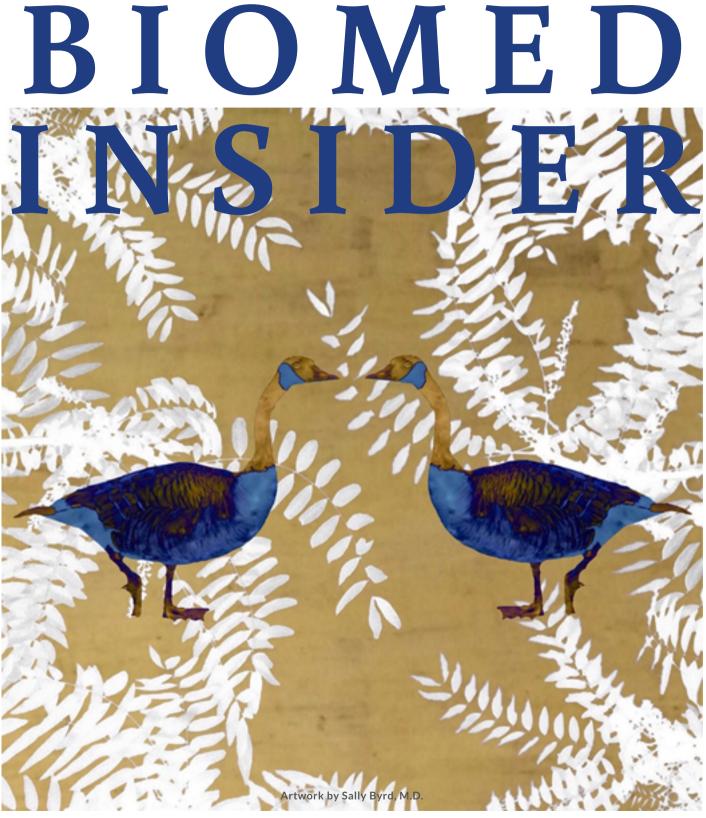
Carlson College of Veterinary Medicine Department of Biomedical Sciences



Oregon State University Carlson College of Veterinary Medicine



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### LETTER from Dr.Luiz Bermudez

Department Head of Biomedical Sciences Carlson College of Veterinary Medicine Oregon State University

This issue of this Biomed Insider features exciting research being carried out at the Carlson College of Veterinary Medicine. In it, one can have a glimpse of the contributions of young scientists to science, which brings up the topic of education for the next generation.

Veterinary education and graduate education are quite important. Many medical professionals (including Veterinarians) became pioneering scientists. Their stories described in many books, are able to convince many of us about the power of science to benefit the world. But, more importantly perhaps, is for the readings to trigger the curiosity about science in students and young professionals. Veterinarian scientists are indeed in need. The percent of veterinarians that pursue research is very small. They are needed to tease apart medical mysteries.

As the public increasingly trust pets as their inseparable companions, there is the need for basic and clinical research aimed to discover the mysteries of biology and medicine, unraveling some stubborn medical questions: Why do some heart fail and not others? Why autoimmune diseases are so capricious? Research in animals are excellent to guide the answers for many diseases, and ultimately help animals themselves and humans as well.

Science is a Sherlock Holmes game. We should create the interest in young veterinarians to play. We are developing one more program to address the need.



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### WESTERN DIET: IMPACT ON GUT HEALTH, METABOLISM, AND MITOCHONDRIA

The typical "Western diet" (WD) is described as high intakes in fat, sugar, sodium, and low in fiber. Well-known for its negative health effects to the human body, WD can lead to increased risk of obesity and metabolic diseases.

Type 2 diabetes (T2D) is the most common metabolic disease, seventh place on the list of "leading causes of death in the U.S." Gut microbiota is the community of microbes within the gut and is an essential role in patients with T2D developed by WD. It is critical to understand how gut microbiota plays a part in metabolic damages triggered by WD and find preventatives for T2D.

Regarding her research on host-microbe interactions under WD, Dr. Natalia Shulzhenko from the Carlson College of Veterinary Medicine decided to find what linked gut microbiota and metabolic changes together. Additionally, her and her colleagues studied how it affects host metabolism.

The study consisted of two mice groups, one group on a normal diet (ND) and the other one on WD. Mice on WD had T2D-like metabolic disease like human T2D. The microbes that were likely to contribute to T2D-related systemic changes in metabolism were measured. They identified microbes into two types: improvers and worseners. Improvers had a negative link with glucose levels and decreased in numbers on WD. Worseners had positive links with glucose levels and increased in numbers on WD. Four microbes identified to corresponding bacterial species:

Lactobacillus johnsonii (LJ), Lactobacillus gasseri (LG), Romboutsia ilealis (RI), and Rumincoccud gnavus (RG). LJ and LG identified as improvers. RI and RG rs, with RG

> linked to obesity. This discovery suggested that microbes and/or their interactions could be significant in T2D.

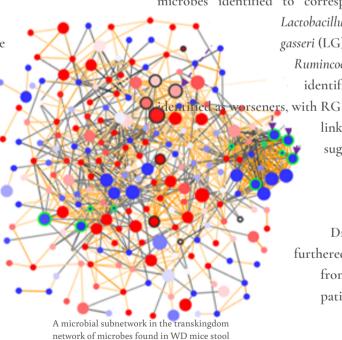
Dr. Shulzhenko and her team furthered their analysis by using data from another study with human patients that had similar WD to assess their predictions.

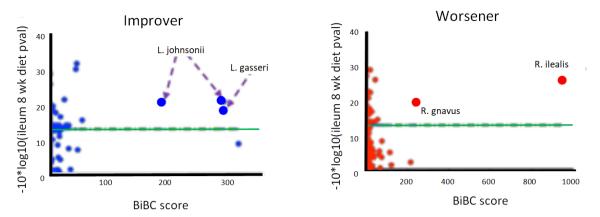
The study results showed a link between

BMI and the amount of microbes in obese humans. The amount of improvers had a negative link with BMI while worseners had a positive link, with RI present in more than 80% of the obese patients. Dr. Shulzhenko and her colleagues predictions had matched the study results and supported their predictions from the analyses in the mouse model on the improvers and worseners.

Previous studies have suggested a relationship

regarding the environment of gut mircobiota and host metabolism. To see if it was true, they performed an analysis on the microbiota found in the intestines and fecal matter from both mice groups. The results showed little correlation so they moved to another hypothesis. They used a transkingdom network analysis to identify specific microbes regulating metabolic parameters.





