

College of Veterinary Medicine
Kansas State University
presents

Phi Zeta Research Day



A.D. 1925

March 2, 2021





The Society of Phi Zeta

The Honor Society of Veterinary Medicine

History of Phi Zeta

Phi Zeta was originated in 1925 by a group of senior veterinary students in the New York State Veterinary College at Cornell University. With the assistance of a group of faculty members, including the Dean of the College, Dr. Veranus A. Moore, the Society was formally organized, and Dean Moore was elected as the first president of the Alpha Chapter. The **Society of Phi Zeta** was organized in 1929 at a meeting in Detroit, Michigan, and Dean Moore became the first president of the Society.

Also in 1929, a charter was granted to the School of Veterinary Medicine at the University of Pennsylvania, and the Beta Chapter was established. In 1931, the Executive Committee approved the petition of a group from Iowa State College, and the Gamma Chapter was established. Since then twenty-four chapters have been chartered, bringing the total number of chapters to twenty-seven. Chapters of the Society may be formed at any recognized veterinary medical college or at any other institution of higher learning.

From its beginning, it has been the aim of Phi Zeta to stand for constant advancement of the veterinary profession, for higher educational requirements, and for high scholarship. As stated in the Constitution, the **Object of the Society** shall be to **recognize and promote scholarship and research in matters pertaining to the welfare and diseases of animals.**

Selection of Membership

Membership in the Society consists of two classifications, Active and Honorary. Those eligible to election as **Active Members** are:

- A. Any candidate for the DVM/VMD degree in a veterinary medical college where a chapter exists, and who has completed at least two years of the professional curriculum, and who meets the following requirements:
 - 1. The candidate must have an acceptable personality, be of good moral character, and possess high ideals regarding professional service conduct.
 - 2. When elected in the junior or third year, students must rank scholastically in the highest 10% of their veterinary medical class.
 - 3. When elected in the senior or fourth year, students must rank scholastically in the highest 25% of their veterinary medical class.
- B. Any veterinarian who has been in possession of a veterinary medical degree for at least two years, and who has displayed ability of high order in dealing with one or more phases of the science of veterinary medicine, and who meets one of the following criteria.

1. The candidate is enrolled as a graduate student in a college of veterinary medicine and has completed at least twenty semester (thirty quarter) hours of graduate credit or has successfully passed preliminary examinations.
2. The candidate has been engaged in an intern or residency program for at least two years or has become board certified in his/her specialty.
3. The candidate has completed two years or more on the faculty of the institution or scientific staff of a scientific institution within commuting distance of the nearest chapter of Phi Zeta and has been involved in veterinary research or service.

Those eligible to election as **Honorary Members** are:

- A. Distinguished veterinarians in possession of their veterinary medical degrees for at least five years and who have rendered notable service to their profession.
- B. Persons not in possession of the veterinary medical degree, who have rendered distinguished service in the advancement of the science relating to the animal industry and particularly of animal diseases.
- C. Only in exceptional instances shall more than two honorary members be elected by any one chapter in any one academic year.

Active members who move from the residence of their chapter may: **1** become known as **inactive members** and not subject to the payment of dues; or **2 transfer** their membership to another chapter.

Name and Symbols of the Society

The organizers of the Society, when seeking a suitable name, sought the help of a learned Greek scholar, **Professor George P. Bristol** of Cornell University. Professor Bristol suggested a Greek word, which in the Latin form is spelled **PHILOZOI** and means "love for animals." The abbreviation of Phi Zeta was adopted as the name of the society. The emblem consists of a pendant formed by the letter Phi superimposed by the letter Zeta. The design was the work of **Louis Agassiz Fuertes**, the great naturalist and artist.

The **Executive Committee**, consisting of the president, president-elect, secretary-treasurer, and the three most past-presidents, oversees and promotes the objectives of Phi Zeta through activities of the various chapters. **Meetings** of the Society of Phi Zeta are held annually in conjunction with the AVMA Convention. All members of the Society are invited to attend these meetings.

Each year the Society sponsors two **Research Awards** and helps fund lectures at various Chapters. Chapters recognize and promote high scholarship and research through an annual initiation ceremony, by sponsoring research days, and by inviting outstanding lecturers to speak on topics relevant to veterinary medicine and the welfare of animals.

Chapters of Phi Zeta

Alpha	Cornell University	1925
Beta	University of Pennsylvania	1929
Gamma	Iowa State University	1931
Delta	The Ohio State University	1934
Epsilon	Auburn University	1948
Zeta.....	Michigan State University.....	1950
Eta	Texas A&M University	1950
Theta.....	Colorado State University	1950
Iota.....	Washington State University.....	1952
Kappa	University of Minnesota.....	1952
Lambda.....	University of California	1953
Mu	University of Illinois	1953
Nu.....	Oklahoma State University	1958
Xi.....	University of Georgia.....	1959
Omicron.....	Purdue University	1962
Pi	University of Missouri	1965
Rho	Tuskegee University.....	1967
Sigma	Kansas State University	1969
Tau.....	Louisiana State University	1977
Upsilon	University of Florida.....	1979
Phi	University of Tennessee.....	1979
Chi	Virginia-Maryland Regional CVM.....	1984
Psi.....	North Carolina State University.....	1984
Alpha Alpha	University of Wisconsin.....	1987
Alpha Gamma	Oregon State University	1987
Omega	Mississippi State University.....	1988
Alpha Beta.....	Tufts University	1991
Alpha Delta	St. George University.....	2006
Alpha Epsilon.....	Western University of Health Sciences.....	2006
Alpha Zeta	Ross University	2014
Alpha Eta.....	Midwestern University.....	2017
Alpha Theta.....	Lincoln Memorial University.....	2018

Each year the Phi Zeta Research Day keynote address is sponsored by a generous endowment by Kenneth D. Olson and his family.

Kenneth Dell Olson was born August 18, 1919, in Frankfort, Kansas, the son of Dell and Gunhild Olson. He graduated from Frankfort High School, then attended Brown Mackie Business College in Salina and Kansas State University. He went on to become a respected and very successful Kansas businessman, and was a loyal K-State Alumnus and member of the President's Club. In 1941, Kenneth married Marjorie Spiller, who was also a K-State graduate. Their son, Dr. Gary Olson graduated from Kansas State University's College of Veterinary Medicine in 1972. He spent two years in Elkhart, Indiana, before moving back to Lawrence in 1974 to establish the Clinton Parkway Animal Hospital. Dr. Olson has been a board member of the Kansas City Veterinary Medical Association, the Kansas Veterinary Medical Association, President of the Douglas County Veterinary Association, and area director for the American Animal Hospital Association.

This year's invited keynote speaker is Dr. Molly McCue.



Dr. McCue is a professor at the Department of Veterinary Population Medicine and the Associate Dean of Research in the College of Veterinary Medicine at the University of Minnesota in St. Paul, Minnesota.

Our speaker is a CVM-KSU alumnus. She completed her Bachelor of Science in Agriculture (1998) and DVM (2000) at Kansas State University, completed an internship in large animal medicine and surgery at the University of Georgia (2001) and a residency in equine internal medicine at Kansas State University (2004). She is a Diplomate of the American College of Veterinary Internal Medicine (Large Animal) and completed her MS degree in Clinical Sciences (epidemiology) at Kansas State University (2004). She then completed her PhD in Comparative and Molecular Biosciences (2007) with a focus on genetics, and postdoctoral studies in genetic epidemiology at the CVM (2008), where she is currently a Professor. She is also a faculty member in the Wellstone Muscular Dystrophy Center, University of Minnesota's Informatics Institute and the Microbial and Plant Genomics Institute. In 2018, Dr. McCue was appointed Associate Dean of Research in the CVM. Her research is primarily focused on equine metabolic syndrome, polysaccharide storage myopathy, recurrent exertional rhabdomyolysis, and performance traits related to metabolic and muscle phenotypes.

Notably, Dr. McCue runs one of the largest equine genetics' laboratories in the world. In addition, to better understanding disease in the horse, Dr. McCue's research focuses on how the horse can be leveraged as a biomedical model. To do that, her laboratory builds tools to conduct network biology to understand cell's function organization, and they are also now applying metabolomics to quantify metabolic differences in the horse, among other genomic and computational biology tools.

For more information, please visit:

<https://vetmed.umn.edu/bio/college-of-veterinary-medicine/molly-mccue>

<https://vetmed.umn.edu/about/people/molly-mccue>

<https://www.equine.umn.edu/research/equine-genetics-and-genomics-laboratory>

The title of her presentation is: ***"A journey in comparative genomics inspired by clinical questions and life's challenges"***



Annual Phi Zeta Research Day
March 2, 2021
The Sigma Chapter of Phi Zeta, est. 1969

Schedule of Events

12:00 (noon)	PLENARY Session¹
BI Auditorium 201 Trotter Hall (livestream) 301 Trotter Hall (livestream) 407 Trotter Hall – Mara (livestream)	Welcome by Phi Zeta President, Dr. Natalia Cernicchiaro Introduction of Keynote Speaker by Phi Zeta Vice President, Abby Ostronic Kenneth D. Olson Phi Zeta Lectureship, Keynote Speaker Dr. Molly McCue , Associate Dean for Research and Professor of Equine Internal Medicine, University of Minnesota "A journey in comparative genomics inspired by clinical questions and life's challenges"
1:15 – 2:30 pm	ORAL Research Presentations²
BI Auditorium	Basic Science Research
201 Trotter Hall	Applied/Clinical Science Research (Small animals/exotics)
301 Trotter Hall	Applied/Clinical Science Research (Large animals)
2:30 – 3:30 pm	Royal Canin POSTER Session³
BI Auditorium (Atrium)	Basic Science Research
407 Trotter Hall - Mara	Applied/Clinical Science Research
3:30 – 5:00 pm	ORAL Research Presentations²
BI Auditorium	Basic Science Research
201 Trotter Hall	Applied/Clinical Science Research (Small animals/exotics)
301 Trotter Hall	Applied/Clinical Science Research (Large animals)
5:30 pm	AWARDS Ceremony⁴
BI Auditorium 201 Trotter Hall (livestream) 301 Trotter Hall (livestream)	Initiation of New Members to Phi Zeta Announcement and Presentation of Awards Recognizing Research and Scholarship Accomplishments Closing Comments

¹ The plenary session (welcome and keynote) will be held in the BI Auditorium and will be live streamed in Trotter Hall rooms 201, 301, and 407 (Mara Conference Center).

² We ask presenters to register and join us for the plenary session in the respective rooms where their presentations will be held in order to comply with the maximum room capacity of the BI auditorium.

Presenters should arrive no later than 1:00 pm to their designated room to upload their presentations. Presentations should be 12-minutes and allow 3 minutes for questions and answers. Given the full schedule, no extensions/exceptions will be made.

³ Poster presenters can register to join any of the rooms during the plenary session.

⁴ We invite all attendees to register and join us for the Award ceremony at the BI auditorium. If we reach maximum room capacity, attendees can join the live stream of the ceremony in rooms 201, 301 or 407 (Mara) Trotter Hall.

Virtual access

Room	Time	Session	Zoom* Session ID and Passcode
BI auditorium	12:00 – 1:00 pm	Plenary and Keynote	ID 254 977 3295; Passcode 2022
BI auditorium	1:15 – 5:00 pm	Basic Research Oral ppts	ID 254 977 3295; Passcode 2022
201 Trotter	1:15 – 4:45 pm	Appl/CS: Small An./Exotics Oral ppts	ID 378 371 3398; Passcode 2023
301 Trotter	1:15 – 4:45 pm	Appl/CS: Large An. Oral ppts	ID 335 804 7142; Passcode 2024
BI auditorium	5:30 – 6:30 pm	Awards Ceremony	ID 254 977 3295; Passcode 2022

Basic Science ORAL PRESENTATIONS
Phi Zeta Research Day
March 2, 2021, 1:15 – 5:00 pm
BI Auditorium

1:15 – 1:30	Upreti, Deepa	Lung cancer prevention by <i>Euglena gracilis</i> extract and the role of intestinal microbiota
1:30 – 1:45	Allison, Molly	Studies on the host selectivity of the staphylococcal innate immune evasion protein, SPIN
1:45 – 2:00	Wang, Yu Shin	Development of reproducible and quantitative tick/tick-borne pathogen infection assays using <i>Dermacentor variabilis</i> and <i>Francisella tularensis</i> subsp. <i>novicida</i>
2:00 – 2:15	Perera, Krishani	Identification and characterization of 3CLpro inhibitors for rabbit hemorrhagic disease viruses
2:15 – 2:30	Adetunji, Shakirat	Porcine macrophage-like cell line CΔ2+ is susceptible to Japanese encephalitis virus infection
2:30 – 3:30	Break for Poster Session¹	
3:30 – 3:45	Balaraman, Velmurugan	Identification of host genes essential for Rift Valley Fever Virus replication
3:45 – 4:00	Schirtzinger, Erin	Early immune response and cytokine induction of PBMC-derived bovine macrophages to infection with Rift Valley Fever Virus in the presence and absence of <i>Culex tarsalis</i> mosquito saliva
4:00 – 4:15	Zabiegala, Alexandria	Coronavirus through time and species: analysis of coronaviruses in relation to the Covid-19 pandemic
4:15 – 4:30	Butterfield, Maddie	Investigation of virus evolution in SARS-CoV-2 clinical samples from experimentally infected cats
4:30 – 4:45	Kwon, Taeyong	Environmental stability of SARS-CoV-2 on different types of surfaces under indoor and seasonal climate conditions
4:45 – 5:00	Smith, MaRyka	Evaluating the value of rapid and accurate traceability in the control of Foot-and-Mouth Disease outbreaks in the U.S.

