parasite egg output by 57%, significantly reducing disease transmission. These findings suggest that VacSIM has the potential to improve both the humoral and cell-mediated immunity of vaccinated livestock while having the potential to reduce commercial losses to producers.

## Skunk adenovirus in three domestic ferrets (*Mustela putorius*)

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Three emaciated domestic ferrets (*Mustela putorius*), from a pet vendor in the Southeastern United States, were submitted for necropsy and histopathology to the Infectious Diseases Laboratory at the University of Georgia. Subject 1 came from a cohort with a 6-week history of multiple cases of respiratory disease and mortality. Subjects 2 and 3 were submitted the following month, after dying within days of arrival at the facility; they were allegedly housed separately from the first cohort. Grossly, all lung lobes of subjects 1 and 2 had multifocal extensive red regions. During histopathological evaluation, all three subjects had evidence of interstitial pneumonia consistent with viral etiology. Subject 1 had moderate subacute multifocal suppurative bronchopneumonia with intralesional gram positive cocci. Subjects 2 and 3 had severe subacute multifocal lymphocytic interstitial pneumonia and complete consolidation of the terminal bronchioles and alveoli. Electron microscopy for samples of all three subjects revealed icosahedral viral particles consistent with Adenoviridae. Genetic sequencing following viral isolation and PCR identified Skunk Adenovirus 1 (SkAdV-1), which was first isolated from a skunk (*Mephitidae*) submitted for rabies testing in Canada in 2015. Adenoviruses are a diverse family of non-enveloped double-stranded DNA viruses that affect almost all known classes of vertebrates. Coevolutionary studies suggest that they are highly species specific, but SkAdV-1 has been found so far across four distinct mammalian families: *Mephitidae*, *Callitrichidae*, *Erinaceidae*, and *Erethizontidae*. Although the skunk in which the virus was identified did not have symptoms, the other species from which SkAdV-1 has been isolated have presented with bronchopneumonia and upper respiratory signs. This study is the first to report SkAdV-1 associated with disease in domestic ferrets. The prevalent finding of cross-species events associated with SkAdV-1 has important implications for wildlife management and for how animals are housed within multi-species facilities. Notably, the three subjects in this case series came from a facility that also houses African pygmy hedgehogs (*Atelerix albiventris*), a species which was associated with a SkAdV-1 outbreak in the Northeastern United States in 2018.

## Analysis of Infectious Bronchitis Virus Survey throughout the Southern United States

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Infectious bronchitis virus (IBV) is a gamma coronavirus that causes an economically significant upper respiratory tract disease in commercial poultry. There are many serotypes of IBV, and it mutates often leading to the emergence of more serotypes, making it difficult to control the disease via vaccination. For this reason, even though nearly every chicken is vaccinated against IBV, disease is prevalent in commercial poultry operations. In an effort to understand how prevalent variant IBV is in vaccinated flocks, a surveillance program was established in the Jordan lab. Southeastern poultry companies were asked to submit samples for PCR analysis. Samples were tested for all known IBV serotypes, and with a “generic” positive/negative test that could detect potentially new serotypes. The results were analyzed by what serotypes were found in each state, region, company, complex, and vaccine program used by the companies. Through the surveillance program, we found that nearly all samples were positive for virus by PCR. This was primarily vaccine origin, indicating that vaccines linger in flocks for much longer than previously expected. Non-vaccine origin viruses were also detected. These potential “challenge” viruses were of multiple serotypes including a group of unknowns. These unknown samples may be novel variants or mutations of vaccines that aren’t detected by the PCR assay, but these findings support the ideology that IBV mutates rapidly. Multiple vaccine programs with many different serotypes were used by companies submitting samples in this survey, yet non-vaccine origin viruses were still detected proving that IBV vaccines are very specific and do not offer full protection against heterologous viruses.

## **Evaluation of oral mycophenolate mofetil (MMF) steroid-sparing effect as an adjunct immunosuppressant for inducing remission of canine pemphigus foliaceus**

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Pemphigus foliaceus (PF) is the most common autoimmune skin disease in dogs. The treatment of canine PF can be challenging and lead to many adverse effects. Oral high-dose glucocorticoid monotherapy has been used for the induction of remission (i.e. induction phase) of canine PF; however, only 20-30% of patients are able to achieve complete remission (CR) within three months of therapy. Steroid-sparing adjuvants are the cornerstone strategy for pemphigus management to induce disease remission with minimal immunosuppression. Selecting the optimal therapeutic option among various first-line steroid-sparing adjuvants (e.g., azathioprine, cyclosporine and mycophenolate mofetil (MMF)) for induction phase of canine PF is often a clinical challenge due to the lack of clinical trials. In this study, we evaluated the therapeutic outcomes of oral glucocorticoid/MMF combination therapy for the induction of remission in canine PF. Twelve canine PF patients treated with oral glucocorticoid/MMF combination to induce PF remission were identified between 2015-2020 through the medical record search from the University of Georgia Veterinary Medical Center. Oral MMF was always administered divided into twice-daily dosages, every 12 hours. Only 2/12 dogs (mean MMF daily dosage 39 mg/kg) achieved CR, whereas 3/12 PF dogs (mean MMF daily dosage 27 mg/kg) achieved near CR (95% lesion improvement) and a single dog (mean MMF daily dosage 23 mg/kg) 80% partial improvement (PR). Five (5/12; mean MMF daily dosage 28 mg/kg) PF dogs achieved the only PR of  $\leq 50\%$  with new PF lesions developing during the treatment. Observed adverse effects included vomiting (1/12) and diarrhea (3/12); MMF was discontinued in a single patient (1/12) due to severe bloody diarrhea. The mean duration of MMF therapy in conjunction with glucocorticoid for canine PF CR and near CR patients was 79 days. Discontinuing oral glucocorticoids while continuing MMF led to the development of new PF lesions in all dogs. In conclusion, MMF seems to offer some benefit to reduce the dose of oral glucocorticoid in the management of canine PF cases during the induction phase of remission. However, our results indicate that oral MMF alone may not be effective during the maintenance phase for canine PF management.