Both graphs show the measurement of microbes likely to contribute to T2D-related systemic changes in metabolism using their BiBC score. The graph on the left shows microbes that are categorized as improvers, with the *L. johnsonii* and *L. gasseri* bacterial species identified. Similarly, on the right side, the graph shows worseners, with the *R. gnavus* and *R. ilealis* bacterial species identified.

Other testing showed improved glucose tolerance in mice without changing insulin levels when WD-fed mice had more LG and LJ. The opposite happened to mice given with RI. They showed impaired glucose tolerance and reduced fasting insulin.

The liver and intestine was examined to see how LG and LJ made an impact in improving systemic metabolism. They analyzed global gene expression changes and focused on the genes that responded similarly to both microbes, identified by the genes expressed differently by LG and LJ, and WD groups. Most of the genes in the liver got upregulated from the LG and LJ supplements. A 'Nfil3 gene' found in the intestine got downregulated in LG and LJ supplemented mice compared to WD mice. The gene deficiency in the intestine was shown to prevent mice from obesity, insulin resistance, and glucose intolerance.

They conducted another analysis in the liver to find any biological pathways enriched in genes that normally would not be. Results displayed enriched genes related to mitochondrial function upregulated by LG and LJ. Genes belonging to the mitochondrial complexes that cells use enzymes to oxidize nutrients got upregulated in liver by LG and LJ supplements. The overall area of mitochondria was larger in WD mice, which suggests that swelling caused by WD can disrupt normal mitochondria functions. Improved mitochondrial health restores fatty acid beta-oxidation, a process that decreases build-up of harmful fatty acids in the liver.

Among the nineteen regulated genes from beta-oxidation, eighteen got upregulated by the increased amount of LG and LJ supplementation. Providing the two bacteria that got decreased by WD was sufficient for improvement of systemic metabolism. LG and LJ reduced lipids and improved mitochondrial function in liver, two inter-related and important processes that can lead to improved systemic metabolism.

The study shows the harmful effects a "Western diet" has on metabolism as demonstrated in improvers decreasing and worseners increasing in the gut microbiota, with each one acting via different host pathways. It has revealed new probiotic strains that have beneficial effects on liver mitochondria.

These findings will lead to future investigations for treatment of type 2 diabetes. It has provided important insights to mechanisms of probiotic behaviors and the opportunity to develop targeted therapies of diabetes.

### A BROCCOLI A DAY COULD



For those unfamiliar with the scientific field and community, there has been stigma towards the idea of clinical trials. There are concerns on pharmaceutical companies and their intent, while other concerns relate to the subjects of the trials.

Behind the scenes of most medical advancements is a group of participants. Before a treatment is approved for clinical trials, it must go through substantial vetting processes. Years of research and preliminary testing must be performed before being evaluated for clinical trials by regulation organizations such as the FDA and CDSCO. Nevertheless, millions of lives are saved from treatments approved through clinical trials - all performed by researchers whose main focus is to fight disease and improve overall health.

Dr. Kaitlin Curran is one of those researchers here at OSU. She is a clinical oncologist and veterinarian who stresses the importance of providing her patients and their owners with comprehensive treatment options. She prioritizes 'quality of life' during treatment process and has been collaborating with other researchers at OSU to conduct clinical trials intended to treat canine cancers. One of these trials involves testing the effect of fasting before and

### CHASE THE CANCER AWAY



after chemotherapy to determine how it improves sideeffects. What is amazing about the trial is that it requires no supplementation of an experimental medication, making it low-risk and cost effective. This can ultimately be something implemented for all canines undergoing chemotherapy.

A significant clinical trial Dr. Curran is working on is the development of natural broccoli seed supplement that may help control tumor cells spreading in canines with lymphoma. It has made mutual partnerships with the College of Pharmacy and the College of Public Health. They found that the broccoli seed supplement has an epigenetic impact on cell division by reducing the availability of DNA, which helps slow down the replication process and delay tumor growth.

Dr. Curran hopes that one day the supplement can assist in the treatment of actively diseased patients and be able to help in the fight for cancer prevention - all accomplished by simply taking your daily vegetables. The work Dr. Curran and her colleagues are performing may save lives one day. She believes that it may one day even translate to human medicine.

#### <u>UPDATE</u>

### RESULTS ON COMMERCIAL PLASMA AS TREATMENT FOR HORSES WITH GASTROINTESTINAL DISORDERS

In the Winter 2018 edition of the Biomed Insider, a study on commercial plasma as a treatment for horses with gastrointestinal disorders was featured. It has now come to an end. Dr. Erica McKenzie's goal was to determine the efficacy of commercial plasma for increasing colloid osmotic pressure (COP) and serum protein concentrations.

She found that transfusions with commercial plasma resulted in small, transient changes in total protein, albumin, and COP in diseased horses. It also found that over 20% of horses had reactions to transfusion. Given the relatively limited benefit and potential for side effects, alternative approaches to treatment may be considered. Dr. McKenzie has been concurrently working on a project evaluating vitamin E and selenium concentrations in horses here in Oregon. Her goal is to create more accurate education strategies for dietary supplementation.

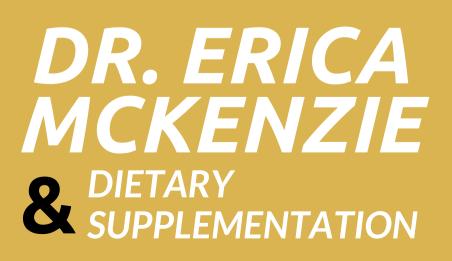
The pacific northwest is known for having a deficiency in soil selenium, resulting in dietary insufficiency and subsequent health issues. Horses are often deficient in vitamin E if they have limited access to green grass. Owners often compensate with selenium and vitamin E supplementation, but the success of these practices are in question.

To determine dietary levels for horses, Dr. McKenzie worked with Dr. Pital, a Master's student and resident in the Carlson College of Veterinary Medicine. They collected whole blood and plasma samples from adult horses and cross referenced the results in each horse's diet. The results concluded that although selenium and vitamin E were supplemented, deficiencies were still common.

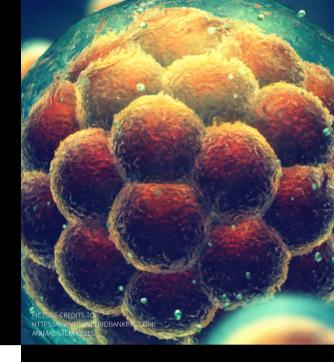
Dr. McKenzie is currently working to implement that knowledge by providing recommendations to Oregon horse owners. The study has been accepted for presentation at the upcoming ACVIM forum and has been presented at continuing education conferences for veterinarians and owners to further improve awareness. Providing educational tips and management strategies helps to not only reduce the prevalence of deficiency-related diseases, but allows owners to accomplish this in a cost-effective way.