¹ Basic research poster presentations will be held at the **atrium of the BI auditorium**

Applied/Clinical Science ORAL PRESENTATIONS

Phi Zeta Research Day

March 2, 2021, 1:15 – 4:45 pm

201 Trotter Hall

1:15 – 1:30	Avellar, Haileigh	Successful treatment of a closed comminuted third metacarpal fracture in a 9-month-old giraffe
1:30 – 1:45	Bosch, Sarah	<i>Theileria</i> piroplasms in a reindeer
1:45 – 2:00	Osipova, Iulia	Tension pneumomediastinum in adult miniature Schnauzer
2:00 – 2:15	Tanner, Matthew	Congenital Gerbode defect in a domestic shorthair
2:15 – 2:30	Rooney, Tess	Evaluation of dexmedetomidine-ketamine-midazolam combination administered intramuscularly in captive ornate box turtles (<i>Terrapene ornata ornata</i>)
2:30 – 3:30	Break for Poster Session¹	
3:30 – 3:45	Aldrich, Lauren	Comparison of plasma tramadol concentration after single-dose oral and transdermal administration in cats
3:45 – 4:00	Komp, Marissa	A pilot study of pain assessment and activity tracking in dogs undergoing radiation therapy
4:00 – 4:15	Dewsbury, Diana	Evaluating canine acceptance of two brands of flavored, chewable carprofen tablets in healthy dogs utilizing a complete cross-over design
4:15 – 4:30	Bold, Dashzeveg	Development of an ELISA for the detection of SARS-CoV-2 specific antibody in cats
4:30 – 4:45	Wang, Zixuan	Comparative analysis of four methods used to assess zones of tissue damage induced by microwave thermal ablation

¹ Applied/Clinical Science research poster presentations will be held at **Trotter Hall room 407 (Mara Conference Center)**

Applied/Clinical Science ORAL PRESENTATIONS**Phi Zeta Research Day****March 2, 2021, 1:15 – 4:45 pm****301 Trotter Hall**

1:15 – 1:30	Curtis, Andrew	A novel subcutaneous implant to deliver vaccine for immunocastration
1:30 – 1:45	Martin, Miriam	A comparison of local anesthetic effectiveness in reducing pain associated with dehorning in dairy calves
1:45 – 2:00	Weeder, Mikaela	CO ₂ laser scalpel evaluation debudding of Holstein bull calves
2:00 – 2:15	Lou, Maria	Evaluating the utility of a CO ₂ surgical laser for piglet tail docking to reduce pain and improve wound healing
2:15 – 2:30	Wise, Payton	Evaluating the efficacy of flunixin meglumine or meloxicam at reducing post-surgical pain in sheep
2:30 – 3:30	Break for Poster Session¹	
3:30 – 3:45	Martin, Miriam	Comparative pharmacokinetics of flunixin meglumine and meloxicam in tilapia (<i>Oreochromis</i> spp.)
3:45 – 4:00	Ishengoma, Victor	Oral antimicrobial administration did not influence the fecal prevalence and antimicrobial susceptibility profiles of <i>Salmonella</i> in piglets
4:00 – 4:15	Roubicek, Cierra	Evaluation of antimicrobial activities of phytophenols against bacterial pathogens that cause liver abscesses in feedlot cattle
4:15 – 4:30	Salih, Harith	Evaluation of sorghum phenolic compounds for their antimicrobial activities against liver abscess causing pathogens in feedlot cattle
4:30 – 4:45	Schnur, Sydney	Relative isolation rates of phages lytic to <i>Fusobacterium necrophorum</i> from ruminal fluid and city sewage samples

¹ Applied/Clinical Science research poster presentations will be held at **Trotter Hall room 407 (Mara Conference Center)**

Basic Science Research POSTERS**Phi Zeta Research Day****March 2, 2021, 2:30 – 3:30****BI Auditorium (Atrium)**

(Posting from 1:00 – 5:00 pm; Q&A for Judging 2:30 – 3:30 pm)

1	Avellar Haileigh	In vitro antimicrobial activity of equine mesenchymal stromal cells and platelet lysate against common clinical pathogens
2	Bieberly Zachery	Effect of proteinase inhibition on glucagon-like peptide-2 concentrations in blood samples from healthy cats
3	Harris Shanice	Human neuron infection with Zika virus and Hybrid IgG4 antibody response to <i>Aedes aegypti</i> salivary proteins
4	Maloney Bailey	Impact of hematophagy on antiviral responses in <i>Culicinae</i> mosquitoes
5	Saunders Danielle	Production of yellow fever virus 17D vaccine strain using overlapping complementary DNA fragments
6	Stewart Savannah	Utilizing a genome-wide screen to identify host genes critical to Japanese encephalitis viral infection
7	Willix Joshua	Genome-wide screen of host genes that enable pathogenesis of Japanese encephalitis virus
8	Wenger MJ	Sublethal effects on motility seen in <i>Amblyomma americanum</i> treated with lotilaner (Credelio®)

Applied/Clinical Science Research POSTERS**Phi Zeta Research Day****March 2, 2021, 2:30 – 3:30 pm****Mara Conference Center, 407 Trotter Hall**

(Posting from 1:00 – 5:00 pm; Q&A for Judging 2:30 – 3:30 pm)

1	Carruth Ariel	Identifying <i>Cytauxzoon felis</i> infections through detection and evaluation of immunodominant <i>C. felis</i> antigens
2	Flowers Macy	Evaluation of enrofloxacin and oxytetracycline to eliminate persistent <i>Anaplasma marginale</i> infection in cattle
3	Toillion Alyssa	Effect of protracted free-choice CTC-medicated mineral for anaplasmosis control on the antimicrobial resistance profile of <i>Escherichia coli</i> in beef cattle on a pasture setting
4	Favreau Laura	A proper place for DREADD: verification of chemogenetic surgery placement in rats
5	Kriley Breanna	Time to failure of prophylactic topical timolol therapy in dogs with primary angle closure glaucoma
6	McCall Jayden	Safety and tolerability of African Swine Fever Virus subunit vaccine candidates in commercial pigs
7	Mark Carolyn	Evaluation of CXCL1, S100A8, MMP8, and TNF as biomarkers of <i>Mycobacterium tuberculosis</i> infection and disease in humans

ORAL PRESENTATIONS REQUIREMENTS:

- Arrive no later than 1:00 pm to your designated room to upload your presentation.
- No specific format required.
- 12-minute presentation time limit; 3 minutes allowed for questions and answers.
- Please give 24-48 hours advance notice if you are unable to attend/present due to unforeseen circumstances.

POSTER PRESENTATION REQUIREMENTS:

- Poster Size: The supplied poster boards can accommodate posters 48 inches wide by 36 inches deep.
- Poster PDFs need to be sent to Ms. Gail Eyestone (geyestone@vet.k-state.edu) by **February 22, 2021**.
- Posters can be printed with the Veterinary Medical Library's Print Graphic Services. Contact Susie Larson at 785-532-4025 or larson@vet.k-state.edu. The cost of poster printing is the responsibility of the presenter or presenter's mentor.
- The BI Auditorium (Atrium) and the Mara Conference Center (407 Trotter Hall), will be open from 9:00 am on the day for set-up.
- Table tents and thumbtacks will be provided.
- The Poster Session question and answer time for judging will be from **2:30-3:30 pm**.
- Posters must be removed from the BI Auditorium atrium and the Mara Conference Center by **5:00 pm**
- Please give 24-48 hours advance notice if you are unable to present due to unforeseen circumstances.

Observing COVID-19 guidelines during Phi Zeta Research Day

This year, and given the current situation, for our most attended sessions, including the plenary and awards sessions, presentations will be live streamed in several rooms (BI Auditorium, Trotter Hall rooms 201, 301 and 407 (Mara)) in order to comply with maximum room capacities. In addition, and with the aim of protecting the health and safety of all our students, staff and faculty, we will require the use of masks or face coverings, as per KSU mandate, and of exercising social distancing. All rooms will be disinfected before and after use, and disinfecting stations will be available in all pertinent spaces for personal use. If you have any questions, concerns, or require additional accommodations, please do not hesitate to contact Dr. Natalia Cernicchiaro (ncernic@vet.k-state.edu) or Gail Eyestone (geyestone@vet.k-state.edu).

Maximum Room Capacity (based on COVID-19 guidelines)

Room	Max. capacity
BI auditorium	62*/ 50**
201 Trotter Hall	37
301 Trotter Hall	37
407 Trotter Hall (Mara)	30

* open doors; ** closed doors

Kansas State University - COVID-19 guidelines

K-state is currently in Phase 3 of the Reopening plan, which consists of:

- Physical distancing remains necessary. Please continue to follow CDC guidelines.
- Maintaining at least six-foot [physical distancing](#) from every other person present at a location.
- [Washing hands with soap and water](#) for at least twenty seconds as frequently as possible, or using hand sanitizer.
- Covering coughs or sneezes — cough into the sleeve or elbow, not into the hands.
- Regularly [cleaning high-touch surfaces](#).
- Avoid shaking hands.
- Mass gatherings are limited to 50 or fewer individuals.
- High-risk individuals restrict activities as much as possible.
- Students, faculty, staff and visitors [must wear face coverings over their mouths and noses](#) while on K-State campuses in all hallways, public spaces, classrooms and other common areas of campus buildings. Face coverings must also be properly worn when in offices or other work spaces or outdoor settings when 6-feet physical distancing cannot be maintained.
- All employees must take the [Come Back 'Cats — Reopening Kansas State University](#) training. Student training will be coming soon.
- Employees are encouraged to work remotely as much as possible.
- Employees, students and visitors should self-assess their symptoms and should not come to campus if they are sick, [have a temperature over 100.4 degrees F](#), or have other illness symptoms of the coronavirus as listed on the [CDC website](#).

Employees, students and visitors should follow [specific guidance](#) if they have:

- Tested positive for COVID-19.
- A sick family member or roommate at home with COVID-19.
- Been in close contact with another person who has COVID-19.
- Been tested for COVID-19 and are awaiting results.
- Recently traveled outside of Kansas.

For more information visit <https://www.k-state.edu/covid-19/return/reopening/phase-3/>

PHI ZETA OFFICERS 2021:

On behalf of the Phi Zeta Executive Committee (2021):



Dr. Natalia Cernicchiaro – President (ncernic@vet.k-state.edu)

Dr. Jessica Meekins – President-Elect (jslack@vet.k-state.edu)

Dr. Sarah Schneider – Secretary/Treasurer (smschneider@vet.k-state.edu)

Abby Ostronic – Vice-President (abbyostronic@vet.k-state.edu)

Gail Eyestone – Administrative Assistant (geyestone@vet.k-state.edu)



2021 Phi Zeta Research Day
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Kansas State University
March 2, 2021



Presenter: Deepa Upreti

Lung cancer prevention by *Euglena gracilis* extract and the role of intestinal microbiota.

Corresponding Author: Masaaki Tamura, DVM PhD, Department of Anatomy and Physiology, College of Veterinary Medicine, Kansas State University, Manhattan, Kansas, USA.

Co-Authors: Deepa Upreti, Department of Anatomy and Physiology, College of Veterinary Medicine, Kansas State University, Manhattan, Kansas, USA.

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Ayaka Nakashima, Central Research laboratory, euglena Co. Ltd., Tokyo, Japan.

Kengo Suzuki, Central Research laboratory, euglena Co. Ltd., Tokyo, Japan.

Jeffrey Comer, Department of Anatomy and Physiology, College of Veterinary Medicine, Kansas State University, Manhattan, Kansas, USA.

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Keywords: Lung cancer prevention; *Euglena* extract; MDSC attenuation; Gut microbiome alteration.

Abstract

Introduction: *Euglena gracilis*, a single-celled alga, is rich in nutrients and thus, used as a nutritional dietary supplement. *Euglena* extracts have shown to stimulate anticancer immunity; however, the anticancer mechanism has not yet been fully elucidated.

Methods: Two different *Euglena* extracts were prepared. First, partially purified water extract (EWE) was prepared by suspending a whole *Euglena gracilis* dry powder in PBS and performing two steps of centrifugation followed by filtration using a membrane with a 0.22 µm pore size. Second, boiled EWE (bEWE) was prepared by immersing unfiltered EWE in boiling water for 12 min followed by high speed

centrifugation and the same filtration procedure. Gut microbiomes were compared between groups using 16S rRNA gene amplicon sequencing.