## Feline leukoencephalomyelopathy in bobcats (*Lynx Rufus*) in Florida, United States: Pathology and Potential Etiologies

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Since 2017, free-ranging bobcats (*Lynx rufus*) and Florida panthers (*Puma concolor coryi*) in Florida have been documented with neurologic disease characterized by ataxia and hind-limb paresis. Our goal was to characterize microscopic and ultrastructural lesions in bobcats affected by this recently documented disease, and to investigate potential etiologies or disease associations. Gross and histopathology were performed on 27 bobcats that either underwent full postmortem examination (n=18) or histopathology after field necropsy (n=9) from 2018-2020. Electron microscopy (EM) was performed on spinal cord from two affected bobcats and a panther. Toxicologic testing on livers (n=6) included heavy metals, vitamin A, and gas and liquid chromatography-mass spectrometry analyses. Six cases underwent anticoagulant rodenticide screening based on gross findings. Testing for potential pathogens was conducted for select cases. Sixteen of the cases were from Florida, five from South Carolina, two from Virginia, one from West Virginia, one from Kansas, one from North Carolina, and one from Alabama. Six bobcats (22%), all from Florida, had diffuse white matter degeneration throughout the spinal cord, most prominently in the lateral and ventral funiculi, with dilated myelin sheaths, shrunken or absent axons, and digestion chambers. Occasionally, less severe lesions were in brainstem and cerebellar white matter, with microgliosis and perivascular, lymphocytic cuffing. A 2-month old female had lesions disseminated throughout the white matter of the brain. This bobcat also had perineuritis of axillary and sciatic nerves. EM revealed condensed and degenerated axons, vacuoles, with no evidence of hypo-, de- or remyelination. Three of six (50%) affected bobcats had copper deficiency and organochlorine detection, and 2/6 (33%) had low vitamin A levels. Thus far, testing for infectious agents (e.g., West Nile virus, rabies virus, bornavirus, feline leukemia virus, and feline panleukopenia virus) has been negative. The age distribution of affected bobcats ranged from 2 months to >32 weeks. Lesions throughout the hindbrain and spinal cord white matter suggest a central nervous system axonopathy of unknown cause. Severe lesions in the 2-month-old suggests potential utero transmission. Continued monitoring, including postmortem examination and toxicologic and infectious disease testing concurrent with environmental assessment, is imperative to understanding this disease.



## Scar revision in the absence of gross disease for incompletely excised mast cell tumors and soft tissue sarcomas in dogs: 101 surgeries (2010-2019)

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Mast cell tumors (MCT) and soft tissue sarcomas (STS) are the most common tumors of the skin and subcutaneous tissues in dogs. Recommended surgical margins for both MCT and STS are lateral margins of 2-3 cm and deep margins including one fascial plane. Despite widely published margin recommendations, incomplete margins are frequently encountered in second opinion practice which increases the risk of tumor recurrence over complete excision. Incomplete margins are associated with recurrence rates of up to 37% for both MCT and STS. The objective of this study was to describe clinical features of dogs undergoing scar revision for incompletely excised MCT and STS in the absence of gross disease and to determine the rate of tumor recurrence after scar revision. Records were searched for dogs undergoing scar revision for incompletely excised MCT and STS without evidence of gross disease at the scar. Retrospective medical record review was performed with information collected on signalment, tumor type/location, diagnostics prior to the first excision, first excision surgical information and pathologic findings, scar revision surgical information and pathologic findings, and follow-up. One-hundred and one scar revisions consisting of 66 MCT and 35 STS were included. Cytology was performed in 42.6% of dogs prior to the first excision. For the first excision, information on lateral and deep surgical margins was reported in 7.9% and 10.9% of dogs; on pathologic evaluation, lateral and deep margins were incomplete in 51.5% and 60.4% of dogs. For the scar revision procedure, information on lateral and deep surgical margins was reported in 83.2% and 90.1% of dogs; on pathologic evaluation, no evidence of tumor was identified in 63.6% and 77.1% for MCT and STS respectively. Tumor recurrence occurred in 3.0% of dogs; none had evidence of tumor in the scar revision pathology. Pre-surgical diagnostics should be used prior to the first excision to plan surgical resection. Surgical reports should include details on lateral and deep margins obtained to guide pathologic interpretation and future scar revision, if required. Scar revision in the absence of gross disease for incompletely excised MCT and STS significantly reduces the recurrence rate from historical reports.

## **A comparison of targeted-Selective and rotational treatment strategies for controlling parasites of horses**

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A common strategy for managing parasites in horses is rotational treatment (RT), where anthelmintics of different classes are administered to all horses in an alternating manner at frequent intervals. Another approach advocated by most equine parasitologists is targeted selective treatment (TST), where horses are dewormed on an individual basis; the decision to treat is based on factors pertaining to the host, parasite, and environment. This study sought to compare these two methods in a herd of horses. Two groups of adult horses were pastured separately and assigned to either RT (n=23) or TST (n=14). Fecal egg counts (FEC) were examined for all horses once monthly for 3.5 years. Horses in the RT group were dewormed every 2 months regardless of FEC results, alternating between pyrantel (PYR) and oxbendazole (OBZ). Horses in the TST group were treated individually with a combination of PYR + OBZ when FEC was  $\geq 200$  eggs per gram. Additionally, all horses were treated once annually with moxidectin (MOX) + praziquantel (PZQ). The effect of these dissimilar treatment frequencies on drug efficacy, egg shedding trends, and cost were evaluated. Excluding the annual MOX + PZQ treatment, the average horse in the TST and RT groups received 1.3 and 5.1 treatments per year, respectively. Despite the greater treatment frequency, RT horses did not have lower FEC. Additionally, more than 85% of FECs from low egg shedding TST horses remained low even without treatment, and no horses showed signs of parasitic disease anytime during the study. Interestingly, the efficacy of PYR and OBZ decreased in both groups similarly, despite the large difference in treatment frequency. These results suggest that TST provides optimal parasite control despite fewer treatments/horse/year, while also decreasing management costs and slowing the rate of development of anthelmintic resistance by encouraging proliferation of anthelmintic-susceptible subpopulations of parasites.

## **Defining vector and host factors predisposing to canine heartworm infection in dogs from communities east and west of the Panama Canal**

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Surveys on canine health were conducted on dog-owning households in the Panama Providence in June 2015 and June-July 2016. Samples include 278 canines belonging to 97 households from 6 communities each differing in forest cover; three to the east and three to the west of the Panama Canal. Veterinary health assessments were performed, and a database was created for each dog. Heartworm testing was performed using commercially available Dirochek Heartworm Antigen Test Kit. Our hypothesis was that heartworm infection rates in dogs, along with their subsequent associated negative impacts on dog health, would increase as a function of deforestation and are highest in deforested sites dominated by cattle pasture. Infection rates east of the Panama Canal were as follows; forested 6.7% (2/30), fragmented 5% (2/39) and deforested 43.5% (10/23) communities. Infection rates west of the Panama Canal were as follows; forested 30.9% (17/55), fragmented 14.8 % (8/54), deforested 0% (0/36) communities. We observed a significant difference between infection status and forestation gradients within communities found to the east and, also within communities to the west of the Canal (Pearson's Chi-squared test for east, X-squared = 19.015, df= 2, p-value = 7.428e-05)(Pearson's Chi-squared test for west, X-squared = 14.92, df = 2, p < 0.001). In regard to canine health assessment, infected dogs on average had a higher body condition score (Mann-Whitney U-Test, W=2925.5, p-value<0.05). There was also a significant difference between age and infection status (Mann-Whitney U-Test, W=2349, p-value=0.02). No association was found between gender and infection status. Of the dirofilarial positive dogs, 60% (18/30) were anemic, 30% (9/30) presented with a leukocytosis and 40% (12/30) presented with an eosinophilia. There was no association between infection status and any of the hematological markers. Performing this survey on canine health assessment and community forest cover allowed us to detect specific host and vector factors predisposing to canine heartworm infection. Ultimately, studying these factors helps to determine reservoir populations, target treatment, prophylactic options and testing for these communities.