# DR. JENNIFER JOHNS & MSCs



Stem cell research has been a hot topic in the scientific community over the past few decades. The ability to understand cellular differentiation has drawn attention for its applications in tissue development, regeneration, and immunological outcomes. Dr. Johns' research relates by showing that various cancers has shown to interact with the patient's own stem cells. From a holistic view, Dr. Johns is trying to figure how the development of osteosarcoma is impacted by the presence of mesenchymal stem cells (MSCs).

To perform this study, she has been collaborating with the oncology group to study tumor cells from the amputated extremities of canine osteosarcoma cells. The tumor signals growth of MSCs. These MSCs can then change the effectiveness of the anti-tumor response of the patient's white blood cells.

The mechanism of cross-talk between MSCs and osteosarcoma cells and the resulting impact on immune response is still under investigation. Dr. Johns is confident that their work will help to elucidate these important interactions. Long-term, the research has the potential to help both canine and human osteosarcoma patients.

The potential for stem cell research is undoubtedly intriguing. As researchers like Dr. Johns delve deeper into their understanding, greater medical applications are simultaneously uncovered for the future of veterinary medicine.

### ABOUT DR. JOHNS

Dr. Jennifer Johns is a veterinarian who has devoted her entire career to finding ways to improve animal health. She is both a DVM and PhD (Comparative Pathology), with a specialty in Clinical Pathology who also teaches histology and pathology for first-year students.

In her earlier years of her career, Dr. Johns continued her initial work on tickborne infections, but today her research involves something a bit different. Her focus has now shifted to research involving stem cells. She is working on finding out how mesenchymal stem cells (MSCs), a stem cell that differentiates into various tissues, impact disease and patient health.

### **ROCKEY LAB: UNDERGRADUATE**

Undergraduate research is a key element of the research community within the Carlson College of Veterinary Medicine (CCVM). These undergraduate students all worked in the lab of Dr. Dan Rockey, a professor in the college who conducts microbiology research. Their stories are great examples of what students can get out of a research experience and how it provides valuable help in addressing both human and animal health and wellbeing.

Will has started working with Dr. Rockey from fall 2011 up until his graduation in Spring 2015. He spent all three summers at OSU in Dr. Rockey's lab. Will began as a biochemistry/biophysics major at OSU. During his first term, he met with his undergraduate advisor to discuss potential labs where he could be involved with research, which led to a meeting with Dr. Rockey.

The time in the lab was important to Will's career. He says, "Coming to graduate school, I was exceptionally well prepared for my coursework as well as the lab work I would be expected to do. Because of the opportunities I received in Dr. Rockey's lab, I was quickly able to become independent and have had a great deal of success." Will has been successful – he has authored several works in graduate school and is lined up for an excellent research opportunity upon completion of his Ph.D.



Will Valiant Honors Bachelor of Science Microbiology, 2015

During his undergraduate research experience, Will was a key participant in research that led to a successful grant proposal and a publication in the laboratory. When asked about memories from his time in the lab he recalls the trip, "Lab-lympics: Competitions included petri-dish Frisbee, dry ice tube rockets, rubber glove sling shots and parafilm art work!". Will also spoke of lab "journal clubs" where undergraduates would add breadth and depth to their understanding of microbiology.



Debbie Kim Honors Bachelor of Science in Mircobiology, 2018

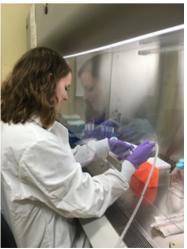
Debbie is currently a medical scribe and is working on the application process for medical school. She worked in the laboratory between 2016 and 2018. Debbie remains an important contributor to the Rockey laboratory's ongoing National Institutes of Healthsupported project in chlamydial biology.

Dr. Rockey says, "Debbie is still active in the group – she comes back to the laboratory

occasionally to assemble genome sequence data important for a publication on which Debbie will be an author. Her contribution to that endeavor is significant and greatly appreciated." Debbie also presented her honors thesis work at the 2018 American Society for Microbiology meeting in Atlanta. She has a long list of memories of her time in the laboratory, but one stands out: "Dry ice volcanoes we made to celebrate Fridays!" Molly Herinckx worked in the Rockey laboratory her entire time as an undergraduate honors student. She graduated in June 2019 with a Bachelor's in Biochemistry and Molecular Biology. Since then, she has been working as a research assistant at PDX Pharmaceuticals, a small biotech company based in Portland.

She plans to pursue a PhD in either immunology or cell biology after another year at the company.

Molly says, "My time in the Rockey lab taught me many essential lab skills and techniques, but more importantly than that, it helped me develop a love for research and prepared me well for starting my research career."



Molly Herinckx Honors B.S. in Biochemistry & Molecular Biology, June 2019

### **RESEARCH HIGHLIGHTS**



Stormy Scharzenberger Honors B.S. in Animal Science, June 2016

Stormy is currently a CCVM student and she will graduate with her DVM in Spring of 2020. She got started in the laboratory through the OSU Pre-Veterinary Scholars program, which works to connect Honors students with researchers in the college. Stormy began her work in the Rockey Laboratory in January 2014, another example of an undergraduate who worked in the lab for her entire undergraduate career. She successfully completed her thesis work and presented her work at a national veterinary conference.

As a DVM student, Stormy has continued the work in the Rockey Lab. She will use her research to complete her senior project, a requirement for CCVM students to complete before graduation. She is currently exploring career opportunities in large animal medicine and veterinary public health.

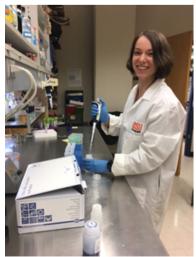
Stormy has a few specific memories of her time in the lab. " I recall having ice cream cake at lab meetings to celebrate our achievements (example: vet school admission). Defending my thesis at the end of my undergraduate career was a highlight, and I valued presenting my work at the 2017 AVMA National Convention in Indianapolis, Indiana."

Dr. Rockey has great memories of Stormy as well. "This is an individual who had very specific goals when she started, and worked hard to both achieve those goals and to be a genuine participant in laboratory science. She is probably the most organized person I have ever met in my life!"