Results: Both EWE and bEWE treatments inhibited the growth of lung carcinoma cells *in vitro*. EWE treatment attenuated granulocytic myeloid-derived suppressor cells (MDSCs) in bone marrow cell cultures. Oral administration of EWE and bEWE (100–200 mg/kg/day) three weeks prior to Lewis lung carcinoma cell inoculation attenuated tumor growth in the lungs of immunocompetent mice. Gut microbiome analysis revealed more diverse microbial compositions in the EWE- and bEWE-treated mouse groups than in the PBS group. Specifically, an increase in the ratio of Bacteroidetes to Firmicutes and a significant increase in *Akkermansia* and *Muribaculum* was observed in EWE- and bEWE- treated mice compared to PBS treated mice.

Conclusions: These studies suggest that oral administration of partially purified water extracts from *Euglena gracilis* altered the intestinal microbiome and this alteration may attenuate host MDSCs, thereby preventing lung carcinoma growth.



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Presenter: Molly Allison

Studies on the host selectivity of the staphylococcal innate immune evasion protein, SPIN.

Corresponding Author: Brian Geisbrecht, PhD, Department of Biochemistry and Molecular Biophysics, Kansas State University, Manhattan, Kansas, USA.

Co-Authors: Molly Allison, MPH, College of Veterinary Medicine, Kansas State University, Manhattan, Kansas, USA

Nitin Mishra, Department of Biochemistry and Molecular Biophysics, Kansas State University, Manhattan, Kansas, USA.

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Keywords: *Staphylococcus aureus*; Myeloperoxidase; SPIN.

Abstract

Introduction: The Staphylococcal Peroxidase Inhibitor (SPIN) is a small, secreted protein from Staphylococci that inhibits myeloperoxidase (MPO) activity. SPIN blocks the MPO active site and prevents production of cytotoxic hypochlorous acid (HOCl) within the neutrophil phagosome. *Staphylococcus aureus* is primarily a pathogen of humans and previous studies have shown that its SPIN protein cannot bind MPO from non-human mammalian species. This raised questions as to whether SPIN proteins from Staphylococci whose preferred hosts are other mammals might likewise exhibit strict binding preference for MPO from these hosts. **Objectives:** (i) To evaluate the interactions between SPIN homologs from Staphylococcal species known to infect dogs and recombinant canine MPO. (ii) To examine whether these SPIN homologs could bind and inhibit human MPO. Preliminary aims were to prepare recombinant SPIN proteins from *Staphylococcus pseudintermedius* and *Macrococcus canis*, as well as to transfect mammalian cells with vectors that direct expression of recombinant human and canine MPO.

Methods: The SPIN proteins were purified by affinity and gel filtration chromatographies, and characterized by electrophoresis and mass spectrometry. Production of recombinant MPO from transiently-transfected HEK293(t) cells was assessed by immunoblot using anti-His mAb, anti-human MPO, and anti-myc mAb as primary antibodies.

Results: Bands specific for both recombinant human and canine MPO were present, indicating positive transfection of mammalian cells and expression of the desired MPO enzymes.

Conclusions: These preliminary results provide evidence that preparation of canine SPIN proteins and MPO was successful for use in further structure/function analyses.



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Presenter: Yu-Shin Wang

Development of reproducible and quantitative tick/tick-borne pathogen infection assays using *Dermacentor variabilis* and *Francisella tularensis* subsp. *Novicida*.

Corresponding Author: Yu-Shin Wang, DVM Candidate Class of 2023, College of Veterinary Medicine, Kansas State University, Manhattan, Kansas, USA.

Co-Authors: Tippawan Anantatat, MS, Department of Diagnostic Medicine and Pathobiology, College of Veterinary Medicine, Kansas State University, Manhattan, Kansas, USA.

Kathryn E. Reif, MSPH PhD, Department of Diagnostic Medicine and Pathobiology, College of Veterinary Medicine, Kansas State University, Manhattan, Kansas, USA.

Corresponding Author: yushinwang@vet.k-state.edu, 785-473-8870.

Keywords: artificial infection; *Dermacentor variabilis*; *Francisella tularensis novicida*; infection assay; tick-borne pathogen.

Abstract

Introduction: In the United States, ticks serve as vectors for numerous bacteria pathogens including *Francisella tularensis* subspecies. Characterization of tick-based infection assay systems are necessary to probe hypothesis-driven questions regarding genetic and functional requirements of tick-borne pathogen infection and transmission success in the tick vector. To address this need, we developed and characterized tick vector-focused infection assay systems by evaluating the infection dynamics of *F. tularensis* subsp. *novicida* in *Dermacentor variabilis* ticks, tissues, and cells.

Methods: In this study, we investigated infection assay systems utilizing embryonically derived *Dermacentor variabilis* (DVE1) cells and tick tissue explants including midgut and salivary gland to evaluate *F. novicida* infection kinetics. For whole tick infection, we evaluated multiple artificial infection methods including submersion in *F. novicida* culture following amputation or puncture; and, microinjection with *F. novicida* culture to characterize the feasibility and effectiveness of each infection route to result in viable infected ticks.

Results: Both DVE1 cells and tick tissue explants were successfully infected and able to support *F. novicida* replication as indicated by increases in bacterial levels. Whole tick infection assays were evaluated with tick viability and bacterial level 7 days post challenge with the results pending.

Conclusions: We demonstrate successful and reproducible *F. novicida* infection in multiple *D. variabilis*-based systems. These infection assays of different host structural complexity will be useful to probe

hypothesis-driven questions about tick/tick-borne pathogen interactions. This model can serve as a basis for other tick-borne pathogen studies to further explore the infection dynamics and accelerate discovery of disease mitigation.



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Presenter: Krishani Perera

Identification and characterization of 3CLpro inhibitors for rabbit hemorrhagic disease viruses.

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Keywords: RHDV; EBHSV; 3CLpro inhibitors.

Abstract

Introduction: Rabbit hemorrhagic disease virus (RHDV) is a Lagovirus in the family Caliciviridae. RHDV is classified into two serotypes, RHDV1 and RHDV2. Infection of RHDV can lead to acute liver failure and death of wild and domestic European rabbits. Although being endemic in most parts of the world, USA has been free of RHDV. However, since 2020 spring, RHDV2 cases have been detected in several states in the US with high mortality rates in domestic and wild rabbit populations as well as some hare species. Unavailability of licensed vaccines or treatment greatly challenges the control efforts and increase the threat of RHDV2 becoming endemic in the US. Calicivirus 3C-like protease (3CLpro) is indispensable for virus replication, and we have previously demonstrated the inhibitory activities of small molecule compounds against calicivirus 3CLpro. Here, we investigated the inhibitory activities of small molecule compounds from an existing 3CLpro inhibitor library against RHDV 3CLpro as well as European brown hare syndrome virus (EBHSV) 3CLpro to identify potent inhibitors against multiple Lagoviruses.

Methods: We generated recombinant 3CLpro of RHDV1, RHDV2 and EBHSV and screened selected compounds using fluorescence resonance energy transfer (FRET) assay. We also compared the homology of RHDV, EBHSV and other Lagovirus 3CLpro sequences from Genbank.

Results: RHDV1 and RHDV2 3CLpro sequences were highly homologous in comparison to that between RHDV and EBSHV 3CLpro. In the FRET assay, the tested compounds showed similar inhibitory trends against all three recombinant 3CLpro, and the inhibitory activities between the three 3CLpro were comparable.

Conclusions: We identified small molecule 3CLpro inhibitors that show similar high potency against RHDV and EBSHV 3CLpro. This proposes the potential applicability of 3CLpro inhibitors as effective therapeutics for multiple Lagoviruses. Financial Support: USDA National Institute for Food and Agriculture (2019-67015-29864); U.S. National Institutes of Health (R01 AI130092)



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Presenter: Shakirat Adetunji

Porcine macrophage-like cell line CΔ2+ is susceptible to Japanese encephalitis virus infection.

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Keywords: porcine; cell line; encephalitis; macrophage; virus.

Abstract

Introduction: Japanese encephalitis virus (JEV) is a zoonotic arthropod-borne flavivirus that is a leading cause of severe neurologic infection in humans. Pigs have a lasting viremia upon natural infection making them important reservoirs of JEV. To understand JEV pathogenesis in pigs, we examined the susceptibility of an established porcine monocyte-derived macrophage-like cell line (CΔ2+) to the attenuated JEV strain, SA-14-14-2.

Methods: Monolayers of CΔ2+ and BHK-21 (positive control) cells were infected with SA-14-14-2 for 5 days at a multiplicity of infection (MOI) of 0.1. Culture supernatants and cells were collected from 0 - 120 hours post infection (hpi), and monolayers were observed for cytopathic effects (CPE). Infectious virus in supernatants was quantified using plaque assays and cells were stained with trypan blue to determine viability. An indirect immunofluorescence assay was used to detect JEV-NS1 antigens.

Results: CΔ2+ cells were susceptible to SA-14-14-2 and produced infectious virus with a mean peak titer comparable to BHK-21 cells which are known to be vulnerable to JEV. The proportion of viable CΔ2+ and BHK-21 cells also declined at a similar rate. Infected CΔ2+ and BHK cells showed time-dependent CPE and intracellular localization of the JEV-NS1 protein was observed at 24hpi.

Conclusions: These findings demonstrate that CΔ2+ represents a relevant cell line for understanding JEV infection dynamics in a natural host species. These data provide a foundation to compare various JEV strains *in vitro* to allow for better understanding of host cell mechanisms critical for viral replication and maintenance in pigs.



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Presenter: Velmurugan Balaraman

Identification of host genes essential for Rift Valley Fever Virus replication.

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Keywords: RVFV; host factor; CRISPR; MP-12; A549 cells.

Abstract

Introduction: Rift Valley fever virus (RVFV) is an emerging zoonotic pathogen that causes Rift Valley fever (RVF) in livestock and humans. RVFV belongs to the family *Phenuiviridae*, genus *Phlebovirus*. Currently, there is no licensed human vaccine or antiviral drug available to control RVF. Multiple species of animals and humans are vulnerable to RVFV infection but the host factors affecting susceptibility are not well understood.

Methods: We utilized a CRISPR-Cas9 system to identify host genes essential for RVFV replication. The CRISPR-Cas9 system was used to generate Genome-scale CRISPR Knock-Out (GeCKO) A549 human cells. Next, GeCKO-A549 cells were selected for resistance to RVFV MP-12 infection. The genomic DNA of

resistant A549 cells was analyzed for single guide RNA (sgRNA) distribution. Bioinformatic analysis of the sgRNA data generated a list of host genes potentially essential for RVFV replication. Respective candidate genes were selected for small interfering RNA (siRNA) gene knockdown, which was confirmed by RT-qPCR assays.

Results: The GeCKO-A549 cell screen with RVFV MP-12 identified 907 host genes. siRNA experiments targeting six host genes were performed. Knock-down of four host genes did not show a reduction of virus replication, whereas siRNA targeting of the other two host genes showed a significant ($p < 0.05$) reduction of RVFV replication.

Conclusions: The CRISPR-Cas9 system was successfully used to identify host genes essential for RVFV MP-12 replication in A549 cells. The role of these host genes on the life cycle of RVFV will be analyzed in future studies, potentially leading to novel antiviral strategies to control RVFV in humans and animals.



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Presenter: Erin E. Schirtzinger

Early immune response and cytokine induction of PBMC-derived bovine macrophages to infection with Rift Valley Fever Virus in the presence and absence of *Culex tarsalis* mosquito saliva.

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Keywords: Rift Valley fever virus; mosquitoes; immune response.

Abstract

Macrophages play a critical role as phagocytes in the innate immune system and produce cytokines that stimulate the adaptive immune system. As in other arboviral diseases, macrophages and dendritic cells are thought to be early infection targets of Rift Valley Fever virus (RVFV). To investigate their role in early immune response, bovine primary macrophages from 3 biological replicates were infected with attenuated RVFV MP-12 in the presence and absence of *Culex tarsalis* saliva. The macrophage lineage of the cells and RVFV infection were confirmed by dual label immunofluorescence for IBA-1 (macrophage marker) and RVFV nucleoprotein. At 0, 8 and 24 hours post infection (hpi) RNA was collected, reverse-transcribed and analyzed by qPCR for 4 reference, 6 early immune response and 9 cytokine genes. Relative gene expression differences between treatments and time points were tested with two-way ANOVAs using Tukey's post-hoc tests with p-value correction for multiple tests. Bovine macrophages infected with MP-12 in the presence of saliva had no statistically significant gene expression differences when compared to virus only at any time point. Conversely, significant differences in gene expression were present when infected samples with and without saliva were compared to uninfected controls. At 8 and 24 hpi, transcription was significantly different than controls for all virus infected samples for 4 early immune response genes as well as for 6 cytokine genes. While *Culex* saliva may not enhance suppression of the early immune response in MP-12 infection, it may still have a role in virulent RVFV strain infections.



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Presenter: Alexandria Zabiegala

Coronavirus through time and species: analysis of coronaviruses in relation to the Covid-19 pandemic.

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Keywords: Coronaviruses; Spike Protein; Tissue Tropism.