## Review of kinematic analysis in dogs

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Objective gait analysis techniques aid investigators in quantifying motion to differentiate normal and abnormal gaits. Historically in veterinary medicine, there has been a great deal of emphasis placed on kinetic analysis (the study of forces occurring during motion) and it has proven to be a very useful objective analysis method for evaluating musculoskeletal conditions as well as therapeutic modalities. Kinematic analysis, alternatively, or the study of motion without forces, allows for the evaluation of joint-specific data such as range of motion, angular velocities, segmental velocities of each portion of the limb, stride frequency, and length. Recent advances in video technology have made the study of kinematics more accessible to investigators. Knowledge of the motions of joints enhances our understanding of limb function. Kinematic analysis can be performed in two-dimensions (2D) or three-dimensions (3D). Differences between these two methods includes equipment as well as mathematical modeling techniques—for example, differing ways to calculate joint angles to report range of motion. Correct use of kinematic analysis requires understanding of fundamental concepts underlying the methodology. The purpose of this report is to provide readers with an overview of kinematic analysis in veterinary patients, particularly the differences between two-dimensional and three-dimensional kinematic modalities.

## Association of equine abdominal fluid neutrophil counts with colic lesion, duration, and prognosis.

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Equine colic can be caused by many different types of lesions, each drastically impacting the course of treatment and prognosis. Identification of clinical markers associated with colic lesion, duration, management, progression, and treatment outcome could aid clinicians in more effectively diagnosing and treating colic patients. Previous studies have evaluated peripheral and abdominal lactate, heart rate, and peripheral white blood cell count as predictors of colic lesion, treatment, and outcome. However, there is little literature specifically analyzing peritoneal fluid neutrophil counts and their correlation to colic disease factors. As neutrophils are an established sign of acute inflammation, and can exert damaging effects within tissues, analyzing their quantity in peritoneal fluid could strengthen colic diagnosis and better guide treatment progression. In this retrospective study, the medical records of 446 colic horses from the past 10 years were analyzed. Peritoneal fluid white blood cell and neutrophil counts will be compared to type of lesion, duration of colic signs, development of complications such as ileus or SIRS following treatment, and treatment outcome. We hypothesize that peritoneal fluid neutrophil counts and percentages will be higher in horses with strangulating and inflammatory lesions, lesions with longer durations (>24 hours), and in cases that develop SIRS or ileus, necessitate surgical management, or require euthanasia due to disease severity or poor prognosis. Identification of such associations in colic patients may allow for earlier or better interventions, provide more information on what to expect during the course of treatment, and help render a more accurate prognosis.

## Pro-inflammatory and pruritogenic transcriptome of compound 48/80-mediated skin lesions in healthy dogs resembles spontaneous atopic dermatitis

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Recently, activation of Mas-related G-protein-coupled receptors (MRGPRs) has been identified to cause itch and IgE-independent mast cell and eosinophil degranulation. Compound 48/80 functions by activating mast cells via MRGPR member X2 (MRGPRX2) in humans and dogs. Compound 48/80 intradermal injections have been proposed histologically to resemble canine AD, but the activation of the associated inflammatory and pruritic pathways is unclear. This study's objectives were to characterize the molecular inflammatory and pruritogenic transcriptome of acute IgE-independent compound 48/80-induced skin lesions in eight healthy male castrated Beagles. All dogs were intradermally injected with compound 48/80 (10 micrograms/site) and buffered saline (diluent). Biopsies were collected at 6h and 24h after intradermal injections of compound 48/80; healthy (non-injected) and saline-injected skin served as controls. One half of the biopsy was used for histopathology, while the second half was used for RNA extraction and transcriptome analysis using RNA-sequencing. Intradermal injection of compound 48/80 resulted in positive wheal and erythema reactions on the injected thoracic side in all dogs. Histologically, there was an influx of inflammatory cells at 6h and 24h post-injection with marked tissue eosinophilia and a mixed infiltrate of neutrophils and lymphocytes. Acute compound 48/80-mediated lesions at 6h had significant upregulation of pro-inflammatory (e.g., IL1B, PTX3, CCL2, IL8), T helper-(Th)1 (e.g., IL12A, OASL, MX1, CXCL10) and Th2 (e.g., IL5, IL13, IL33, IL4R) genes. A phenotypically unbiased Gene Set Variation Analysis (GSVA) of compound 48/80-induced skin lesions revealed significant enrichment of Th1 and Th2 and Th1, Th2 and Th22 pathways at 6 and 24h, respectively. There was a significant upregulation of genes encoding known pruritogenic proteins and pathways, such as proteases cathepsin S (CTSS), CTSC, chymase, tryptase and mastin, nerve growth factor (NGF), leukotriene-B4 (LTB4) and histamine-synthesis enzyme and receptors (HDC, HNMT). Pathway analysis revealed strong significant upregulation of JAK-STAT, amphoterin and TREM1 signaling. Acute compound 48/80 lesions at 6h showed high homology with spontaneous canine (174/362 DEGs; Spearman's rho=0.6) and human AD transcriptome (1395/7889 DEGs; Spearman's rho=0.54). In conclusion, compound 48/80-mediated skin lesions exhibit a multipolar T-cell axis upregulation (Th1, Th2, and Th22) in healthy dogs, resembling canine, and human AD inflammation.

## Increased incidence of keratoconjunctivitis sicca in a mixed breed research colony.

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Over 20 dogs within a colony were diagnosed with Keratoconjunctivitis Sicca (KCS) over 5 years, with no previous history of KCS within that colony prior to this period. The signalment of dogs included: 3 intact female and 3 intact male hounds (weight range of 27-35 kg); 3 intact male, 6 castrated male, and 2 intact female beagles (weight range 9.1-14.2 kg) ; 3 intact female mixed breed dogs (weight range 10.3-11kg ). Schirmer tear test 1 values at diagnosis were in the following ranges in individual dogs: Hounds 2mm/min OS, 2 mm/min OD to 10 mm/min OS, 14 mm/min OD; Beagles 0 mm/min OU to 6 mm/min OS, 18 mm/min OD; Mixed breed dogs 0 mm/min OU to 7 mm/min OS, 10 m/min OD. The dogs were on various studies under multiple investigators and purchased from multiple vendors. A retrospective epidemiological analysis on records of the cleaning agents, methods of sanitization, housing conditions, housing positions within rooms, facility, vaccination history, room feeding logs and food purchase logs was performed to identify any possible factors that changed over the time period when the incidence of KCS occurred. We hypothesized that this environmental change may be linked to the incidence of KCS. No common factors were readily apparent with the exception of diet being switched within a few months of the first case of KCS being identified. In a subset of animals, further diet change for unrelated reasons, resulted in hounds having no further incidence of KCS over the following years, even in similar housing conditions with dogs where KCS was diagnosed. The diet was changed on the remaining colony as this was the only identified common link between all the dogs with the KCS diagnosis. In the following one and a half years, no further animals have been diagnosed with KCS in this colony. While this does not fulfill Koch's postulates for the cause of KCS, diet changes in other dog colonies may be prudent when higher than expected incidence of KCS is observed.