Four undergraduates- Michael, Savanna, Molly, and Addison work in the laboratory. They are pictured with Steven Carrell, who has had a presence in the laboratory as an undergraduate researcher, a faculty research assistant, and a graduate student during his current doctoral work in the Comparative Health Sciences graduate program. As Dr. Rockey says, "As faculty members, one of our major responsibilities is career development. How can we assist students in identifying career goals and making sure we contribute to their achieving these goals? Undergraduate research in the Carlson College of Veterinary Medicine is a major way we can help students move these objectives forward."



Left to Right: Michael, Savanna, Molly, Addison, and Steven



Savanna Avila-Crump B.S. in Biochemistry, June 2019

Savanna Avila-Crump worked in the Rockey lab for two years from the end of her sophomore year until graduation. Savanna graduated in the spring of 2019 with a BS in biochemistry and a minor in chemistry.

Since then, she has worked as a lab manager in a biochemistry laboratory on campus. Savanna is an example of an individual who did not quite know what she wanted to do in life, but used undergraduate research to help sharpen her focus and build confidence in a career as a scientist.

"When I first started working in a lab, I struggled to believe in my ability to succeed in science-- I always felt like I was a little behind, or not good enough-- I experienced what many refer to as imposter syndrome. In the Rockey lab, I was supported by my fellow lab members who not only taught me valuable technical skills but also believed in me and helped me build confidence as a scientist."



Andrew Schlueter B.S. Zoology 2015 DVM June 2019

Andrew graduated from the CCVM in June 2019 and is currently an intern at Louisiana State University, which will lead to a career in veterinary oncology. He worked with Dr. Rockey from 2013 to 2015. When asked about how he ended up working with Dr. Rockey, Andrew says, "I wanted experience to help my vet school application and I heard from a friend that there was an opening in the lab. This was important, because the opportunity helped get me into veterinary school on my first application and allowed me to present my data at the American College of Veterinary Internal Medicine Forum."

Andrew has a few memories from his days in the group. "During the summer of 2014 the entire lab went down to the river and had a barbecue and floated down the river. Scientifically, there was a moment when our PCR sequencing results came back unusual. We had identified a genus of bacteria that had not been not seen in the US. It wasn't a "Eureka!" moment, but it was memorable because we all said 'Hmmm... that's really weird." That event led to a publication on which Andrew is an author.

Dr. Rockey says, "Andrew is an excellent example of how research can positively affect the path of an individual as they sort out there career. I came to depend on him as a very productive and interested member of the laboratory."

Emaan is currently working as a medical scribe in both a pediatric clinic and an emergency department. She has attended the Wake Forest University Physician Assistant program this last May. Emaan completed her honors thesis research in the laboratory from January 2015 to December 2017. She says that her research in the laboratory prepared her for a future career as a health sciences professional.

"Although I am not pursuing a career in research, I know that my time in the Rockey Lab helped me grow as an individual and made me a stronger candidate for Physician's Assistant schools. Much of medicine is evidence based, so having an understanding of research methodology is necessary to providing effective patient care. I also learned important skills in teamwork, leadership, and public speaking. I have many amazing memories from my time in the Rockey Lab, but one of my favorites was a lab trip to Sahalie Falls over the summer of 2017. Another is presenting my honors thesis project at the 2017 American Society of Microbiology Conference in New Orleans, LA."



Emaan Khan Honors Bachelor of Science In Mircobiology, 2017

Dr. Rockey has high praise for the Emaan's work. "Emaan's work in the laboratory was critical to our groups development of preliminary data for a project involving Oregon sheep farmers and chlamydia-associated apportion. She was a significant contributor in the laboratory."



Current undergrads in the laboratory: Makenna Browne (senior), Addison Deboer (Junior) and Jake Roetcisoender (Freshman), all in the Biochemistry/Biophysics Department.

At the Carlson College of Veterinary Medicine at OSU, continuous assistance is offered to developing scientists to improve their technical skills, broaden their exposure to the biology of infection, and clarify their personal goals as they move to a career in the health sciences.

As faculty members, one of their major responsibilities is career development. Undergraduate research in the CCVM is a major way we can help students move toward their goals.

"Conducting biomedical research in the Rockey laboratory taught me to contribute to human and animal welfare simultaneously, directly through husbandry and indirectly via zoonotic disease control. My early research experiences opened the door to a wealth of opportunities, ranging from presenting at the AVMA National Convention to participating in the UDSA Smith-Kilborne Program on foreign animal diseases. I also developed important skills in critical thinking, leadership, and public speaking through my roles in the

laboratory."

STORMY SCHARZENBERGER Honors BS in Animal Sciences, OSU

### FISHING FOR ANSWERS

Commonly known for its role as a sexually transmitted disease, Herpes Simplex Virus (HSV) has become somewhat of an epidemic in recent decades. As of 2017, the Center for Disease Control notes that genital HSV infects one in six people between the ages of 14-49. Genital HSV is not the only type of herpes an individual can contract. Dr. Ling Jin, a professor of virology in the Department of Biomedical Sciences, studies herpesvirus pathogenesis and viral-host interactions. Her research has been focused on HSV in patients infected via non-sexually transmitted mechanisms and how they permanently remain within the body.

HSV can be contracted by various means and as of right now, HSV is not curable. The host immune system may have the ability to resolve active symptoms, but it does not actually clear the infections. Instead, the virus goes into a state



of hiding. Understanding how HSV remains latent is a crucial aspect of Dr. Jin's research. Though not completely understood, research has shown that HSV infects neuronal tissue, aiding in its evasion of the hosts immune system. For non-sexually transmitted infections, latent HSV resides primarily in the trigeminal ganglion. It resides until the environment is suitable for the virus to 'reactivate.' Symptoms of reactivated HSV from the trigeminal ganglion include lip sores and tissues damage to the eye, commonly causing blindness in children and elderly.

#### "THE BIGGEST OBSTACLE TO ADVANCING HSV RESEARCH IS FINDING AN ADEQUATE HOST TO STUDY IT."



Mice have been the standard model for HSV research. However, analysis of infected host cells requires dissection of neruonal tissue and samples of peripheral blood, neither of which are excess in a mouse model. A mouse's immune system is also very susceptible to HSV, killing a number of them during the infection process. With such a constraint, Dr. Jin sought out a more suitable host. Coincidentally, while helping to study a herpes outbreak in late 2000, she stumbled upon an unusual host that mimics human herpesvirus infection in the immune system - Koi fish. These fish also mimic humans in their ability to survive the infection, allowing better research samples without termination of the host.