Abstract

Coronaviruses, positive-sense, single stranded, RNA viruses, are a large group of viruses in the *Coronaviridae* family that cause a wide variety of diseases in humans and animals. From the avian IBV, the first coronavirus isolated from poultry with respiratory disease during 1930s to current SARS-CoV-2, coronaviruses continue to present challenges to animals and humans. Animal coronaviruses can cause asymptomatic to fatal infections in the respective target host including avian, porcine, bovine, canine, feline, or murine species. However, interspecies jumping of coronavirus has occurred periodically. Typical human coronaviruses such as 229E and OC43 are associated with common cold causing mild upper respiratory infection. More recently found human coronavirus including NL63 and HKU1 have an increasing tendency to cause lower respiratory disease. Since 2003, highly virulent coronaviruses including SARS-CoV, MERS-CoV, and SARS-CoV-2 have emerged with severe lower respiratory disease with systemic signs. Coronavirus S protein is a major structural protein and is responsible for viral attachment and entry to target cells. This protein is involved in cross-species transmission, tissue tropism, and virulence and can serve as a vaccine component and potential therapeutic target. In this literature review, various animal and human coronaviruses are analyzed with focus on S protein including interactions with the cellular receptor, subdomain arrangement, functional mutations and alterations in virulence, and receptor affinity. Understanding this fundamental biology should help finding solutions for preventive and therapeutic measures for current and future coronavirus pandemics.



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Presenter: Maddie Butterfield

Investigation of virus evolution in SARS-CoV-2 clinical samples from experimentally infected cats.

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Keywords: SARS-CoV-2; COVID-19; felines; cats; next-generation sequencing.

Abstract

Introduction: Understanding mutation rates of RNA viruses, including coronaviruses like SARS-CoV-2, is critical for disease control measures. High mutation rates in RNA viruses can result in accumulation of genetic mutations during infection, potentially altering the viral geno- and phenotype. Here, we evaluated the SARS-CoV-2 genome after inoculation of cats with the human USA-WA1/2020 SARS-CoV-2 isolate to determine if mutations would occur due to host adaptation.

Methods: Clinical samples (nasal, rectal, and oropharyngeal swabs, bronchoalveolar lavage fluid, and mucus) were obtained after intranasal/oral challenge of cats with SARS-CoV-2 isolate USA-WA1/2020. SARS-CoV-2-RNA from 29 clinical samples was isolated and sequenced via next-generation sequencing. Consensus sequences from samples were determined and compared to reference USA-WA1/2020 sequence to identify nucleotide changes. SARS-CoV-2 genes analyzed included nucleocapsid (N), envelope (E), Orf1ab, and spike (S) genes (~88.9% of the genome).

Results: Consensus sequences deviated from the reference USA-WA1/2020 sequence in the Orf1ab gene, ranging from 1-6 nucleotide changes per consensus sequence when compared to reference Orf1ab. No mutations were observed in N and E genes. Interestingly, a 12-nucleotide insertion was found in the N-terminal domain of the S gene, adding Lys-Leu-Arg-Ser (KLRS) residues in a predicted

surface-exposed region. Subsequent research indicates the KLRS mutation results in a significant increase in viral replication in VeroE6 cells compared to wild-type SARS-CoV-2.

Conclusions: Mutations observed after cross-species transmission of SARS-CoV-2 will be further analyzed for their phenotypic impact. This work is crucial in our understanding of mutational changes in the SARS-CoV-2 genome after cross-species transmission and novel host adaptation.



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Presenter: Taeyong Kwon

Environmental stability of SARS-CoV-2 on different types of surfaces under indoor and seasonal climate conditions.

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Keywords: SARS-CoV-2; environmental stability; fomite; virus decay.

Abstract

Introduction: Transmission of SARS-CoV-2 mainly occurs through direct contact with an infected person via droplet. The potential role of contaminated surfaces in SARS-CoV-2 transmission has been suggested since the virus has been extensively detected on environmental surfaces. These findings are the basis for our investigation on virus stability on multiple surfaces under different climate conditions in order to predict the seasonality of SARS-CoV-2. Therefore, the aim of this study was to estimate the virus stability and its biological half-life on various types of surfaces under indoor and seasonal climate conditions.

Methods: A total of 5×10^4 TCID₅₀ of SARS-CoV-2 was added onto 12 different materials, dried for 4.5 hours and incubated under indoor, summer, spring/fall and winter conditions up to 21 days post contamination. At various days post contamination, infectious virus was recovered and titrated to calculate the respective biological half-life values.

Results: This study revealed that SARS-CoV-2 survived the longest on surfaces under winter conditions with a survival on most surfaces post-contamination up to 21 days, followed by spring/fall conditions with a survival up to 7 days. Infectious virus was isolated up to 4 days post-contamination under indoor conditions, whereas no infectious virus was found at 3 days post-contamination under summer conditions.

Conclusions: Our study demonstrates the remarkable persistence of SARS-CoV-2 on many different common surfaces, especially under winter conditions, and raises awareness to the potential risk of contaminated surfaces to spread the virus.



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Presenter: MaRyka Smith

Evaluating the value of rapid and accurate traceability in the control of Foot-and-Mouth Disease outbreaks in the U.S.

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Keywords: Foot-and-Mouth Disease; livestock traceability; infectious disease modeling.

Abstract

Introduction: Improving livestock traceability in the United States would support the demand for transparency in product origins and control of foreign animal disease introductions. This study addressed the effect of rapid and effective tracing of livestock and the impact of time to detection of the index herd on the size of a simulated Foot-and-Mouth Disease (FMD) outbreak in the United States.

Methods: We used a spatially explicit stochastic disease transmission model to simulate FMD outbreaks. Our simulated outbreaks began in Southwest Kansas, an area with a high density of beef feedlots and cow-calf production. We modeled the time delay to detect the index farm and the speed and accuracy of tracing direct contacts of infected herds. The time to detection of the index farm was 8 or 14 days from the first infection. Direct contact tracing levels were: within 7 to 10 days, with approximately 85% accuracy (current performance), or within one day with 99% accuracy (optimal electronic I.D. performance).

Results: The models suggested that detecting the first farm earlier (day 8) decreased the outbreak size compared to later detection (day 14). Increasing the speed and accuracy of direct contact tracing resulted in fewer large outbreaks but had minimal to moderate effect on the median outbreak size.

Conclusions: These results support the value of early detection and improved animal traceability to control an FMD outbreak. Further development of animal traceability in the United States is necessary to achieve the modeled optimal results.



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Presenter: Haileigh K. Avellar

Successful treatment of a closed comminuted third metacarpal fracture in a 9-month-old giraffe.

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Keywords: giraffe; fracture; third metacarpal.

Abstract

A captive 9-month-old male intact *Giraffa tippelskirchi* and *Giraffa camelopardalis* mix presented to the Kansas State University Veterinary Health Center (KSU VHC) for consultation on a closed comminuted fracture of the right third and fourth metacarpal bones with moderate displacement. Surgical treatment included placement of a transfixation pin cast with five 3/16" centrally threaded pins diverging 30 degrees performed under general anesthesia. The giraffe was discharged 2 days after surgery with no complications to be managed by zoo staff. Radiographic monitoring of fracture healing was completed at 5 and 8-weeks. The healing of the fracture was classified as a functional malunion. Removal of the transfixation pins and replacement of a half limb fiberglass cast was performed under general anesthesia at 11 weeks post operatively. Following the second anesthetic event the giraffe experienced 3 re-sedation episodes within 24-hours and was successfully treated with atipamezole. Five days after cast replacement the giraffe was discharged from KSU VHC. The final cast was removed standing at 60 days when the pin tracts were no longer radiographically evident. One year following the initial fracture injury the giraffe is on full pasture turn out, able to ambulate normally, and has excellent cosmetic appearance of the limb. This is the first documented case of successful treatment of a long bone fracture in an adolescent or mature giraffe. Reporting the anesthetic protocols, alpha-2 recycling, and fracture management is important to veterinarians not solely focused on zoological medicine as private exotic wildlife ownership becomes more prevalent across the country.



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Presenter: Sarah Bosch

Theileria piroplasms in a reindeer.

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Keywords: *Theileria*; piroplasm; reindeer.

Abstract

A 1-year-old, castrated male, reindeer (*Rangifer tarandus*) from Missouri was presented to the Veterinary Health Center at Kansas State University for difficulty breathing. Physical exam revealed a poor body condition, tachypnea, chemosis, tacky mucous membranes, a prolonged capillary refill time, and a tick near the patient's jaw. Upon review of a blood smear, erythrocytes occasionally contained one to two, pale blue, 1-1.5 um, signet ring to angular shaped, intra-erythrocytic piroplasms consistent with *Babesia* spp. or *Theileria* spp. Total genomic DNA was extracted from whole blood collected into EDTA tubes. PCR amplification and subsequent sequencing of the piroplasm small subunit rDNA revealed a 96% identity with known *Theileria cervi* sequences in the NCBI database. The tick found on the patient was identified, using gross morphology, as *Amblyomma americanum*, commonly known as the lone star tick. *T. cervi* is a tick-transmitted protozoan that can cause anemia, weight loss, and death in infected patients. The organism has been found in many members of the cervidae family, including white-tailed deer and reindeer. The primary vector of *T. cervi* is *A. americanum*. While usually not pathogenic in healthy white-tailed deer, *T. cervi* infection can be fatal in reindeer. This reindeer was treated with medical supportive care and is reportedly doing well at home.



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Presenter: Iulia Osipova

Tension pneumomediastinum in adult miniature Schnauzer.

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Keywords: Pneumomediastinum; tension; spontaneous; atraumatic; canine.

Abstract

A 1.5-year-old female spayed Miniature Schnauzer presented for a one-month history of progressive tachypnea, exercise intolerance, weight loss, and lethargy. Radiographs identified progressive pneumomediastinum and subcutaneous emphysema. Thoracic computed tomography revealed accumulation of gas within the mediastinum, subcutaneous emphysema, and no pulmonary pathology. No tracheal or esophageal lesions were identified during esophagoscopy and bronchoscopy. No gross pathologic lesions were visualized during the exploratory thoracotomy. The patient died 6 days following surgery. The final diagnosis was atraumatic spontaneous tension pneumomediastinum. This is the first case of canine spontaneous tension pneumomediastinum reported and the first documentation of its characterization with computed tomography.



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Presenter: Matthew Tanner

Congenital Gerbode defect in a domestic shorthair.

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Keywords: cat; left ventricle to right atrial shunt; ventricular septal defect.

Abstract

A 5-month-old, intact female Domestic Shorthair presented to the Kansas State Veterinary Health Center for evaluation of a murmur and exercise intolerance. Physical exam revealed a grade V/VI right, parasternal, holosystolic murmur with no other abnormalities. On echocardiogram, there was an abnormality in the membranous interventricular septum and tricuspid valve, allowing blood to shunt from the left ventricle to the right atrium. This lesion is consistent with an infravalvular Gerbode defect. The Gerbode defect is a rare, left ventricle-to-right atrial shunt that comprises 0.08% of all cardiac shunts in humans and has only been documented in veterinary medicine five times. This lesion can be congenital or acquired, and in veterinary medicine, it has only been reported in the canine species. To the author's knowledge, this is the first reported instance of a Gerbode defect in the feline species. At the time of submission, the patient is alive and is maintaining a good quality of life.



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Presenter: Tess Rooney

Evaluation of dexmedetomidine-ketamine-midazolam combination administered intramuscularly in captive ornate box turtles (*Terrapene ornata ornata*).

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Keywords: anesthesia; chelonian; dexmedetomidine; ketamine; midazolam.

Abstract

Introduction: The objective of this study was to characterize the effects of a combination protocol of dexmedetomidine-ketamine-midazolam (DKM) administered intramuscularly (IM) in ornate box turtles (*Terrapene ornata ornata*).

Methods: Each turtle was immobilized with dexmedetomidine (0.1 mg/kg), midazolam (1 mg/kg) and ketamine (10 mg/kg). Time to first response, time to maximal effect, the plateau phase, and time to recovery from reversal administration were recorded. Vitals, muscle tone, reflexes, and the ability to perform endotracheal intubation were recorded at five-minute intervals. Response to an IM injection was assessed every 15 minutes. Atipamezole (0.5mg/kg, IM) and flumazenil (0.05mg/kg, SC) were administered 60 minutes after the initial DKM injections.

Results: The mean time to first response, time to maximal effect, the plateau phase, and time to recovery were 2.1 minutes, 14.9 minutes, 38.7 minutes, 7.8 minutes, respectively. Respiratory rate was not observed in most animals. The body temperature significantly increased over time. Animals lost reflexes in their forelimbs and neck prior to loss of reflexes in their hindlimbs and tail, and recovered them in the opposite direction. Throughout the study, the palpebral reflex was persistent in 43% of turtles and the tail pinch reflex remained intact for 13% of turtles. All turtles recovered with no adverse effects.

Conclusions: This study showed that this DKM protocol may be safely used in ornate box turtles to produce rapid-onset, deep sedation for roughly 40 minutes, long enough to perform many clinical procedures or to facilitate intubation for inhalant anesthesia, followed by a smooth, reliable recovery.



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Kansas State University
March 2, 2021



Presenter: Lauren Aldrich

Comparison of plasma tramadol concentration after single-dose oral and transdermal administration in cats.

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Keywords: tramadol; transdermal; cat.

Abstract

Introduction: Tramadol is a centrally-acting analgesic with weak opioid agonist and monoamine reuptake inhibitor activity and good oral bioavailability in cats. However, tramadol is bitter and many cats refuse oral dosage. A retail transdermal tramadol gel product is marketed as a low-stress alternative to pilling cats but there are no published reports confirming transdermal absorption of tramadol in live cats. **Hypothesis:** Transdermal application of a tramadol gel in cats would result in measurable and clinically relevant plasma concentrations of tramadol and its metabolites.