## Abundance and diversity of ticks in different habitat types within parks in Athens-Clarke County, Georgia

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Tick abundance and diversity data provide important information about tick-borne disease risks for people and pets. In Georgia, several tick species are vectors of pathogens of medical and veterinary concern. However, there have been few studies on ticks in Athens-Clarke County, GA, particularly within parks and natural areas where humans and pets may have contact with ticks. The objectives of this study were to 1) determine the number and diversity of ticks in parks/natural areas in Athens-Clarke County, 2) determine their phenology and habitat preferences, and 3) determine if species abundance or diversity are related to any spatial associations or habitat connectivity. From May–July 2020, we conducted over 1,000 tick drags in eight parks/natural areas to collect host-seeking ticks from the environment. Each tick drag was 100 meters long and 6 replicates of three different habitat types (field, edge, and forest) were sampled. We found two tick species: *Amblyomma americanum* (lone star tick) and *A. maculatum* (Gulf coast tick). Of note, we did not find the invasive tick *Haemaphysalis longicornis* (Asian longhorned tick) which was recently found in border counties of TN and NC. Nearly all ticks (97.5%, n=435) were *A. americanum* and remainder (2.5%, n=11) were *A. maculatum*. Although *A. americanum* were found in all sampled habitat types, *A. maculatum* were primarily found in grass/field habitats. These data are an important first step in educating the public on ticks in local community parks and where tick prevention strategies should be implemented to decrease the risk of tick-borne diseases.

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## Effect of antibiotic growth promoter administration on the intestinal microbiota and virus shedding post avian influenza virus infection in chickens

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Outbreaks of avian influenza virus (AIV) can have detrimental effects in poultry flocks resulting in mortality and indirect costs because of eradication efforts, control and loss of markets. The role of the microbiota in essential physiological processes and disease progression has expanded. Recently, chickens infected with low pathogenicity avian influenza virus (LPAIV) showed an intestinal microbiota population shift, with an increase in *Escherichia* and decrease in probiotic organisms like *Lactobacillus*. Further, similar studies reported that the absence of commensal gut microbiota in chickens increased virus shedding and compromised immune responses toward LPAIV infection. While it is shown that the intestinal microbiota affects AIV infection, it is not well known how altering the intestinal microbiota will affect AIV replication and shedding. Extensive literature reports showed that a variety of antibiotic growth promoters (AGPs) change the composition and diversity of the microbiota at different parts of the avian intestinal tract. While the literature is rich in the relationship between AGPs and the intestinal microbiota, little is known about the effect of AGP-induced microbiota changes on AIV shedding. Therefore, we explored the potential effect of a widely used AGP (Bacitracin methylene disalicylate (BMD)) on LPAIV shedding and weight gain in broiler chickens. Results showed that there were no significant differences in oropharyngeal virus shedding among groups; however, the BMD-fed group displayed higher virus shedding via the cloaca compared to the non-BMD group. Understanding the relationship between AGP induced microbiota changes and AIV infection will further increase our comprehension of the impact that the microbiota plays in AIV infection in poultry.

## Parainfluenza virus 5 (PIV5) expressing 3-cysteine-like proteinase and RNA-dependent RNA polymerase as novel vaccines against SARS-CoV-2

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The outbreak of COVID-19 has caused a pandemic that has spiked widespread concern and jeopardized global health security. The causative agent of COVID-19, SARS-CoV-2 (Severe Acute Respiratory Syndrome Coronavirus 2), is a newly emerged human coronavirus (HCoV) with similarities in the epidemiology, clinical features, and genetics to the SARS-CoV pandemic in 2003. This incident highlighted the importance of developing effective vaccine designs and antiviral therapies. Recently, our lab published on the generation of a PIV5-based (parainfluenza virus 5) MERS (Middle East Respiratory Syndrome) vaccine, indicating our PIV5-MERS-S as a promising vaccine candidate against MERS-CoV and potentially other emerging HCoVs, such as SARS-CoV-2. With this data, we aim to develop a PIV5-based vaccine for SARS-CoV-2 focusing on 3CL-pro (3-cysteine-like proteinase) and RNA-dependent RNA-polymerase (RdRp) of ORF1ab. Both 3CL-pro and RdRp are highly conserved among SARS CoVs and HCoVs. 3CL-pro is essential for the majority of the cleavage events during polyprotein processing and Nsp (nonstructural protein) maturation for betacoronaviruses. RdRp is the protein responsible for the formation of the replication and transcription complex, essential for viral genome replication. Furthermore, there is evidence to support the presence of T Cell epitopes in these enzymes, which have been linked to eliciting increased immune responses and long-term immunity to CoV infection when compared to B Cell responses. The high genetic and structural similarities and conservation, along with the potential to elicit T Cell responses, encourages the pursuit of engineering a PIV5-based SARS-CoV-2 vaccine expressing Nsp novel antigen targets.

## Nephrocystostomy as a novel ureteral bypass procedure in cats

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Ureteral obstruction is common in cats. Traditional ureteral surgery is technically demanding and associated with complications. Implantable subcutaneous ureteral bypass devices offer a palliative option and have recently become popular. While these devices have fewer acute complications, chronic complications including infection and encrustation are common. Identifying a novel technique to bypass the ureter which utilizes native tissues and is less technically demanding is a critical challenge in feline surgery. The objective of this study was to develop a novel nephrocystostomy (NCT) technique, as a ureteral bypass procedure, which employs native tissues and could be used clinically to treat cats with proximal ureteral obstructions. Nine adult, purpose-bred cats underwent 1 of 2 surgical techniques. All cats had the proximal ureter ligated on the operated kidney. In group 1 (n = 3), a NCT catheter was placed from the caudal pole of the kidney into the renal pelvis and the apex of the bladder was sutured around the catheter in 2 layers. In group 2 (n = 6), a 6 mm defect was made in the caudal pole of the kidney and the bladder mucosa was sutured into the renal pelvis to create the NCT. All cats had the NCT catheter in place for at least 45 days prior to a second surgery to remove the catheter. Cats were evaluated for 30 days after catheter removal by serum creatinine levels, ultrasound, CT and renal histopathology. With the catheter in place, all cats maintained a patent NCT. After catheter removal, all cats in group 1 developed obstruction of the NCT and hydronephrosis within 7 days, whereas all cats in group 2 had patent NCT's at 30 days. Abdominal CT scans of the cats in group 2 revealed evidence of renal degeneration in the caudal pole which was confirmed on histopathology; the cranial pole appeared normal. NCT is a viable procedure in cats and incorporation of the bladder mucosa into the NCT defect dramatically improved patency up to 30 days. The cause of degenerative changes in the caudal pole is unknown but may be related to vascular impairment from the bladder mucosa sutures.