So far, Dr. Jin has found that stress factors are a key environmental marker for latent herpes reactivation. Furthermore, a number of genes have been uncovered in latency and reactivation. The capabilities of the Koi model may now place Dr. Jin as the frontrunner of herpesvirus research with regards to latent infections. Although it may take some time, Dr. Jin's studies could lead to the development of a treatment against latent infection of herpesvirus.

# PREPARING AN ATTACK

*Mycobacterium tuberculosis* may be the most notable mycobacterial pathogen in humans, but non-tuberculosis (NTBs) bacteria plays a role in important diseases in a wide range of animal species.

At the Department of Biomedical Sciences, a large portion of research focuses on studying different species of mycobacteria. Dr. Lia Danelishvili is a leading researcher in the study of NTBs, investigating the molecular These immune cells are specialized phagocytes that ingest and kill invading organisms and process bacterial components to create a larger immune response to the host.

For tuberculosis, the macrophage engulfs the bacteria, but is unable to kill it. This allows the pathogen to reside in it and continue to promote pathogenicity. Dr. Danelishvili began with investigating immune responses that can promote the killing of So far, the study has produced a number of promising antigens that can initiate immune responses to weaken the pathogenic mycobacterial growth within macrophages.

While antigens are continuing to be isolated, Dr. Danelshvili and her group tests these antigens across different pathogenic mycobacterial species to come up with universal strategies to eliminate infections caused by both human and animal pathogens.

"MORE ANTIGENS NEED TO BE UNCOVERED TO REPLICATE THAT INNATE IMMUNE RESPONSE AS CLOSE AS POSSIBLE TO THE ONE THAT WE OBSERVE DURING *M. SMEGMATIS* KILLING."

system of intracellular infection and the host-pathogen interaction. Recently, her projects have focused on uncovering bacterial surface molecules that involves mycobacterial recognition and activation of the host innate immune system.

Mycobacteria has an evolutionary ability to infect the host's immune system and make it inactive. How it happens is unclear, however it is known that mycobacteria resides and grows within macrophages. mycobacteria. Her studies led her to a species called Mycobacterium smegmatis. It can be recognized by macrophages and killed within it. They wanted to characterize the cellular processes of the host response to find what aspects of M. smegmatis triggers these killing mechanisms. The process can be somewhat challenging and to challenge, overcome this Dr. Danelishvili used a complex technique that makes mycobacterial antigen isolation more stable and easy to work with.

The results have been promising, but Dr. Danelishvili says, "more antigens need to be uncovered to replicate that innate immune response as close as possible to the one that we observe during *M. smegmatis* killing."

Her hope is to create an 'antigen bank' that encompasses the most of proteins involved in macrophage activation as it is seen during *M. smegmatis* killing. It is a complicated process, but it may lead to possible treatments that can help to fight a large variety of mycobacterial diseases.



## THESE HIPS DON'T LIE, RIGHT?



An x-ray of a dog with CHD before surgery

A recent addition to the Carlson College of Veterinary Medicine is Dr. Jeffrey Biskup, a veterinary surgeon who focuses on small animal orthopedic and neurologic surgeries. As such, one of his primary interests is improving the care for patients with orthopedic injuries. Recently, his focus broadened towards a common disease in dogs - canine hip dysplasia (CHD).

CHD is looseness in the hip that leads to arthritis and abnormal stances or movements (lameness). It can be from genetic predisposition and other factors such as being overweight. Patients can develop chronic lameness that can be painful and lead to an inability to perform normal activities if untreated. Thanks to their bilateral nature, patients with CHD can still be active during development, showing no symptoms or abnormalities. This can be challenging for dog owners to recognize and can lead to a late diagnosis of the disease, making treatment difficult. "The challenge is awareness," says Dr. Biskup. Fortunately for patients with CHD, treatment options are available.

There are two primary treatment types: 'medical management' and surgical repair. Medical management is common for dogs with mild disease that involves adjusting the lifestyle of the patient to accommodate for CHD. It includes modifying diet and exercise habits to improve body mass and strengthen the lamed or atrophied muscles. It can also involve providing medications to reduce inflammation and manage pain.

Patients often show improvement but require frequent check-ups. If medical management no longer improves clinical signs, surgery is considered. One of the most common surgeries performed is a 'femoral head ostectomy'. It involves the removal of the femoral head to eliminate bone contact and decrease pain. However, the function of the limb will not be normal beacuse the joint is removed.

Dr. Biskup recommends a total hip replacement for more active dogs. The surgery can be difficult, but it has the highest efficacy in overall improvement for pain and function. He emphasizes the importance of educating pet owners on hip dysplasia and obtaining an early diagnosis if possible. CHD is the most common orthopedic disease in dogs but it continues to be under-diagnosed. This involves having an early baseline exam, especially if a puppy is an at-risk breed or showing clinical signs. They include lameness or abnormal movements at a young age, difficultly rising, stiffness, and decreased activity.

Dr. Biskup hopes in the future he will be treating more dogs with CHD, not beacuse of increased incidence, but because of increased recognition. This in turn should increase early treatment, allowing more dogs to live and have more fulfilled lives.

#### An x-ray of a dog with CHD after surgery



# Student Research Spotlight

#### ANNABEL MARTIN-VARNUM

Annabel worked with Dr. Kathy Magnusson and Dr. Maude David on the oral administration of Chaetomium elatum in mice to induce changes in They anxiety-related behaviors. discovered the effects of C. elatum to be sex-dependent, with males exhibiting greater anxiogenic effects. They will work on finding the underlying effects by assessing and analyzing different testing methods. In addition, samples will be collected from the intestines of selected subjects and used to analyze host tissues.

Mice brains will get dissected and recordings will be performed in one brain hemisphere to characterize functional correlates of the observed anxiety phenotype. Synaptic strength and plasticity will be tested using multielectrode array recordings. The other brain hemisphere will undergo RNA sequencing.

#### MARILYN VO TRAN

Marilyn examined how a small protein, termed NEDD8, causes degradation of another protein within a cell when the two proteins got fused together. Often, NEDD8 is coupled with particular proteins within the cell which can change the target proteins' functions.

She worked with PhD student Kartikeya Vijayasimha and found that under certain circumstances, NEDD8modification could target protein destruction. Her results got submitted for publication.



#### SHANNON KATHLEEN QUINN

Autism Spectrum Disorder (ASD) is caused by a combination of genetics and environmental factors. A link between the gut microbiome and ASD has been found in mice. Among several potential biomarkers, *Clostridium celatum* is a gram-positive anaerobic bacteria enriched in children with ASD.