Methods: **Animals:** Eight clinically normal client-owned domestic cats. A 20 gauge through-the-needle central venous catheter was placed in the medial saphenous vein of each cat for repeat sampling. One cat received a single oral tramadol dose (2 mg/kg) and seven cats received a single Lipoderm-based, commercially-prepared, transdermal tramadol application (10 mg). Concentrations of tramadol and metabolites were determined in plasma by liquid chromatography with mass spectrometry. Plasma tramadol concentrations were measured at fixed times over 24 hours. Tramadol content of the commercial gel was analyzed to confirm content and dosage.

Results: Transdermal plasma concentrations were undetectable or very low (<1 to 4.3 ng/mL) relative to the oral plasma concentrations (261.3 ng/mL maximum) and transdermal pharmacokinetics were not possible to determine. Tramadol content exceeded the target dose in all gel samples analyzed, with 12/13 above the 90-110% USP standard for compounded drugs.

Conclusions: A single Lipoderm-based transdermal tramadol dose at 2 mg/kg does not result in clinically relevant plasma concentrations in cats.



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Presenter: Marissa Komp

A pilot study of pain assessment and activity tracking in dogs undergoing radiation therapy.

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Keywords: oncology; biosensor; radiotherapy.

Abstract

Radiation therapy is an important modality for cancer treatments in dogs and is effective for localized tumors. However, monitoring treatment response to cancer therapy is challenging with subjective assessments. The aims of this pilot study were to 1) better assess radiation therapy response and monitor side effects including pain and distress, 2) understand the changes in the radiation patients' activity levels, and 3) investigate overall quality of life throughout radiation therapy and follow-up. Eight healthy control dogs without cancer or radiotherapy, eleven dogs receiving palliative radiotherapy for cancer, and four dogs receiving definitive radiotherapy for cancer were enrolled at the Veterinary Health Center, Kansas State University. In this study, two methods were used for monitoring changes: an owner questionnaire and an activity tracking device (FitBark® 2, Kansas City, MO). The online questionnaire for pain assessment and quality of life was developed and an activity tracking device was attached to each dog's collar to provide minute-by-minute data. This noninvasive method of timely remote data acquisition encourages owner compliance which is a common challenge. The benefits of the biosensor also extend to the owner by involving them in the treatment evaluation process. Preliminary results revealed a slight decrease in activity and a marginal increase in overall well-being of treated dogs compared to control dogs. These results indicate that activity tracking with a biosensor and a novel questionnaire can be used as excellent remote assessment tools and stimulate proactive communication regarding quality of life, which has potential to improve patient care for dogs.



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Presenter: Diana Dewsbury

Evaluating canine acceptance of two brands of flavored, chewable carprofen tablets in healthy dogs utilizing a complete cross-over design.

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Keywords: canine; osteoarthritis; cross-over; carprofen.

Abstract

Introduction: Canine osteoarthritis (OA) affects nearly one of every five canines with symptoms including but not limited to pain, discomfort, and lameness. While there is no cure for OA, non-steroidal anti-inflammatory drugs (NSAIDs), including carprofen, provide an effective treatment in managing symptoms of OA. Highly palatable and convenient formulations at an affordable cost can increase owner compliance in treatment administration, especially for long-term chronic diseases such as OA. In this study, the acceptability between two carprofen products (Rimadyl® (Zoetis; Kalamazoo, Michigan) and Carprieve® (Norbrook Laboratories Limited; Newry, Northern Ireland) liver-flavored chewable 25mg carprofen tablets) were evaluated utilizing 37 healthy, purpose-bred canines in a 2x2 complete cross-over design.

Methods: Acceptability was evaluated by allowing canines to voluntarily apprehend the half or full-sized tablet out of their clean food bowl within 60 seconds. If the tablet was not apprehended, the tablet was then offered by hand for an additional 60 seconds, without encouragement or coercion to ingest the tablet. The acceptability outcome was documented as full or partial/none. A McNemar's Chi-squared test was performed to determine differences in acceptability for paired data.

Results: Twenty-six (70.3%) and 27 (73.0%) canines voluntarily accepted Carprieve® and Rimadyl®, respectively. Considering the acceptability outcome paired by individual, the majority of canines in this

study (64.9%; 24/37) voluntarily accepted both Carprieve® and Rimadyl®. Eight canines (21.6%) denied or partially accepted both formulations whereas three (8.1%) and two (5.4%) dogs fully accepted Rimadyl® and Carprieve®, but not the other product, respectively.

Conclusions: In this population of healthy purpose-bred canines, the acceptability between a single-dose of Carprieve® and Rimadyl® chewable carprofen tablets did not significantly differ ($P = 0.65$).



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Presenter: Dashzeveg Bold

Development of an ELISA for the detection of SARS-CoV-2 specific antibody in cats.

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Keywords: SARS-CoV-2; serology; ELISA; felines.

Abstract

Introduction: Companion animals are susceptible to a variety of coronaviruses and recent studies indicate felines are highly susceptible to SARS-CoV-2. Reliable diagnosis is an integral part of disease control. RT-PCR diagnostics to detect SARS-CoV-2 RNA is currently been utilized as gold-standard method to diagnose COVID-19 infections. In addition, serological assays are critical to determine the effectiveness of vaccine administration and the herd immunity of populations; they can also be a useful tool in combination with RT-PCR when applied at least one week after onset of clinical signs.

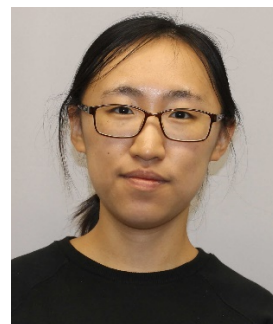
Methods: Recombinant nucleocapsid (N) protein and the receptor binding domain (RBD) of the spike protein of the SARS-CoV-2 (GenBank accession #: MT380725.1) were expressed in *E.coli* (N) and mammalian cells (N, RBD). The recombinant proteins were utilized to develop indirect ELISA tests using well characterized SARS-CoV-2 positive and negative cat serum panels from previous studies.

Results: The optimal conditions of the indirect ELISA (iELISA) tests were established based on checkerboard dilutions of antigens and antibodies. These tests revealed an optimal concentration of recombinant antigens and serum samples in both the N and RBD iELISAs of 100ng antigen/well and a positive serum dilution of 1:800. The threshold of the iELISA tests was set at the average optical density (OD) of negative cat sera plus three times the standard deviation. The diagnostic sensitivity of the iELISA tests was 86- 96%, the diagnostic specificity 90-98%, depending on the respective antigen.

Conclusions: The detection of cat antibodies specific for the N or RBD proteins of SARS-CoV-2 by iELISA is possible. The iELISAs can be used for high throughout screening of cat sera for SARS-CoV-2- specific antibodies in a low biocontainment environment, whereas virus neutralization tests with live virus have to be performed in BSL-3 laboratories.



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Presenter: Zixuan Wang

Comparative analysis of four methods used to assess zones of tissue damage induced by microwave thermal ablation.

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Keywords: microwave ablation; transmission electron microscopy; nitroblue tetrazolium staining; HSP-70; HMGB-1.

Abstract

Introduction: Microwave thermal ablation (MTA) is an emerging therapy for localized tumors. Defining the boundary between viable and non-viable cells is critical for evaluation of MTA safety and efficacy. Due to MTA-induced thermofixation, standard H&E staining alone has limited use in defining this boundary. In this study, we comparatively assessed the use of immunohistochemistry (IHC), transmission electron microscopy (TEM), and nitroblue tetrazolium (NBT), in addition to H&E staining, in analyzing the margin of cell viability following MTA in normal bovine and swine tissue.

Methods: *Ex vivo* and *in vivo* MTA was performed on normal tissue from bovine (liver) and swine (liver and lung), respectively. NBT staining was performed on cryostat sections of fresh tissue. TEM analysis was performed on tissues fixed in Trump's fixative. Formalin-fixed paraffin-embedded sections were used for H&E staining and IHC for vimentin, HMGB-1 and HSP-70 antibody markers.

Results and Conclusions: NBT staining and immunohistochemical staining for vimentin and HMGB-1 was reduced in the ablation center, and there was a gradient change towards normal staining intensity through the transition or hemorrhagic zone and into the periphery zone. The translocation of HMGB-1 from nucleus to cytoplasm indicates damage and inflammatory change in the transition zone and periphery. TEM confirmed cellular organelle damage in ablation centers and transitional zones while cell membranes were maintained, supporting thermofixation. These four methods, in combination, provide a promising approach to acute MTA ablation analysis in future studies.



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Presenter: Andrew Curtis

A novel subcutaneous implant to deliver vaccine for immunocastration.

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Keywords: Immunocastration; vaccines; cattle.

Abstract

Introduction: In cattle, benefits of castration include a reduction in unwanted pregnancies, improved meat quality and fewer injuries in confinement operations. Nevertheless, physical castration, particularly in older calves, can result in pain, hemorrhage, infection and death. Immunocastration could offer a safer and nearly pain-free alternative, but current strategies can be impractical as they require multiple doses of vaccine to be effective. Thus, a study was conducted to test the ability to immunocastrate bulls with a single-dose subcutaneous implant using gonadotropin releasing hormone (GnRH) as the antigen.

Methods: Twelve young (13-46 days old) Holstein bulls were randomly assigned to 1 of 2 implant groups. Six calves received the treatment implant (GnRH) and 6 received the control implant under the skin in the back of the left ear. Calves were followed for 28 days to determine the immune response to implantation. Inflammation was monitored via infrared thermography of ears and scrotums at days 0, 14, and 28. The presence of anti-GnRH antibodies was also established at days 0 and 28.

Results: Calves in the treatment group were all (6/6) found to have produced anti-GnRH antibodies 28 days after implantation. Conversely, no calves in the control group (0/6) were positive for GnRH antibodies. There was no treatment effect on ear or scrotal temperatures.

Conclusions: Preliminary data support the ability of vaccine implants to stimulate humoral immune response in young bull calves. Temperature changes in ears and scrotums were not impacted by treatment. Thus, further long-term study of this method appears justified.



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Presenter: Miriam Martin

A comparison of local anesthetic effectiveness in reducing pain associated with dehorning in dairy calves.

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Keywords: analgesia; cattle; dehorning; pain.

Abstract

Concern for animal welfare has led to investigating pain mitigation during dehorning. The objective was to compare the effectiveness of bupivacaine liposome suspension, lidocaine, or lidocaine + meloxicam administered at dehorning. Fifty male Holstein calves were randomly assigned to 1 of 5 treatment groups: 1) bupivacaine liposome suspension block, oral placebo, cautery dehorn (BUP); 2) lidocaine block, oral placebo, cautery dehorn (LID); 3) lidocaine block, oral meloxicam, cautery dehorn (LID + MEL); 4) saline block, oral placebo, cautery dehorn (CON); and 5) saline block, oral placebo, sham dehorn (SHAM). Biomarkers were collected from 0 to 120 hours post-dehorning and included infrared thermography (IRT), mechanical nociceptive threshold (MNT), and pressure mat gait analysis.

Biomarkers were statistically analyzed using repeat measures with the calf being the repeated measure. A treatment effect was observed for the MNT mean difference of the right horn bud minus a control point which were -1.21 kgF, -1.41 kgF, -1.56 kgF, -1.65 kgF, and -1.68 kgF for the SHAM, CON, BUP, LID +

MEL, and LID groups, respectively ($P = 0.004$). The BUP group did not differ from CON ($P = 0.78$) or SHAM ($P = 0.07$). A treatment effect was observed for gait distance means which were 182.05 cm, 189.69 cm, 195.77 cm, 199.54 cm, and 200.59 cm for the SHAM, BUP, LID + MEL, LID and CON groups, respectively ($P = 0.04$). These data show that administration of bupivacaine liposome suspension at the time of dehorning was not different than lidocaine or lidocaine + meloxicam.



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Presenter: Mikaela Weeder

CO₂ laser scalpel evaluation debudding of Holstein bull calves.

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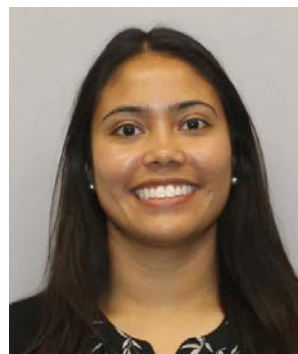
Keywords: laser; Holstein; calf; debudding; CO₂.