## Identifying Genes of the Shikimate, Isoprenoid & Ubiquinone Pathways from *T. gondii*: Current and Novel Drug Targets

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The protozoan parasite, *T. gondii*, is one of the leading causes of foodborne illness in humans and one of five neglected parasitic infections in the US. To date, no efficacious chemotherapeutic treatments exist against the entire spectrum of its life stages. The pathways leading to the production of ubiquinone appear to be divergent between humans and *T. gondii* and harbour known and potentially novel targets for therapeutics. In outlining these pathways, this review seeks to determine and organise the genes predicted to express the enzymes from the shikimate, polyprenoid and ubiquinone biosynthetic pathways. We analysed the literature describing homologous pathways in humans, yeast and other apicomplexans including *T. gondii* in addition to analyzing -omic data for *T. gondii*. The aim is to postulate a roadmap of the biosynthetic pathways involved in producing ubiquinone. Using these methods, it has been possible to outline similarities and differences between *T. gondii* and other eukaryotes in an effort to identify areas where further research is needed to fill in knowledge gaps. Of particular interest is the pentafunctional AROM complex involved in the shikimate pathway and the *T. gondii* homologue of Coq2. The ultimate goal of investigating these pathways in *T. gondii* is to identify genes that may serve as novel drug targets in the search for effective treatment of toxoplasmosis.

## Pathologic cardiovascular lesions associated with experimental anti-fibrotic therapies

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Currently therapeutic options for fibrotic diseases such as liver cirrhosis and idiopathic pulmonary fibrosis are limited. Tadalafil, a phosphodiesterase 5 inhibitor prescribed for erectile dysfunction with additional anti-fibrotic properties, and the investigational anti-fibrotic drug IPW-5371 were examined for therapeutic fibrosis therapy in an irradiated mouse model. The combination of these agents unexpectedly resulted in increased mortality in C57 B6/N male mice, but not female mice. This incidental finding indicates the lack of information regarding the possible relative contraindications between these two drugs in both mouse and human models. Death appeared to result from gross hemorrhage into body cavities and this study aimed to evaluate the pathological findings relating to these deaths. We investigated the effects of tadalafil and IPW-5371 administration on the cardiac and left ventricular outflow tract tissue in 134 irradiated C57 B6/N male and female mice. Tissues were collected, fixed, and sectioned using hematoxylin and eosin and Masson's trichrome stains in serial sections. These slides were then evaluated and scored by lesion frequency. We hypothesized that an increase in inflammation, hemorrhage, degeneration, and erosion would be noted more frequently in mice that had the combination treatment compared to other mice. In total, 61 6-month-old male mice were statistically analyzed. Mice that were administered drugs had significantly higher frequencies of pathologic lesions than mice that were not administered drugs. In particular, mice that were given IPW-5371 either alone or in combination with tadalafil had higher frequencies of pathologic scores than mice that were not administered the drug. Tadalafil alone was not found to be a large driver of the frequency of pathologic lesions. Irradiation did not have any significant effects on the frequency of lesions. These findings indicate that subclinical lesions do exist in mice administered this drug combination at a greater frequency than mice that were not administered these drugs. The prescription of this drug combination in humans should be greatly reconsidered, and patients currently taking anti-fibrotic agents should be closely monitored for pathologic cardiovascular changes.



## Fish host susceptibility influences myxozoan community composition of Proliferative Gill Disease in catfish aquaculture

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The myxozoan *Henneguya ictaluri* is the cause of proliferative gill disease (PGD), an important parasitic disease of US farm-raised channel (*Ictalurus punctatus*) and channel (*I. punctatus*) × blue (*Ictalurus furcatus*) hybrid catfish. Research indicates arrested sporogenesis occurs in hybrid catfish, yet PGD persists in hybrid catfish production systems, suggesting other myxozoans besides *H. ictaluri* may be associated with PGD. Further, it is hypothesized host susceptibility drives myxozoan diversity in catfish pond aquaculture systems. This work investigated the influence of catfish host on myxozoan community composition within 1) naturally infected gill tissues from clinical PGD cases and 2) pond water associated with channel and hybrid catfish monoculture. For three years, DNA extracted from gills of diagnostic case submissions with clinical PGD, as well as water from experimental ponds dedicated to either channel or hybrid catfish monoculture, were analyzed by metagenomic sequencing to compare myxozoan community composition and diversity between catfish species and assess year-over-year trends. Myxozoan community composition significantly differed between channel and hybrid systems in both gill and pond water datasets for all three years examined. Channel catfish gills had greater relative abundance of *H. ictaluri* in 2017 and 2019 and unclassified taxa in 2018 compared to hybrid catfish. *H. ictaluri* was present in all channel and hybrid catfish PGD cases, although in nearly half of these clinical PGD cases *H. ictaluri* was not the most abundant myxozoan. In the pond experiment, *H. ictaluri* relative abundance was significantly greater in channel catfish ponds in years 2 & 3. In hybrid catfish ponds, *H. ictaluri* never exceeded 20% average relative abundance. Both datasets revealed hybrid catfish monoculture can selectively lower *H. ictaluri* proliferation. This work suggests crop rotation strategies could mitigate disease by preventing *H. ictaluri* from reaching levels associated with catastrophic losses. Further, detection of numerous known and unclassified myxozoan sequences provides evidence PGD may involve mixed species infections and indicates the number of described species vastly underestimates the true myxozoan diversity associated with catfish aquaculture. Future work will investigate the potential contribution of other myxozoans to gill pathology in PGD outbreaks in channel and hybrid catfish production systems.

## Development of a molecular genetic key for the identification of *Haemaphysalis* ticks in North America

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In public and veterinary health, the accurate identification of tick species is critical to the detection and control of associated tick-borne diseases. For ticks in the family Ixodidae, differentiating species is based on mouthpart morphology; however, these features are often damaged during tick collection from hosts, making morphological identification difficult or impossible. The Asian longhorned tick, *Haemaphysalis longicornis* (Acari: Ixodidae), is a tick of recent One Health importance to the U.S. Native to East Asia, the Asian longhorned tick has become invasive in multiple regions of the world, including the U.S., and is a known vector of a wide variety of pathogens. In order to monitor and control the spread of *H. longicornis*, we explored molecular techniques for accurate identification and differentiation between the morphologically similar species within the *Haemaphysalis* genus that are native to North America. To do this, we developed a restriction fragment length polymorphism (RFLP) assay utilizing both the 16S ribosomal RNA and the cytochrome *c* oxidase subunit I (*COI*) gene sequences to differentiate between the invasive and native *Haemaphysalis* spp. We found that genetically similar species of *Haemaphysalis* have unique RFLP cutting patterns, in both the 16S and *COI* sequences. These results reveal a rapid and cost-effective method for identifying the presence of this invasive tick, even if specimens are damaged. Furthermore, this RFLP assay can be applied to *Haemaphysalis* species endemic to other regions of the world for the rapid identification for most species tested to date. The work presented in this study serves as the foundation for region specific PCR-RFLP keys for *Haemaphysalis* species can likely be further applied to other morphometrically challenging taxa.