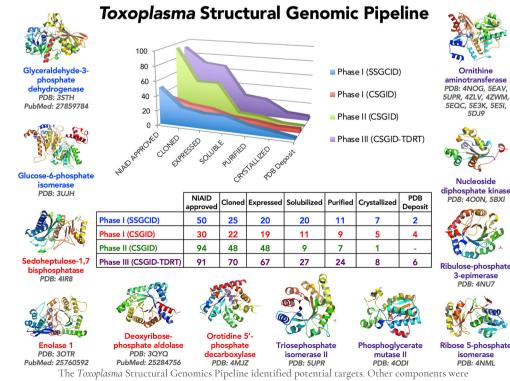
Over the summer of 2018, Shannon worked as an undergraduate intern under Dr. Muade David and Dr. Kathy Magnusson. She worked on a project titled: Behavioral effect of *C. celatum* on anxiety. She would help mate the mice, assist with injecting the mother's PolyIC or NACl, and wean the pups. Once the mice were ready for testing, Shannon fed the mice with apple juice or apple juice laced with bacteria. She also performed various behavioral tests on each mouse, analyzed the results, and assisted in dissections.

### PARASITE ALERT: FUTURE ERADICATION OF TOXOPLASMA GONDII

*Toxoplasma gondii* (*T. gondii*) is a single-celled parasitic organism that can infect animals and humans. It is commonly found in contaminated foods and cat feces. The Centers for Disease Control and Prevention states that over 40 million people in the U.S carry the parasite.

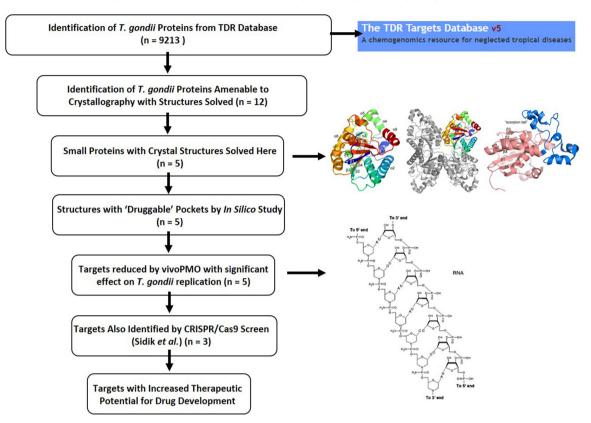
*T. gondii* infection leads to a disease called toxoplasmosis. Typically, people will not experience any symptoms since a healthy immune system keeps the parasite from causing the illness. Even so, anyone with a weak immune system can have serious symptoms. *T. gondii* can grow and reproduce inside the cells of a host when the host is infected. This parasite has two vital life stages in humans, tachyzoites and bradyzoites. The tachyzoite stage causes acute

infections, but treatment is available. That is not the case for the bradyzoite stage. This stage is found within tissue cysts and is the dormant life stage responsible for reactivation of clinical disease. New therapeutic targets and medicines are needed as current treatment is limited by toxicity and hypersensitivity. Dr. Hong Moulton and her colleagues have decided to investigate that idea and work on furthering the possibilities. They hypothesized that using PMOs (nucleic acid-like molecules with no charge that can inactivate expression of specific genes) and linking them to a cellular delivery moiety would decrease expression of these enzymes. That down regulation would decrease the replication of those molecules. They could determine if an enzyme contributed to parasitic replication and could use it as a potential therapeutic target.



integrated to find specific target enzymes.

A summary figure showing drug discovery pipeline of *T. gondii*. The TDR (Tropical Disease Research) database gave important initial insights of potentially vital parasite proteins. Integrating that information to the other components shown in the boxes identified the targets with improved therapeutic potential.



Thev began by constructing a Toxoplasma Structural Genomics Pipeline and combining that data with several other components to identify and characterize potential drug targets. Of all the potential drug targets matched, five soluble enzymes were chosen: phos-phoglyerate mutase II, nucleotide diphosphate kinase, ribulose phosphate 3-epimerase, ribose-5phosphate isomerase, and ornithine aminotransferase. They cloned the gene coding of the enzymes,

crystallized them, and characterized their structures. From there, they performed a study via computer simulation to discover the possibility of the enzymes having a binding association to a drug.

They also used vivoPMO for efficacy testing and toxicity testing. VivoPMOs can decrease gene expression by disrupting the interactions between snRNP and RNA. Using vivoPMOs could help them determine if particular enzymes take part in the replication of the parasite. Subsequently, it would indicate them as potential therapeutic targets.

A survey was conducted to find the average phenotypic scores that were completed in a screening with a family of DNA sequence. Negative scores indicated a likelihood of the target enzymes being a significant contributor to the parasite's fitness and viability.

The results exhibited the five enzymes having a successful predicted binding association to a drug and parasite replication. The study created an effective method to find molecular targets and could be useful for recognizing small molecule inhibitors. This information could help figure a therapy with a less toxic molecular transporter. Developing new methods for the treatment of both active and latent infection may make it possible to cure human *Toxoplasma gondii* infection and Toxoplasmosis.

### **CATCH OF THE DAY**

The zebrafish as a model for study and research began in the 1960s. Their significance has increased since. Check out these two studies below regarding the zebrafish!

#### A NOVEL ZEBRAFISH MODEL

Morpholino oligomers are known research tools. These uncharged acid-like molecules can be useful in molecular biology for changing gene expression. Nonetheless, they lack proper systemic delivery into cells. Although advancements have been made to counteract delivery problems, it remains as the leading obstacle of morpholinos to be used as effective treatment. These slow advancements are partly because of the cost of the animal models used for screening and the cost of materials to use. There is a demand on costeffective vertebrate models for evaluating in-vivo (performed inside of a living organism) delivery of morpholinos.

Dr. Hong Moulton and her colleagues were able to constructe a 'transgenic zebrafish model that included a dual reporter cassette.' It could determine in vivo delivery, safety of morpholinos, and tracking the location of the compounds. Measuring the amount of morpholino delivered to various tissue cells can be found by the changes in reporter gene expressions. They did this successfully in the blood stream to have exon skipping in the heart of an adult zebrafish.