Abstract

Cautery hot-iron disbudding is a painful routine husbandry practice done on many dairy farms and calf rearing facilities. Refinements to eliminate or reduce the pain associated with disbudding are desired. Carbon dioxide laser scalpels cut and ablate tissue using high power light energy. The objective of this project was to test the utility of a CO₂ laser scalpel in bovine disbudding; and to compare healing and pain measures to cautery hot-iron disbudding. Twelve Holstein bull calves, aged six to 39 days of age were enrolled onto the study. Calves were randomly assigned to be disbudded with a CO₂ laser scalpel (Laser, n = 6) or cautery hot-iron (Hot-iron, n = 6). Calves were sedated with xylazine for the procedure; and given oral meloxicam and a local anesthetic block for analgesia. Outcome measures were maximum surface temperature by infrared thermography (IRT), mechanical nociception threshold (MNT) tests, and digital images for wound healing. The IRT and MNT measures were collected prior to disbudding and out to 72 h post-procedure. Images for wound healing were collected prior to disbudding, and at 6, 24, 72 h, and 7, 14, 28, 42 days post-disbudding. Maximum surface temperatures were not different between groups (Laser 35.3 ± 0.3°C vs. Hot-iron 36.0 ± 0.3°C). No differences in MNT measures were noted between the Laser calves (2.28 ± 0.19) compared to Hot-iron calves (2.42 ± 0.19 kgf). All six calves in the Laser group were completely healed by day 42 whereas only 4/6 Hot-iron calves were fully healed. These results suggest disbudding using a CO₂ laser scalpel is painful. Further research focusing on pain associated with later time points is needed to fully evaluate its utility.



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Presenter: Maria Lou

Evaluating the utility of a CO₂ surgical laser for piglet tail docking to reduce pain and improve wound healing.

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Keywords: animal welfare; tail docking; pain; piglet; CO₂ surgical laser.

Abstract

In the United States, piglets are routinely tail docked using side plyers to decrease the prevalence of tail biting. This method causes significant post-procedural pain. Refining the surgical procedure by using a CO₂ laser may reduce tail docking-associated pain and inflammation, improving on-farm piglet welfare. The objectives of this preliminary study were to evaluate the ability of a CO₂ surgical laser to 1) reduce pain and 2) improve wound healing of piglets undergoing tail docking. Thirty piglets (3 days old; Yorkshire x Landrace) were randomly allocated to one of three treatments (n = 10 piglets/treatment group): tail docking with side plyers, tail docking with a CO₂ laser, or sham-tail docking (control). Piglet vocalizations were recorded at the time of tail docking and the maximum frequency, amplitude, and energy of vocalizations were quantified. Piglets were video recorded in their pens for 30 min. pre-procedure and at 0-2 and 6-8 h post-procedure for behavior scoring. Digital and infrared thermography (IRT) images were collected at baseline, 0, 8, 24, 48, 72, 96, 120, 144, and 168 h post-procedure, and at baseline 0, 0.5, 8, and 24 h post-procedure, respectively for inflammation and wound healing assessment. Other outcome measures collected in this study included: salivary cortisol, blood cortisol, facial grimacing, and body weight. Data are currently being analyzed. Results for vocalizations and wound assessment (digital and IRT) will be available prior to conference day to determine if using a CO₂ laser was able to reduce pain and inflammation and improve wound healing of piglets' post-tail docking.



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Presenter: Payton Wise

Evaluating the efficacy of flunixin meglumine or meloxicam at reducing post-surgical pain in sheep.

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Keywords: analgesia; livestock; NSAID; behavior.

Abstract

Pain management is lacking in U.S. sheep production systems, partially attributed to the limited amount of scientific studies assessing sheep pain responses after analgesia treatment. This study evaluated the ability of flunixin meglumine (FLU) or meloxicam (MEL) to provide pain relief to sheep after soft tissue surgery. Thirty ewes were randomly assigned to one of three treatment groups: FLU (intravenous): 2.2mg/kg pre-, at 24 and 48h post-laparotomy; MEL (oral): 2.0mg/kg pre- and 1.0mg/kg at 24 and 48h post-laparotomy; or control (CON): sedation only. Outcome variables were recorded pre-procedure and up to 48h post-surgery. Video cameras collected behavior data of sheep in their pens, while facial

grimacing, vocalization and gait were assessed as sheep walked across a pressure mat. Average temperature and mechanical nociceptive threshold (MNT) around the incision site were collected using an infrared thermography camera and an algometer, respectively. CON sheep spent less time eating and more time walking than FLU and MEL sheep. CON sheep had lower abdominal temperatures (less inflammation) and higher MNT (less pressure/pain sensitivity) at all sites near the incision than FLU and MEL sheep. CON sheep had greater front/hind limb stride length and greater hind limb force/contact pressure than FLU and MEL sheep. There were no significant differences in behavior, gait, abdominal inflammation, or pain when FLU and MEL were compared, suggesting both drugs provided similar levels of post-surgical analgesia. However, post-operative pain and inflammation were still evident in sheep provided FLU or MEL. More work is needed to optimize sheep pain management on-farm.



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Presenter: Miriam Martin

Comparative pharmacokinetics of flunixin meglumine and meloxicam in tilapia (*Oreochromis* spp.).

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Keywords: pharmacokinetics; pain; fish; analgesia.

Abstract

Aquaculture is one of the largest growing sectors of the world food supply. There is evidence of pain perception in fish but analgesic use in aquaculture is limited. The objective was to investigate the comparative pharmacokinetics of flunixin and meloxicam in tilapia. Two hundred-seventy fish were assigned to 1 of 3 treatment groups 1) flunixin meglumine administered intramuscularly at 2.2 mg/kg; 2) meloxicam administered intramuscularly at 1 mg/kg; or 3) meloxicam administered orally at 1 mg/kg. Blood samples were collected from 6 fish per treatment group at 14 timepoints out to 10 days post-drug administration. Plasma drug concentrations were determined using ultra-high pressure liquid chromatography coupled with mass spectroscopy. The plasma concentration versus time data were analyzed with a non-compartmental approach using commercially available software (Phoenix®, Version 8.3, Certara, Inc., Princeton, NJ, USA). Flunixin reached a mean maximum concentration (C_{max}) of 4826.7 ng/mL at 0.5 h, had a terminal half-life ($T_{1/2}$) of 0.5h, and an area under the concentration time curve

extrapolated to infinity (AUC_{INF_obs}) of 25,261.62 h*ng/mL. Intramuscular meloxicam had a $T^{1/2}$ of 9.4 h after reaching a C_{max} of 11.3 ng/mL at 2 h, with an AUC_{INF_obs} of 150.31 h*ng/mL. Oral meloxicam has a $T^{1/2}$ of 1.9 h after reaching a C_{max} of 72.2 ng/mL at 2 h, with an AUC_{INF_obs} of 400.83 h*ng/mL. When administered intramuscularly, flunixin reaches sufficient plasma concentrations to potentially have an effect, while meloxicam when administered intramuscularly or orally at the given dosage likely does not due to the low plasma concentration.



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Presenter: Victor Ishengoma

Oral antimicrobial administration did not influence the fecal prevalence and antimicrobial susceptibility profiles of *Salmonella* in piglets.

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Keywords: *Salmonella*; Antimicrobial; AMR; In-feed; In-water; Chlortetracycline; Tiamulin.

Abstract

A total of 1,296 weaned piglets were used in a 35-d study to assess the impact of in-feed vs in-water administrations of chlortetracycline (CTC) and or tiamulin on prevalence and antimicrobial resistance (AMR) profiles of *Salmonella enterica*. Piglets were allocated to 48 pens (27 pigs per pen) and pens were assigned randomly to six treatment groups: control, in-feed CTC, in-water CTC, in-feed tiamulin, in-water tiamulin, and in feed CTC and tiamulin. Fresh fecal samples were collected randomly from 5 of 27 piglets

from each pen on days -7, 0, 7, 14, 21, and 28. *Salmonella* isolation and identification were done by enrichment, plating on selective medium and species confirmation of putative colonies by PCR. Antimicrobial susceptibility and resistance of the isolates were determined using premade antimicrobial panel (Sensititre™ CMV3AGNF and BOPO7F). Whole Genome Sequencing (WGS) was performed on an Illumina HiSeq platform. All isolates were identified as serotype Typhimurium by WGS. The overall prevalence of *Salmonella* was 3.0% (43/1,440) with no treatment effect ($P > 0.05$). All isolates were resistant (100%) tetracycline and tiamulin. Additionally, the isolates were resistant to ampicillin (100%), streptomycin (100%), sulfisoxazole (100), ciprofloxacin (95.4%) and nalidixic acid (74.4%). Only a few isolates were resistant to trimethoprim/sulphamethoxazole (4.7%), ceftriaxone (7.0%), and ceftiofur (7.0%) were observed. PCR assays indicated the presence of *tetB* gene in all isolates, while 11(25.6%) and 4 (9.3%) isolates were positive for *tetD* and *tetA* genes, respectively. Neither in-feed nor in-water administration of CTC or tiamulin impacted the fecal prevalence and antimicrobial susceptibility of *Salmonella* in nursery piglets.



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Presenter: Cierra Roubicek

Evaluation of antimicrobial activities of phytophenols against bacterial pathogens that cause liver abscesses in feedlot cattle.

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Keywords: Liver abscess; Feedlot cattle; Phytophenols; Antibiotic Alternatives.

Abstract

Liver abscesses occur in finishing cattle fed high-grain, low-roughage diets. Cattle with abscessed livers do not show any clinical signs and are detected only at slaughter. Liver abscesses, which account for 67% of all liver abnormalities in cattle slaughtered in the US, are of major economic concern to the beef industry. *Fusobacterium necrophorum*, *Trueperella pyogenes*, and *Salmonella enterica*, particularly the serotype Lubbock, are the main etiologic agents. Currently, the control of liver abscesses is based on in-feed use of antibiotics. The emergence and dissemination of antimicrobial resistance to antibiotics use in animals are a public health concern. Plant-based phenolic compounds, called phytophenols, are known to have antimicrobial properties. Our objectives were to evaluate antimicrobial activities of phytophenols on the liver abscess bacterial pathogens. Phytophenols extracted from rosemary, green tea, grapeseed, organic goji berry, and green coffee were selected for testing. The phytophenols were extracted using 75% aqueous acetone and total phenolic content was determined by spectrophotometric analysis. Bacteria were cultured in Mueller-Hinton broth (*S. Lubbock* and *T. pyogenes*) or anaerobic brain-heart infusion broth (*F. necrophorum*) with and without phytophenols (1

mg/ml), at 6, 12, 24, and 48 hours and bacterial concentrations were determined by spread plate technique. If phytophenol was inhibitory, a micro-broth dilution method was used to quantify the inhibition. Phytophenols from green tea, grape seed, and rosemary inhibited *T. pyogenes*. Further studies are ongoing to investigate different concentrations of phenolic compounds. Phytophenols that inhibit the pathogens may have the potential to be used as a feed additive to prevent liver abscesses.



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Presenter: Harith Salih

Evaluation of sorghum phenolic compounds for their antimicrobial activities against liver abscess causing pathogens in feedlot cattle.

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Keywords: Liver abscess; Feedlot cattle; Sorghum phenolic compounds; Antibiotic Alternatives.

Abstract

Liver abscesses occur in finishing cattle fed high-grain, low-roughage diets. Infected cattle are asymptomatic and abscesses are not detected until at slaughter. Liver abscesses are of significant economic concern to the feedlot industry because of liver condemnation and their impact on cattle performance and carcass yield. The causative agents include *Fusobacterium necrophorum* subsp. *necrophorum*, *F. necrophorum* subsp. *funduliforme*, *Trueperella pyogenes*, and *Salmonella enterica*. Tylosin, a macrolide antibiotic, is supplemented in the feed to reduce liver abscesses. Because of the concern with emergence of potential antimicrobial resistance, there is a need to find antibiotic alternatives. Sorghum grain phenolic compounds, which are antimicrobial, have the potential to be an antibiotic alternative to control liver abscesses. Our study investigated the efficacy of phenolic compounds extracted from black, sumac, brown, and burgundy sorghum varieties on liver abscess pathogens. Phenolic compounds were extracted by 75% aqueous acetone and total phenolic content was determined spectrophotometrically. Muller-Hinton broth (for *S. enterica* and *T. pyogenes*), and

anaerobic Brain–Heart infusion broth (for *Fusobacterium*) with and without sorghum extracts (1 mg/ml) were used. Growth was measured at 24 and 48 hours to determine bacterial concentration. A micro-broth dilution method was used to quantify the inhibition, if the compound was inhibitory. Black and sumac sorghum phenolics inhibited growth of both *Fusobacterium* subspecies, *T. pyogenes* and *S. enterica*. Further studies are ongoing to investigate different concentrations and phenolic compounds from varieties of sorghum grains on the liver abscess pathogens. Sorghum phenolic compounds that inhibit the pathogens may have the potential to be supplemented in the feed to control liver abscesses.



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Presenter: Sydney Schnur

Relative isolation rates of phages lytic to *Fusobacterium necrophorum* from ruminal fluid and city sewage samples.

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Keywords: bacteriophages; *Fusobacterium necrophorum*; cattle; liver abscesses; antimicrobial alternative.