## ***Bordetella pseudohinzii*'s Pertussis Toxin-Like Genes Contribute to Persistence in Otitis Media Infections**

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Infections causing otitis media (OM) are a major health concern in young children that can lead to multiple doctor visits and prescriptions for antimicrobials. *Bordetella bronchiseptica* and *Bordetella pseudohinzii* are murine pathogens that mimic the natural infection process of OM in humans; *B. bronchiseptica* causes acute infections and *B. pseudohinzii* causing chronic infections. Here, the genome sequences of *B. bronchiseptica* and *B. pseudohinzii* were compared for dissimilarities in the sequence to identify putative candidate genes contributing to the persistence of *B. pseudohinzii* in OM based on a similarity score (H-value) ranging from 0-1. Guided by previously studied virulence factors of the Bordetellae species, we identified four genes that appear to encode the ability to assemble a pertussis toxin-like toxin. Subunit A (psxA) is the catalytic domain and had a higher similarity score (H= 0.5), while subunits BC, D, and E had a lower similarity to *B. bronchiseptica* genes. *Bordetella pertussis* produces and secretes pertussis toxins, which contribute to the colonization and pathogenesis of the disease whooping cough through immune modulation. We hypothesize that in the case of *B. pseudohinzii*, the pertussis toxin-like genes will contribute to its persistence in OM. In vivo experiments showed that when the psxA gene is knocked out, *B. pseudohinzii* is no longer able to persist at high levels in the lungs, trachea, and middle ears, compared to wild type *B. pseudohinzii*. This gene analysis is a starting point for future studies analyzing the function of the pertussis toxin-like genes in *B. pseudohinzii* and the role these genes may play in immune evasion, resulting in persistent OM.

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## **Effect of meperidine on equine blood histamine, tryptase, and immunoglobulin-E concentrations**

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Meperidine has been reported to cause adverse effects related to histamine release including wheals, ataxia, and hypotension in horses. The objective of this study was to determine the effect of meperidine on plasma histamine, tryptase, and immunoglobulin E (IgE) levels in horses. Six healthy adult horses, weighing  $494 \pm 33$  kg, were enrolled in a prospective randomized crossover design study. Each treatment consisted of a subcutaneous (SC) and intramuscular (IM) injection, which included meperidine 1 mg kg<sup>-1</sup> SC with saline 6 mL IM (MSC), meperidine 1 mg kg<sup>-1</sup> IM with saline 6 mL SC (MIM), saline 6 mL SC with saline 6 mL IM (P). Each horse received all treatments with a seven day washout period. Blood was obtained for plasma histamine analysis and serum IgE at baseline (0), 5, 10, 15, 30, and 60 minutes and tryptase at baseline (0), 15, 30, 60, 120, and 240 minutes. Histamine was evaluated with liquid chromatography-tandem mass spectrometry. Tryptase and IgE concentrations were evaluated with enzyme-linked immunosorbent assays. All variables were evaluated with a mixed-effect ANOVA with alpha set at 0.05. Neither SC nor IM administration of meperidine caused a statistically significant increase in histamine ( $p > 0.06$ ), tryptase ( $p = 0.296$ ), or IgE ( $p = 0.336$ ) from baseline. There were no differences between treatment groups ( $p > 0.486$ ). An immune response in the MSC or MIM group was not observed in this study as measured by histamine, tryptase, or IgE.

## Pharmacokinetics and pharmacodynamics of meperidine in healthy horses

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The pharmacokinetics and anti-nociceptive effects of subcutaneous (SC) and intramuscular (IM) meperidine in horses has not been well described. Six adult horses, weighing  $494 \pm 33$  kg, were enrolled in a prospective randomized crossover blinded design study. Three treatment protocols were used, each consisting of a SC and IM injection described as follows: meperidine 1 mg kg<sup>-1</sup> SC with saline 6 mL IM (MSC), meperidine 1 mg kg<sup>-1</sup> IM with saline 6 mL SC (MIM), saline 6 mL SC and 6 mL IM (P), with a seven day washout between treatments. Plasma meperidine concentrations and pharmacodynamic data (thermal and mechanical thresholds, vital parameters) were collected at various time points for 24 hours. Accelerometry data was obtained for 8 hours to measure locomotor activity. Data were analyzed using a mixed effects model. Alpha was set at 0.05. Meperidine terminal half-life ( $t_{1/2}$ ), maximal plasma concentrations, and time to maximal concentration ( $T_{max}$ ) were  $164 \pm 56$  minutes and  $186 \pm 59$ ,  $243.1 \pm 80.1$  and  $265.7 \pm 47.2$  ng mL<sup>-1</sup> at  $24 \pm 13$  and  $17 \pm 6$  minutes for MSC and MIM, respectively. No effect of treatment or time was observed on thermal or mechanical thresholds, heart rate, respiratory rate, locomotor activity, frequency of urinations or defecations, and fecal weight ( $p > 0.2$  for all variables). Both SC and IM administration of meperidine exhibited rapid absorption with a short  $t_{1/2}$  and  $T_{max}$ . Neither SC nor IM meperidine administration increased thermal or mechanical thresholds nor caused any changes in vital parameters.

## Characterization of the serum cytokine profile in feline non-flea hypersensitivity dermatitis

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Feline non-flea hypersensitivity dermatitis (HD) is a pruritic and inflammatory skin disease commonly encountered in cats. Investigations of the pathogenesis of feline non-flea HD have been limited to histopathologic cell-type evaluations of skin lesions, with only two reports evaluating two serum cytokine levels. In the present study, we evaluated 13 cytokine and chemokines from the serum of 14 client-owned cats diagnosed with non-flea HD and 12 healthy cats using a commercially available feline-specific multiplex assay. We hypothesized that the serum cytokine profile in feline non-flea HD would demonstrate significant upregulation of the T helper (Th)-2 cytokines interleukin (IL)-4 and IL-13 compared to the healthy controls. Cats were diagnosed based on previously published criteria for feline non-flea HD. The two groups were compared using a one-tailed Mann-Whitney-U test where  $P < 0.05$  was considered significant. Patients with feline non-flea HD had a significant increase in serum T helper (Th) 1 (interferon gamma (IFN $\gamma$ ), IL-2, IL-18) and Th2 (IL-13) concentrations. In addition, chemokines involved in inflammation and chemotaxis (CCL5, CCL2 and CXCL12), as well as the growth factors, stem cell factor (SCF) and fms-related tyrosine kinase 3 ligand (Flt3L), were also significantly increased. To investigate whether the extent of feline HD skin disease influenced the cytokine and chemokine concentrations in affected patients, we correlated serum markers with the clinical feline HD measures Scoring Feline Allergic Dermatitis (SCORFAD) and the pruritus Visual Analog Scale. The growth factor Flt3L showed a significant positive correlation with SCORFAD. This is the first study to evaluate a broad array of serum secretory immune markers in non-flea HD cats. These markers are largely associated with a mixed Th1 and Th2 inflammatory response, which has also been reported in human and canine patients with atopic dermatitis. Further studies are needed that compare the disease-associated lesional skin immunologic markers to the serum markers as well as assess whether such serum biomarkers can be modulated by pharmacological/therapeutic interventions.