#### **PARASITE DETECTION**

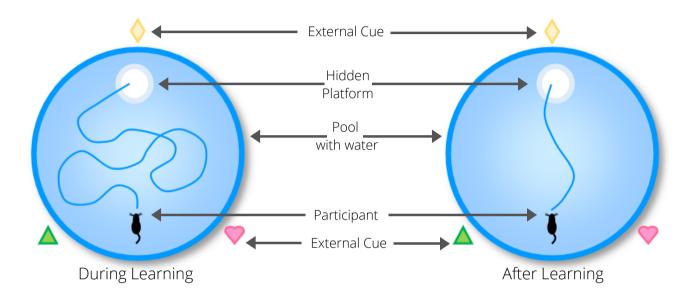
Getting accurate environmental screening for pathogens relies on lots of different components. For an environmental screening to have useful data, it is vital to have a fitting sample size and know when to collect them - especially in the aquatic environment. To figure the dynamics of pathogenic evolution and transmission in screening tank water for the pathogen, *Pseudocapillaria tomentosa*, the timing of the infected zebrafish host has direct indications on environmental detection testing sensitivity.

Many studies have measured eDNA (environmental DNA) shed by organisms in the environment to distinguish and quantify species in that area. When using that measure with other methods combined, it can quantify eDNA to make a comparable amount of a particular organism in a sample with precision. Dr. Justin Sanders and his colleagues has come up with a 'droplet digital PCR based' test to find and quantify *Pseudocapillaria tomentosa* eDNA through the course of the diagnostic setting. They found a relationship between the parasite development in the intestines of the zebrafish host, the amount of eDNA present in tank water, and the transmission dynamics.



### MEMORIES IN THE WATER

A Morris Water maze (MWM) is a common task used in behavioral neuroscience to study spatial learning and memory. Commonly used with rodents, participants are placed in a pool of opaque water and must find a hidden platform using external cues.



Professor Dr. Kathy Magnusson of neuroscience at OSU conducted a study between cognitive functions tested across ages. Female and males participants were grouped into young (18-31) and old (60-86). They were seated in front of a computer and took several tests that measured their cognitive levels. Additionally, they conducted a virtual MWM that included several trials: long-term memory, working memory, reversal, and visible control trials.

For the long-term memory, participants were unaware the platform remained at the same spot for the first 12 hidden platform trials. They performed another 12 trials after being aware. In the working memory trials, they were aware of the platform moving every two trials. After finding the platform, they would have 15 seconds to look for it again. In the reversal trials, they were aware the platform would be placed somewhere else.

The results showed that awareness on the platform during the hidden trials corresponded with various cognitive functions,

similar to the working memory trials. The average time spent near the platform area correlated with Logical Memory immediate recall. Reversal trials showed a relationship to Logical Memory recall after 50 minutes. The study suggested the virtual MWM tasks are applicable for memory testing.

Her team conducted another study with virtual MWM tasks to examine the age-related differences and the impact of noncombat military service in older males on cognitive flexibility and spatial memory. The tests remained the same and the results showed that older males performed noticeably worse than young males on Logical Memory.

Veterans did way worse than civilians on the same task. Older males performed worse than young males in the visible trials. For long-term memory, veterans performed worse than civilians. Older male veterans did worse in reversal trials than older male civilians. Their findings from the study suggests negative impacts that military service has in studies of cognitive aging and can be a hidden variable to it.

# **BEARS HAVE HERPES?**

Herpesviruses are members of *Herpsviridae*, a family of DNA viruses. They can cause infections and diseases in animals and humans. Although the number of human herpesviruses are low, many other types of herpesviruses affect animals.

Seldom has there been record of herpesvirus infection in bears. There has been a study that found presence of a gammaherpesvirus in several captive sun bears, but no successful gammaherpesvirus culture from bears has been recorded.

Dr. Ling Jin and her colleagues took on the challenge to investigate herpesvirus in bears. They were able to detect and find partial characterization of gammaherpesvirus from multiple black bears with and without neurological diseases from Oregon, Nevada, and California, USA.

They sampled various tissue cells from the bear and purified any virus particles of infected cells to view under an electron microscope. Tissue DNA was separated using a tissue DNA extraction kit. When they collected the tissue cells of black bears from Nevada, they cultured them to find out whether infectious viruses were present. There were no visible structural changes caused by viral invasions in those cells. However, they did find out bears with central nervous system (CNS) clinical signs from Neveda had herpesvirus-like capsids (protein shells of a virus) in the infected cells. Herpesvirus-like particles was also present in the infected cells from a bear in Oregon with no CNS clinical signs. They analyzed DNA samples from the bears

for herpesviral genome and detected pieces of amplicons. More black bears were tested to see if herpesviral genome was in bears without clinical neurological signs.

The team found positive amplicons, but they were not present in all the tested tissues and tested bears. They matched the DNA sequence alignments of amplicons and found a 94% identity to bear gammaherpesvirus 1 (UrHV-1). Black bears from California and Nevada were tested for amplicons from lymph node DNA. The DNA sequence alignments of amplicons also matched to UrHV-1. To find how common this UrHV-1-like virus is in black bear population, they used a technique to analyze various tissues in the bears collected from Oregon, Nevada, and California. Most of them tested positive, which indicated that UrHV-1 and black bear herpesviruses have similar DPOL (DNA polymerase) DNA.

Interestingly, they found that human rectal epithelial cells are susceptible to this bear herpesvirus infection. Under an electron micro-scope examination, herpesvirus-like capsids and particles were observed in infected cells found in the colon. Capsids were also observed in tissue culture infected with bear fecal samples. Many of them had similar form to rotavirus and coronavirus infection, hinting that coronaviruses may be common in black bears. They found positive amplification from all the tested tissues in bears with neurological signs. Yet, fewer tissues tested positive in bears without clinical signs, and in some instances, no tissue tested positive in bears without clinical signs.

The tissues which tested positive in the brains of non-neurological bears were often spleen and lymph nodes. It is possible this black bear herpesvirus persists in these lymphoid rich tissues, like many other gammaherpesviruses. Dr. Jin and her colleagues tested that hypothesis using white blood EDTA samples collected from three other bears from Nevada and Oregon with Positive no neurologic signs. amplication with UrHV-1 specific primers was observed in total DNA of white blood cells.

Dr. Jin and her colleagues speculate that the virus may merely have been present in lymphocytes associated with the carcinomas. They believe at least two different black bear herpesviruses exist in free-ranging black bears and they may be close to UrHV-I. However, further investigation will be needed to determine if a specific black bear herpesvirus is associated with neurological disease in black bears.

# HUMPS, BUMPS, & LUMPS

Feline injection-site sarcoma is one of the more serious adverse effects following vaccination in cats. They are tumors that occur in bones and soft tissues. They appear at locations where a cat has had an injection.