Abstract

Liver abscesses are a major problem in the beef cattle industry, currently controlled by in-feed tylosin. Phages that lyse *Fusobacterium necrophorum* in the rumen, the primary etiologic agent, are a potential antimicrobial alternative for prevention of liver abscesses. Our aim was to isolate phages that lyse *F. necrophorum* from bovine ruminal fluid and city sewage samples and perform comparative analysis on relative frequencies of isolations between the two sample sources. Pooled ruminal fluids at slaughter and pooled untreated city sewage samples were each collected on five separate occasions. Each sample, enriched with lysine and brain heart infusion medium, was screened for presence of phages on 48-59 *F. necrophorum* strains. Presumptive phage plaques were harvested, and the viruses purified. The phage isolation frequencies were compared between the source samples, sampling dates, and *F. necrophorum* strains. Thirty-one (11.5%) phages were isolated from ruminal fluid samples and 58 (22.7%) from city sewage samples. From each sampling date of pooled rumen fluid, phages were isolated with relative frequencies of 28.9, 16.4, 3.7, 0, or 6.3%. The relative frequencies of phages from each sampling date of city sewage were 24.0, 32.1, 12.0, 29.4, or 15.7%. Of the 50 *F. necrophorum* strains, 29 of the bacterial strains did not yield any phages, while the remaining yielded 1 to 6 phages. A few bacterial strains

yielded phages more frequently than other bacterial strains. In conclusion, city sewage samples had higher isolation rates of phages lytic to *F. necrophorum* compared to bovine ruminal fluid.



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Presenter: Haileigh K. Avellar

***In vitro* antimicrobial activity of equine mesenchymal stromal cells and platelet lysate against common clinical pathogens.**

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Keywords: equine; mesenchymal stromal cells; platelet lysate; antimicrobial.

Abstract

Septic arthritis is considered a medical emergency in equine patients. Disease following bacterial colonization can lead to significant morbidity and mortality while requiring costly treatment. Antimicrobial properties of regenerative therapies including mesenchymal stromal cells (MSC) and platelet products have been researched extensively in human medicine and show promising results. The purpose of this study was to evaluate bacterial suppression by equine platelet lysate (EPL) and adipose derived mesenchymal stromal cells (aMSC) *in vitro*. We hypothesized that both products would significantly inhibit the growth of *Staphylococcus aureus* (Sa) and *Escherichia coli* (Ec). Pooled blood from ten horses was used for production of EPL. MSCs were isolated from adipose harvested from the gluteal region of three horses. The study evaluated three treatment groups 10X EPL, 1.6 million aMSCs, and positive control (PC). The incomplete unbalanced block design with repeated measurements was

implemented. Optical density readings and colony forming units/ml were calculated at hours 0, 3, 6, 9, 12, 18, and 24. Decreased bacterial growth was seen at multiple time points for the *Sa*-MSC and *Sa*-EPL treatments supporting our hypothesis. Increased bacterial growth was appreciated in the *Ec*-EPL group with no difference in the *Ec*-MSC treatment which opposed our hypothesis. A clear conclusion of antimicrobial effects of EPL and MSCs cannot be made from this in vitro report. Although it appears MSCs have a significant effect on decreasing the growth of *Sa*, further studies are needed to explore these effects particularly in gram-positive bacteria.



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Presenter: Zachery Bieberly

Effect of proteinase inhibition on glucagon-like peptide-2 concentrations in blood samples from healthy cats.

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Keywords: Enteroendocrine; Peptide hormone; Proteinase inhibitor; Cats.

Abstract

Introduction: Glucagon-like peptide-2 (GLP-2) is a 33-amino-acid peptide hormone secreted by gastrointestinal (GI) L-cells in response to enteral nutrition. Measurement of circulating active (1-33) GLP-2 is challenging due to rapid cleavage by the enzyme dipeptidyl peptidase IV to inactive (3-33) GLP-2. Previous studies evaluating methods of GLP-2 collection in humans showed the use of proteinase inhibitors within the sample as the only method that delayed peptide degradation and resulted in higher measured concentrations. The objective of this study was to compare concentrations of GLP-2 in feline plasma collected with or without proteinase inhibitors.

Methods: Six healthy, client-owned cats were enrolled prospectively. A pre-prandial blood sample was obtained after at least a 10-hour fast, cats were fed a standardized meal, and a second 1-hour post-prandial sample was collected. Blood was collected into chilled EDTA tubes on ice and immediately centrifuged (temperature controlled), separated, and stored at -80° C. At the time of collection, half of each blood sample was mixed with 10% volume of the proteinase inhibitors Aprotinin and Diprotin A per sample volume. Plasma GLP-2 was measured with a commercial ELISA marketed for cats; all samples were run in duplicate. GLP-2 concentrations were compared between samples collected with or without proteinase inhibition using a paired t test.

Results: There was no significant difference in GLP-2 concentration between samples with and without proteinase inhibition (pre-prandial $p=0.96$; post-prandial $p=0.55$).

Conclusions: Findings may be due to differences in feline GLP-2 degradation compared to humans, ELISA specificity, or other sample collection variables.



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Presenter: Shanice Harris

Human neuron infection with Zika virus and Hybrid IgG4 antibody response to *Aedes aegypti* salivary proteins.

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Keywords: Zika; Dengue; virus; mosquito; salivary.

Abstract

Zika virus (ZIKV) and Dengue virus (DENV) are flaviviruses carried by the mosquito vector *Aedes aegypti*. ZIKV has been linked to the development of microcephaly in fetuses of infected pregnant women, along with other serious neurological complications. DENV is endemic in many of the regions affected by ZIKV, and it has been suggested that prior immunity against DENV may have an impact on ZIKV infection. The aim of this study is to determine whether pre-existing DENV antibodies bispecific for viral particles and *Ae. aegypti* salivary proteins act to decrease ZIKV infection of human neuron cells by activating anti-inflammatory pathways or increase infection through antibody dependent enhancement. Our results have shown that IgG antibodies against total *Ae. aegypti* salivary gland extract are much higher in exposed individuals living in DENV endemic areas than in non-exposed samples. In addition, there were fewer antibodies expressing λ light chain in exposed patients, indicating a bias in the immune response that is different than that seen in non-exposed individuals. Neuroblastoma cells will be infected with ZIKV strain MR-766 (original African strain) in the presence or absence of hybrid IgG4 antibodies in order to determine antibody effect on viral entry and replication inside the cells. It is anticipated that this study will suggest a vital role of pre-existing DENV immunity on ZIKV neuronal infection through hybrid antibodies and establish a better understanding of the neurological pathology of this disease.

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Student Support: Boehringer Ingelheim Veterinary Scholars Program



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Presenter: Bailey Maloney

Impact of hematophagy on antiviral responses in *Culicinae* mosquitoes.

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Keywords: hematophagy; mosquitoes; flaviviruses; Toll; Imd.

Abstract

Introduction: A majority of pathogenic flaviviruses are transmitted by mosquitoes in the *Culicinae* subfamily. These mosquito-borne flaviviruses initiate infections in competent mosquito species through engorgement of infectious blood meals. It has long been known that hematophagy is a critical step for the establishment of flavivirus infections; questions such as how blood feeding can impact the antiviral immunity of mosquitoes and the effect of blood feeding in the establishment of effective flavivirus infections have rarely been examined.

Methods: In this study, meals consisting of either bovine serum albumin protein solutions or defibrinated sheep blood were fed to female mosquitoes to evaluate the effects of hematophagy on antiviral gene expression in *Aedes* and *Culex* species mosquitoes.

Results: Significant differences in expression levels of Cactus and Caspar genes were observed between mosquitoes receiving sheep blood versus protein meals. These findings are consistent with previous reports of noted differences between infectivity of flaviviruses presented after ingestion of infectious blood and protein meals.

Conclusions: Our observations suggest that the immunological responses of mosquitoes may be modulated by blood feeding. The feasibility of applying this model to the investigation of vector-host interactions that modulate antiviral response mechanisms and the kinetics of flavivirus infections in mosquitoes is supported by the results of our study.



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Presenter: Danielle Saunders

Production of yellow fever virus 17D vaccine strain using overlapping complementary DNA fragments.

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Keywords: yellow fever virus; flavivirus; reverse genetics.

Abstract

Reverse genetic systems are powerful tools that enable the genetic manipulation of RNA viruses. Most existing reverse genetics systems depend on the insertion of complementary DNA (cDNA) fragments into bacterial vectors, which are then replicated using high-fidelity DNA-dependent DNA polymerase. This process is often laborious and technically challenging due to the toxicity associated with viral genetic material in bacterial hosts. Recently, the infectious subgenomic amplicons (ISA) method has proven to be an efficient and rapid reverse genetics system by transfecting overlapping cDNA fragments covering full-length genomes of positive-sense RNA viruses. The objective of this study is to evaluate the feasibility of applying the ISA method for the rapid production of live-attenuated vaccines for positive-sense RNA viruses. Using the yellow fever virus (YFV) 17D vaccine strain as a proof-of-concept, the successful recovery of recombinant viruses will demonstrate that live-attenuated vaccines for positive-sense RNA viruses can be produced using relatively simple molecular virological techniques. Different strategies were evaluated for the successful amplification and assembly of overlapping YF viral cDNA fragments and *cis*-acting regulatory sequences. Techniques that lead to the recovery of infectious viruses from mammalian cell lines suitable for vaccine production will be discussed in this presentation.



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Presenter: Savannah C. Stewart

Utilizing a genome-wide screen to identify host genes critical to Japanese encephalitis viral infection.

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Keywords: JEV; CRISPR; GeCKO; flavivirus.

Abstract

Japanese encephalitis virus (JEV) is a mosquito-borne flavivirus endemic to many Asiatic countries which circulates within swine and many species of wild birds. Japanese encephalitis virus is the leading cause of human encephalitis in endemic areas, and infections can result in long-term, neurologic impact in up to 50% of symptomatic patients. Apoptosis and redox cell-stress responses have been identified as major pathological factors within flavivirus infection. A better understanding of these genes, and their respective pathways, within the context of JEV infection, could pinpoint specific genes for future use in therapeutics. The goal of this project is to generate a genome-wide knockout library within two different cell lines: HEK293T and A549. HEK293T cells are less permissive to JEV infection in the early-stages, whereas A549 cells are permissive in all stages of JEV infection, which will allow for a comparison analysis for early infection. Libraries will then be exposed to JEV 14-14-2, an effective vaccine strain of JEV, and will screen knockout cells that survive the infection. This will help identify genes that play a role in JEV invasion, as well as targets for viral neutralization. Identification of genes that exacerbate the pathogenesis of JEV could lead to novel therapeutic targets.



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Presenter: Joshua Willix

Genome-wide screen of host genes that enable pathogenesis of Japanese encephalitis virus.

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Keywords: JEV; CRISPR; GeCKO; flavivirus.

Abstract

Japanese encephalitis virus (JEV) is a mosquito-borne flavivirus that exist in a zoonotic cycle involving swine and many species of wild birds. Japanese Encephalitis Virus is the leading cause of human viral encephalitis in endemic areas accounting for up to 68,000 clinical cases each year. Between 13,600 to 20,400 cases are fatal, most of them are children who suffer more adverse effects of the infection. Those that survive infections may have long-term, neurologic impacts in up to 50% of clinical patients. Currently, there are no antiviral treatments available for patients suffering from JEV infection. Apoptosis, inflammation, and endoplasmic reticulum responses have been identified as major pathological factors associated with flavivirus infection. Identifying these host factors and their respective genes that aid the pathogen during JEV infection could uncover novel targets for treatment. A technology that could identify these genes is Genome-Scale CRISPR Knock-Out (GeCKO) libraries. Cell lines that undergo library construction will generate knockouts affecting almost every gene within the genome. We will generate a GeCKO library within two different cell lines: HEK293T and A549. We will then infect the libraries with JEV and will screen knock-out cells that survive the infection. We hypothesize that the cells that survive the infection will have inactivated host genes that are deleterious during JEV infection. Further analysis will be conducted to determine if the identified genes will be involved with either viral invasion, replication, or viral exit. These target genes could uncover new targets for novel pharmaceuticals and antivirals to treat JEV infections.



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Presenter: M.J. Wenger

Sublethal effects on motility seen in *Amblyomma americanum* treated with lotilaner (Credelio®).

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Keywords: Tick; Acaricide; Lotilaner; Motility; Dog.

Abstract

Isoxazolines are a novel class of systemically-acting acaricides such that ticks must feed on the host to ingest the acaricide. We expect the pre-lethal effects of the isoxazoline lotilaner (Credelio®) to shut down normal physiological processes, which will result in reduced speed and duration of movement. To test the pre-lethal effects of lotilaner, six beagles were treated according to labeled dose bands and six were left untreated. *Amblyomma americanum* ticks were applied and groups of five ticks were removed after 2, 4, and 8 hrs. Upon removal, movement of each tick was recorded and motility parameters were analyzed in EthoVision XT software. A significant difference was observed at all three time points between treated and untreated ticks ($p < 0.0413$). Significant differences were observed between treated and untreated ticks for average mean velocity and mean maximum velocity only at 4 and 8 hrs ($p < 0.0001$). Within the treated group, motility of the 2 hr ticks was significantly different from 4 and 8 hr ticks for all three parameters ($p < 0.05$). The untreated ticks showed no significant motility differences between the time points ($p > 0.31$). Ticks treated with lotilaner showed a time-dependent decrease in motility as compared to untreated ticks. These data exhibit evidence that, while still attached to the animal, the neuromuscular processes of the tick appear to be shutting down, potentially reducing the risk of pathogen transmission to the host.



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Presenter: Ariel Carruth

Identifying *Cytauxzoon felis* infections through detection and evaluation of immunodominant *C. felis* antigens.