## Effects of oral RRR- $\alpha$ -tocopherol (vitamin E) on plasma oxidative stress and endocrine markers in healthy horses

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Equine Pituitary Pars Intermedia Dysfunction (PPID) is a common, progressive, neurodegenerative disorder resulting from oxidative damage to the hypothalamus. Currently, PPID therapy is limited to the dopamine agonist pergolide mesylate, which does not stop ongoing oxidative damage. Anti-oxidant therapy with RRR- $\alpha$ -tocopherol (vitamin E) might slow PPID progression if it can reduce systemic oxidative stress. The objective of this study was to assess the effect of two RRR- $\alpha$ -tocopherol formulations on plasma oxidative stress and endocrine markers in healthy horses. We hypothesized that oral administration of RRR- $\alpha$ -tocopherol alters basal plasma oxidative stress and endocrine markers in healthy horses, and the degree of this effect varies between formulations. Ten healthy horses received 5000 IU (~10 IU/kg) of RRR- $\alpha$ -tocopherol acetate powder or water-dispersible liquid, or placebo orally once daily for 21 days in a randomized crossover design. Horses underwent a  $\geq 28$ -day washout between treatments. Blood was collected at baseline and on days 3, 7, 14 and 21 of treatment for measurement of plasma oxidative stress markers and at baseline and day 21 for assessment of endocrine parameters. Plasma reactive oxygen metabolites (dROMs) and plasma antioxidant capacity (PAC) were quantified using a validated photometric assay. Plasma total cortisol, adrenocorticotrophic hormone (ACTH), and leptin concentrations were measured with validated immunoassays. Oxidative data was analyzed using a repeated measures ANOVA or a Friedman Test as appropriate to compare days 1, 3, 7, 14, and 21 within each treatment. Endocrine data was analyzed using a paired t-test or a Wilcoxon test. Statistical significance was set at  $P < 0.05$ . Endocrine parameters did not differ significantly between days 1 and 21 in any of the three treatment groups. There was also no effect of RRR- $\alpha$ -tocopherol treatment on dROMS or PAC. This RRR- $\alpha$ -tocopherol dosing regimen did not alter oxidative stress or endocrine parameters in healthy horses. Previous research suggests this dosing regimen is sufficient to significantly increase plasma concentrations of RRR- $\alpha$ -tocopherol, so it is unlikely that a larger dose or longer course would change outcomes. However, further study is needed to assess if treatment could have an effect on animals with deranged hormones or high levels of oxidative stress.

## Detection of *Atp6v0d2* and *Mcoln1* mRNAs in the mouse female reproductive system using *in situ* hybridization

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The lysosome is the most acidic organelle in the cell. Lysosomal acidity is primarily maintained by Vacuolar-type H<sup>+</sup>-ATPase (V-ATPase) and counter ion channels (e.g., Transient receptor potential cation channel, Mucolipin subfamily1 (TRPML1, encoded by gene *Mcoln1*)). V-ATPase is comprised of 14 different subunits, and gene *Atp6v0d2* encodes one tissue-specific V0d2 subunit for V-ATPase. *Atp6v0d2* is dramatically upregulated in the uterine luminal epithelium upon embryo implantation initiation. Embryo implantation is the critical first embryo-maternal interaction for successful pregnancy in mammals. Our *in vivo* study in *Mcoln1*<sup>-/-</sup> female mice shows reduced fertility at 2 months old (2M) and embryo implantation failure, leading to infertility at 5M. Embryo implantation failure is accelerated by progesterone deficiency, which is caused by degenerating corpus luteum, the main site for progesterone synthesis during embryo implantation. On the other hand, *Atp6v0d2*<sup>-/-</sup> female mice have overall normal fertility. Since both channels regulate lysosomal lumen pH in opposite directions, we hypothesize that the deletion of both *Atp6v0d2* and *Mcoln1* could potentially alleviate the adverse effects of TRPML1 deficiency in cells that have co-expression of both genes. One critical step for testing this hypothesis is to demonstrate the co-localization of their respective proteins, ATP6V0d2 and TRPML1, in the cells. Due to the lack of effective antibodies for immuno-colocalization, we turned to an alternative strategy: *in-situ* hybridization (ISH), which detects mRNAs in the cell. ISH involves cRNA probe generation, hybridization, and detection. The tissue positive controls are from the post-coitus day 4.5 uterus and intestine of wild-type mice, where the genes are normally highly expressed; the tissue negative controls are from *Mcoln1*<sup>-/-</sup> and *Atp6v0d2*<sup>-/-</sup> counterparts. Both sense and anti-sense probes are generated to confirm the signal specificity. I will share my experience with the ISH process, involving sectioning of frozen mouse tissues, designing optimal cRNA probes, and optimizing the signal development process. The improvements of optimizing the probe template concentration, modifying the rate-limiting steps in cRNA probe synthesis, and heating the tissue sections to preserve RNAs before hybridization all contributed to successful results of strong signaling. Our study will fill in the knowledge gap about functions of lysosomal channels in female reproduction.

# Temporal and spatial patterns in Canine Distemper Virus cases in wildlife diagnosed at the Southeastern Cooperative Wildlife Disease Study (SCWDS), 1975-2019

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Canine distemper (CDV) is an important infectious disease that can affect many mammal species, particularly carnivores, and is responsible for substantial carnivore population declines. The primary objective of this study was to identify long-term spatial and temporal patterns in CDV cases in wildlife diagnosed at the Southeastern Cooperative Wildlife Disease Study (SCWDS). Additionally, we aimed to identify how outbreaks in different species may be related, as raccoons are thought to be the primary wild reservoir. Finally, we aimed to identify spatial patterns of infections with potential associations with human activity. Analysis was conducted on passive surveillance diagnostic data of wild mammals diagnosed with CDV that were submitted to SCWDS between January 1975 and December 2019. Overall, 964 cases were submitted to SCWDS from 17 states that were identified as CDV positive, comprising raccoons (n=646), gray foxes (n=254), striped skunks (n=33), coyotes (n=18), red foxes (n=4), gray wolves (n=3), black bears (n=3), two mink, and one long-tailed weasel. Raccoon and gray fox case data from the state of Georgia (n=441) were selected for further analysis based on these forming the considerably greater part of the data set. An autoregressive integrated moving average model used the numbers of gray fox CDV cases from the previous two months and of raccoon cases in the current month to predict the numbers of gray fox cases in the current month. There were temporal trends in CDV cases for both species, with cases more likely to occur during the breeding season. Cases were more likely to occur in areas of medium to high human population density. This pattern was most prominent for raccoons, which may correspond to high transmission rates in suburban areas, where population densities are highest, possibly due to a combination of suitable habitat and supplemental resources. There are enough potential relationships suggested from this passively collected data which would support more comprehensive sampling of wild carnivores over time and space to try to elucidate spillover trends between species, particularly in suburban areas. This could inform decision making regarding vaccination and other strategies aimed at reducing transmission among both domestic and wild animals.