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Keywords: Cytauxzoon felis; Cytaux; cat; parasite; tick-transmitted.

Abstract

Cytauxzoon felis is a tick-transmitted apicomplexan parasite that infects mononuclear cells and erythrocytes of domestic cats. The mortality rate of cytauxzoonosis in domestic cats is close to 90%, with the cats that survive remaining persistently infected for life without displaying clinical signs. These carrier cats can serve as additional infection sources of *C. felis* for naïve ticks and further increase the risk of domestic cat exposure to *C. felis*. Our goal is to develop a diagnostic assay to identify carrier cats whose low level of circulating parasitemia lead to inconsistent results with nucleic acid detection methods. Our experimental approach includes probing protein extracts from *C. felis* infected tissues with serum from carrier cats, identifying immunodominant *C. felis* antigens using LC-MS/MS analysis, followed by expressing, purifying and evaluating select immunodominant antigens by western blot and ELISA. Currently, we have developed a real-time quantitative PCR assay against Cf76 to quantify infection levels in *C. felis* infected tissues and probed protein extracts from *C. felis* infected tissues with serum from carrier cats. We are also expressing and purifying Cf76 to use as a positive control to compare with novel immunodominant proteins identified by LC-MS/MS. The ideal immunoassay will detect carrier cats, including those with low levels of parasitemia that may go undetected by using

molecular assays, and rapidly diagnose *C. felis* infections earlier in the parasite's lifecycle so that the veterinarian can make informed decisions regarding patient treatment and welfare.



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Presenter: Macy Flowers

Evaluation of enrofloxacin and oxytetracycline to eliminate persistent *Anaplasma marginale* infection in cattle.

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Keywords: *Anaplasma marginale*; Cattle; Enrofloxacin; Infection clearance; Oxytetracycline.

Abstract

Introduction: Bovine anaplasmosis is a bacterial disease of cattle caused by the rickettsial pathogen *Anaplasma marginale*. Common clinical symptoms include anemia, lethargy, pyrexia, and death. Antimicrobial treatment of anaplasmosis historically relied on oxytetracycline; however, Baytril 100-CA1 (enrofloxacin) was recently granted conditional approval for the treatment of clinical anaplasmosis. In most cases, anaplasmosis challenged animals survive infection with or without treatment intervention and remain carriers of *A. marginale*, serving as disease reservoirs. This study was designed to investigate the efficacy of enrofloxacin and oxytetracycline to eliminate *Anaplasma marginale* infection in persistently infected steers.

Methods: Fourteen Holstein steers previously inoculated with a Virginia or KS2 *A. marginale* strain and confirmed positive using a PCR test targeting the *A. marginale* Major Surface Protein 5 gene were allocated to treatment groups receiving injections of enrofloxacin (Baytril100-CA1) or oxytetracycline (Bio-Mycin 200). The recommended dosage for each treatment was administered subcutaneously for five consecutive days (Bio-Mycin 200 at 4.5mL/100,10 mL/injection site; Baytril100-CA1 at 5.7 mL/100 lb, 20mL/injection site). Blood samples were collected daily during treatment and twice weekly following the last treatment to monitor *A. marginale* infection status using the quantitative *msp5* PCR assay.

Results/Conclusion: All seven steers receiving oxytetracycline and five of the seven steers receiving enrofloxacin remained positive for *A. marginale* infection. Injection site reactions were observed for some steers treated with oxytetracycline. Identification of effective antimicrobial-based protocols to clear anaplasmosis infection would provide producers options to reduce anaplasmosis transmission potential in their herd and retain valuable stock.



2021 Phi Zeta Research Day
College of Veterinary Medicine
Kansas State University
March 2, 2021



Presenter: Alyssa Toillion

Effect of protracted free-choice CTC-medicated mineral for anaplasmosis control on the antimicrobial resistance profile of *Escherichia coli* in beef cattle on a pasture setting.

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Keywords: anaplasmosis; antimicrobial resistance; cattle; chlortetracycline; *Escherichia coli*.

Abstract

Introduction: Anaplasmosis is a hemolytic, tick-borne disease caused by *Anaplasma marginale* which can cause clinical anemia and death in cattle. This is a disease of economic importance in not only the United States, but worldwide. Current control options are limited, and FDA-approved antimicrobial control options do not have a defined duration of use. A practical and routinely used anaplasmosis control method involves feeding free-choice chlortetracycline-medicated mineral in a pasture setting for several months. However, sub-therapeutic antimicrobial use poses the risk of expediting the development and dissemination of antimicrobial resistance in off-target commensal bacteria in the bovine gut. The objective of this study was to determine the antimicrobial susceptibility of *Escherichia coli* isolated from anaplasmosis endemic beef cattle herds provided different FDA-approved free-choice chlortetracycline-medicated mineral formulations.

Methods: A closed-herd, comprised of Hereford-Angus cows, naturally endemic for anaplasmosis, were grazed in four different pastures. Each cattle herd was randomly assigned one of four FDA-approved CTC-medicated mineral formulations (700, 5,000, 6,000, 8,000 g/ton) labeled for 'the control of active anaplasmosis' and provided their respective CTC-medicated mineral formulation for five consecutive months. Fecal samples were collected monthly from a subset of cows (n=6 or n=10) per pasture. *E. coli* was cultured and isolated from fecal samples and the micro-broth dilution method was used to determine the tetracycline minimal inhibitory concentrations.

Results/Conclusions: Despite prolonged administration of free-choice CTC-medicated mineral, tetracycline susceptibility of *E. coli* isolates were not significantly different between treatment groups/herds provided different CTC-medicated mineral formulations over the treatment period.



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Presenter: Laura Favreau

A proper place for DREADD: verification of chemogenetic surgery placement in rats.

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Keywords: Prelimbic area; impulsivity; DREADD; stereotaxic surgery.

Abstract

Introduction: A behavioral pilot study was completed to examine the role of the prefrontal region (PL) in rodents that had previously received a time-based intervention. The experimental group (n=6) received an inhibitory DREADD (Designer Receptors Exclusively Activated by a Designer Drug) virus to decrease activity in the region while the control group (n=6) received a sham control virus that did not effect activity. The experimental group made more impulsive choices, suggesting that the PL may be involved in behavioral impulsivity following intervention. However, the surgical placement and expression of the viruses must be evaluated to confirm these results. This project aimed to verify and evaluate the viral expression of both viruses in the PL.

Methods: Rats were euthanized and perfused, after which the brains were immediately extracted and soaked in paraformaldehyde for 24 hours followed by sucrose for 48 hours. Brains were then frozen with dry ice. Later, the brains were sectioned using the Rat Brain in Stereotaxic Coordinates Atlas (Paxinos & Watson) at 40µm thickness. Slices were stained with DAPI (4',6-diamidino-2-phenylindole) as a counterstain and viewed under fluorescence (Green Fluorescent Protein for sham group, mCherry for the active DREADD). We assessed the accuracy of placements and percentage of expression in the PL.

Results: While the data show promising trends, the placements were somewhat mixed in accuracy and expressions.

Conclusions: This suggests the need for replication and refinement of the current study.



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Presenter: Breanna Kriley

Time to failure of prophylactic topical timolol therapy in dogs with primary angle closure glaucoma.

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Keywords: glaucoma; canine; dog; prophylactic; timolol.

Abstract

Introduction: Primary angle closure glaucoma (PACG) is a heritable ocular condition in dogs manifesting as a sudden elevation in intraocular pressure (IOP) that ultimately results in irreversible blindness. While PACG initially occurs as a unilateral condition, the fellow eye is at risk for developing glaucoma at some point in the future. At the time of PACG diagnosis, the fellow (pre-glaucoma) eye should be treated prophylactically with topical medication to delay the onset of disease. Selective topical beta blockers are effective for such prophylaxis, but there has been minimal research on the efficacy of nonselective beta blockers.

Methods: Medical records from the Kansas State University Veterinary Health Center (2009-2020) were retrospectively reviewed to determine the time to failure of prophylactic treatment with timolol maleate 0.5% in the healthy eye of dogs diagnosed with PACG. Treatment failure was defined as the time when additional medications became necessary to control IOP.

Results: Thirty-four client-owned dogs, diagnosed with PACG based on histopathology or clinical signs and gonioscopy of the pre-glaucoma eye, were identified. Mean age at diagnosis was 7.8 years. Twenty-three cases experienced failure of prophylactic therapy at a mean of 7.2 months (range, 0.5-37.6 months); however, 11 cases had not yet experienced failure, based on KSU records (mean time since diagnosis: 30.1 months, range: 8.2-65.3 months) at the time of this abstract submission.

Conclusions: Preliminary results indicate that timolol therapy is effective at delaying the onset of glaucoma when used prophylactically in the healthy pre-glaucoma eye of dogs with PACG.



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Presenter: Jayden McCall

Safety and tolerability of African Swine Fever Virus subunit vaccine candidates in commercial pigs.

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Keywords: ASFV; subunit vaccine; protective antigens.

Abstract

Introduction: African Swine Fever Virus (ASFV) is currently the greatest threat to the global swine population. It has decimated the pork industry in much of Asia and developing a vaccine against it is of the utmost importance. While previous studies have shown that protection can be conferred using attenuated ASFV, safety issues have yet to be addressed. This study aims to identify the antigens responsible for conferring immune protection in order to inform development of safe and effective subunit vaccines for protection of domestic and feral pigs.

Methods: ASFV genes were PCR amplified from pcDNA constructs, restriction enzyme-digested, and then ligated into pDonR shuttle plasmid. The genes were validated by sequencing, shuttled into Adenovirus plasmid backbone (pAd5), and protein expression was evaluated using anti-FLAG antibody and confirmed by immunocytoometric analyses using ASFV convalescent serum. The pAd5 constructs were transfected into HEK 293A cells for virus assembly. The recombinant viruses were expanded to produce virus stock for immunization of swine.

Results: Forty-four recombinant adenoviruses encoding multiple ASFV antigens were successfully assembled and confirmed to contain the correct ASFV genes. The recombinant adenoviruses were shown to express the encoded antigens and the expressed antigens were shown to be authentic using ASFV convalescent serum. Safety, immunogenicity, and protective efficacy of a prototype ASFV subunit vaccine formulated using a cocktail of the recombinant adenoviruses will be evaluated in domestic pigs.

Conclusions: The assembled viruses will be used to formulate an experimental vaccine for immunization of pigs. Following prime-boost immunizations, the pigs will be challenged with wildtype ASFV.

Antibodies and T cells from survivors will be used to identify the ASFV antigens involved in stimulating immune responses that correlate with protection. Identification of the protective antigens will allow development of a safe and efficacious subunit vaccine for protection of domestic and feral pigs.



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Presenter: Carolyn Mark

Evaluation of CXCL1, S100A8, MMP8, and TNF as biomarkers of *Mycobacterium tuberculosis* infection and disease in humans.

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Keywords: Tuberculosis; biomarkers; CXCL1; S100A8; MMP8; TNF.

Abstract

The disease active pulmonary tuberculosis (TB) is caused by the infectious bacterium *Mycobacterium tuberculosis*. According to the World Health Organization (WHO), *M. tuberculosis* infects 2-3 billion people across the globe including 13 million in the United States alone. Unfortunately, there are not currently any blood-based, or serum biomarker, diagnostic tests that can differentiate between active pulmonary TB and latent tuberculosis infection (LTBI). Utilizing specific serum biomarkers as diagnostic tools to differentiate LTBI and active pulmonary TB can benefit a large variety of patients infected with *Mycobacterium tuberculosis*. Diagnostic tests using serum biomarkers can aid in the surveillance and maintenance of LTBI, and help these patients avoid unnecessary antibiotic use. This can help prevent the adverse effects of prolonged antibiotics and limit the development of antibiotic resistance. Patients with active TB will benefit by receiving a faster diagnosis and treatment. Our study tested 144 human serum from patients with active pulmonary TB, LTBI, and healthy patients for the biomarkers MMP8, CXCL1, S100A8, and TNF using antigen-capture enzyme linked immunosorbent assays (ELISAs). Our results show that MMP8, CXCL1 and S100A8 have potential as serum biomarkers to differentiate between active tuberculosis infections, latent infections, and healthy patients while TNF does not appear to have significant differences between the three groups. Once reference ranges are established, MMP8, CXCL1, and S100A8 can be used in conjunction with clinical signs and/or the TB skin or blood tests to quickly distinguish if the patient has an active or latent TB infection.

The Sigma Chapter of the Society of Phi Zeta wishes to acknowledge and thank our volunteer moderators and judges for the 2021 Phi Zeta Research Day.

Judges for Basic Science Research oral presentations:

Matthew Basel, moderator; Masaaki Tamura, Paige Adams

Judges for Applied/Clinical Science Research oral presentations (Small animals/exotics):

Raghavendra Amachawadi, moderator; Brian Herrin, Ronnie Elmore

Judges for Applied/Clinical Science Research oral presentations (Large animals):

Kathryn Reif, moderator; Andrea Dixon, Michael Sanderson

Judges for Basic Science Research posters:

Sally Davis, Moderator; Roman Pogranichniy

Judges for Applied/Clinical Research posters:

Katherine KuKanich, moderator; Michael Kleinhenz