## **In vitro modeling of clinical dosing regimens of Adequan® in Chilean flamingoes (*Phoenicopterus chilensis*) for assessment for potential anticoagulant effect**

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Adequan® is the only parenteral polysulfated glycosaminoglycan (PSGAG) FDA-approved for degenerative joint disease management in veterinary species. Its active glycosaminoglycan is chondroitin sulfate, which has a chemical structure similar to heparin, potentially conferring anticoagulant properties. While used extra-label for many species, case reports have described hemorrhagic diathesis in treated birds at high doses. It is hypothesized that hypocoagulation, evaluated by changes to thrombin clotting time (TCT), would be associated with maximal systemic plasma concentrations achieved by doses of 10 mg/kg, with dosages below this showing no effects.

For this *in vitro* study, citrated plasma samples were opportunistically collected from 42 Chilean flamingoes (*Phoenicopterus chilensis*) and baseline coagulation was assessed via TCT and fibrinogen assays. Repeated TCT analysis was performed on plasma aliquots from each bird containing the maximum plasma concentration expected to be achieved from one of three Adequan® doses (1mg/kg; 5mg/kg; 10mg/kg). These doses were chosen as the dose at the housing institution considered clinically effective for avian species, the on-label canine dose, and the dose associated with published cases of hemorrhagic diathesis in avian species, respectively. Due to inherent variability in coagulation values among birds, each subject's treated samples were compared with its own baseline values to determine statistically significant differences. Results support clinical observations that Adequan® at 1 mg/kg does not have an anticoagulant effect in Chilean flamingoes and can be used safely with appropriate clinical judgement, while doses of 5 mg/kg and greater are associated with hypocoagulation and should not be used in avian species.



# Reconstructing the evolutionary history and global spread of Leptospirosis using genomic data: preliminary results

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Leptospirosis, caused by the spirochete genus *Leptospira*, is one of the most prevalent zoonotic diseases in the world resulting in significant morbidity and mortality. Nevertheless, factors leading to the emergence and transmission dynamics of the pathogen remain unknown. We aim to reconstruct the evolutionary history and global spread of *Leptospira* strains using genomic and spatiotemporal data to better understand the pattern of emergence and transmission of this pathogen.

We collected over 388 Whole-Genome Sequenced (WGS) *Leptospira* isolates from both the National Center for Biotechnology Information (NCBI)'s Sequence Read Archive (SRA) and Assembly databases. This dataset includes genomes from 52 species of *Leptospira*, covering 35 unique regions/countries from all the 7 Global Burden of Disease (GBD) regions within a collection period of 102 years (1915 -2017). To reach the same level of completion for all genomes, we assembled the 36 SRA collected genomes with SPAdes. We also extracted metadata of these isolates from both the NCBI and the Pathosystems Resource Integration Center (PATRIC) databases. To avoid high rates of horizontal gene transfer (HGT), gene duplication, and gene reduction events biasing the result of Maximum Likelihood (ML) phylogenetic relationship inference, we used PIRATE to identify and extract the isolates' core genome, which is composed by the alignment and concatenation of the core genes. We then used the ML tree estimated by IQtree to explore the temporal signal of the dataset with Tempest, as well as *Leptospira*'s evolutionary rate.

Our analyses identified 759 core genes that were conserved by 95% of all collected isolates. The maximized correlation coefficient between the root-to-tip divergence of the isolates based on the ML tree and the collection dates was estimated to be 0.4283, which unravels the temporal signal within our dataset, and the evolutionary rate was estimated to be 1.209E-2 substitution/site/year. These results provide the foundation for our future analysis, which will be the reconstruction of the evolutionary history and global spread of *Leptospira* using phylodynamic approaches.

We expect that our studies will provide insights into *Leptospira*'s evolution and transmission characteristics, which are essential guidelines for future disease control and prevention.

# **Intrinsic traits and extrinsic factors predictive of RNA viral spillover between wildlife and livestock**

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As environmental change brings wild and domestic animals in increasingly close contact, disease spillover at the wildlife-livestock interface is an emerging threat to food production systems. This study aims to identify critical extrinsic factors and intrinsic traits of host-viral interactions which are predictive of viral spillover between wildlife and livestock. Following the PRISMA 2009 systemic review guide, we searched PubMed using designed search keywords to retrieve RNA viral spillover and relevant topics published between 1968 and 2020. Titles and abstracts of articles were screened by the metagear package in R. Article content was examined, with intrinsic traits and extrinsic factors identified in each spillover event. In total, we retrieved 1,950 literature records, and among those 266 (13.6%) were classified as relevant, 670 (34.4%) were potentially relevant and 1,014 (52.0%) were non-relevant. To date, we examined 98 relevant literature and obtained 106 recorded RNA viral spillover events. Our preliminary results showed 25 RNA virus species, including 94 (88.7%) single-stranded, 85 (80.2%) segmented, 62 (58.5%) enveloped, 60 (56.6%) narrow host range and 34 (32.1%) zoonotic as important intrinsic traits associated with spillover events. Key extrinsic factors identified include, 62 (58.5%) livestock- and domestic-wildlife interface, 50 (47.2%) local livestock production, 30 (28.3%) reservoir epidemic, 23 (21.7%) incomplete vaccination coverage and 19 (17.9%) human-animal contact. The host range of viral pathogens that can spillover from wildlife to domestic livestock is determined by viral and host ecology, genetic traits, and pathogenesis within a host. Preliminary results show that livestock management system scale, host source epidemic and frequency of animal contact appear to be critical determinants of spillover likelihood.

# The effect of sampling bias in genomic surveillance

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Genomic epidemiology of emerging viruses has proven to be a useful tool for outbreak investigation, for tracking virus evolution and spread, and informing control measures. However, spatial and temporal sampling biases and existence of infection clusters during the epidemic spread, direct applications of existing approaches can lead to biased parameter estimations and data misinterpretation.

In this study, we illustrate extensive sampling biases in pandemic scenarios, using the severe acute respiratory syndrome coronavirus 2 (SARS-Cov-2) pandemic as a model system. To address this issue, we propose a new subsampling methodology that explicitly accounts for spatiotemporal sampling heterogeneity between confirmed cases and available genomes.

SARS-Cov-2 sequences (n=17,440) from the all US states and 5 of its territories, as of August 11th, 2020, were downloaded from GISAID (Global Initiative on Sharing All Influenza Data). Sequences missing accurate collection date and location were removed. Genomes were aligned using MAFFT v7.453 and variants recalled using Nextclade. We examined relationships between the total number of mutations and available sequences per state. To further characterize sampling heterogeneity, the spatiotemporal distributions of confirmed cases and available sequences were compared. We developed a subsampling methodology, with weight proportional to the number of cases confirmed in the month.

We found a striking positive linear correlation with  $p < 2.2e-16$  and  $r = 0.995$  between SARS-Cov-2 genetic diversity and sequence availability. Overall, most states experience an exponential growth in confirmed case numbers. However, genomic surveillance efforts did not reflect the increased incidence.

Our findings illustrate that taking into consideration the imbalanced relationship between confirmed cases and available viral genomes across time is paramount to accurately measure regional genetic diversity. For future analysis, we will replicate several hallmark SARS-Cov-2 phylogenetic studies by using our subsampling scheme and compare the results. If limited viral diversity were not detected as demonstrated, previous results should be carefully validated and conclusions adjusted.