Feline injection-site sarcoma (FISS) is rare but can be clinically challenging to manage with the current standard of care. Cats will typically have to undergo surgery that involves skin removal or amputation along with chemotherapy and radiation for effective treatment. There is not enough knowledge on the tumors so it is vital to learn about FISS and how to effectively treat and manage it. Dr. Stephen Ramsey and his colleagues worked on the first mRNAsequence study of feline neoplasm (abnormal growth of tissue) to be documented. It can lead to more crossspecies approaches to study other types of feline cancer.

Using tumor transcriptome profiling can identify the molecular basis of cancers which can be useful for new therapeutic targets. Dr. Ramsey and his colleagues used that approach of profiling to profile normal tissue samples and FISS tumors to compare against each other using a sequencing technique. Using this approach, they found over 3000 transcripts with changed abundance compared to the normal skin and FISS tumors.

They analyzed the mRNA sequence data to compare cancer to normal gene expression levels and find changes to chromosome structures of DNA sections in FISS. They also wanted to find molecular pathways and biological processes linked with FISS development. The analysis found 17 molecular pathways or biological processes with genes enriched in gene sets expressed differently in FISS that got upregulated and downregulated. Furthering their study, they performed a cross-species analyses at the level of cancer to normal gene expression ratios to compare FISS transcriptome to those of soft-tissue sarcomas in dogs and humans. They found a three-way overlap between the tumor and normal tissue; 53 genes got upregulated and 38 genes got downregulated.

With those genes, Dr. Ramsey and his colleagues analyzed them using data from a screening and found 11 drugs and four drug targets that could potentially provide valuable information for the treatment of FISS.

Their study shows the potential of comparing oncology to improve knowledge and treatment of FISS. It also opened up cross-species studies for future research opportunities.

# THE GOOD AND THE BAD

African Buffalo are one of the wildlife species where infections are found among their population. The infections can transfer by direct contact from animal to animal. One of the known infections they have is bovine tuberculosis, a disease that typically affects the lungs.

A fundamental obstacle in disease ecology is incorporating biological process across scales. Epidemiologists work hard to find methods to predict and control disease patterns in natural populations that result from different host immune responses influenced by genetic variation and integrating environmental factors.

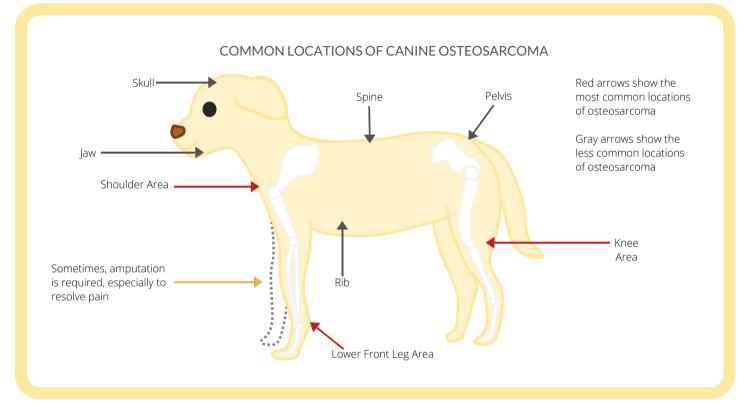
What is interesting is that disease has an influential role to evolution, but the effects of it can be different for individuals. Throughout history, there are trade-offs to resisting infections in evolution. It can contribute to the reproductive and immune interests of individuals. However, observing patterns of disease transmission and the effects require studies in natural populations unlike immunology and genetics, which are commonly studied in model systems. Dr. Anna Jolles and Dr. Brianna Beechler both conducted a study with their colleagues on the resistance of disease. They focused on bovine tuberculosis, a chronic bacterial disease, in African buffalos.

Dr. Anna Jolles and her colleagues studied wild African buffalo and found the genetic coordinates of two genes. They are close to genes that contribute to large white blood cell activation and pathogen degradation that increases the risk of bovine tuberculosis infection up to nine times. They got measurable variation in resistance at multiple scales through different patterns of disease in the buffalo. Dr. Brianna Beechler and her colleagues focused on the pros and cons of traits that resist bovine tuberculosis in a group of free-ranging African buffalo. They observed two heritable forms of resistance to the disease. The phenotype that allow the hosts to prevent or delay infection identified as 'infection resistance'. The phenotype that limits the infected lesions from spreading identified as 'proliferation resistance.'

However, the downside of having those phenotypes include reduced body conditions and survival once infected but linked to higher reproductive rate overall. The study was evident to a connection of heritable disease resistance traits and life history variation.



### MAN'S BEST FRIEND AND SIDEKICK



Osteosarcoma is the most common type of bone cancer that starts off in the cells that form bones. It affects humans and dogs. Canine osteosarcoma is aggressive in dogs. Current treatment methods often yield poor results. By the time of diagnosis, the cancer cells will have typically reached to other body parts. To tackle down this disease for both humans and dogs, understanding the factors that contribute to the disease's progression is crucial. There has been evidence that indicated that serotonin plays an important part for normal bone physiology.

Dr. Patrick Chappell, an assistant professor at the Carlson College of Veterinary Medicine, demonstrated the importance of serotonin receptors. With prior knowledge, his team sought an approach that regarded to other developing tumor subtypes. They hypothesized that osteosarcoma tumors may develop a neuroendocrine phenotype. They used canine osteosarcoma cell lines that were isolated in a controlled environment and cells from spontaneous tumors in dogs. They investigated two neuropeptides that was associated closest with re-production: kisspeptin and gonadotropin-releasing hormone. Neuropeptides were assessed and looked into their gene expression. The assessment showed several canine osteosarcoma cell lines that secreted gonadotropin-releasing hormones and kisspeptin. These two neuropeptides also wield notable effects on growth and gene expression.

Both neuropeptides were discovered to increase expressions of a particular serotonin receptor, which has been shown to associate with cellular growth. Using serotonin and gonadotropin-releasing hormone antagonists successfully reversed the growth effects. Those neuroendocrine hormones could be the next targets to look into for further research and potentially for osteosarcoma treatment.

## RISING PATHOGENS

*Mycobacterium avium* subspecies *hominissuis* (MAH) is a subspecies from the genus *Mycobacterium*. MAH is a nontuberculous mycobacterial pathogen that infects humans and other mammals. In humans, it affects the lungs and will lead to lung disease. People who have a weak respiratory system or respiratory disease are more susceptible to MAH infection; yet, it is not to say that healthy individuals will not get affected. Nonetheless, the number of patients with nontuberculous mycobacterial lung infections are increasing.

Treatment for MAH infection requires an extended time period that can include therapy and multidrug treatments. Even with prolonged treatment, complete elimination of MAH only occur in 40% to 60% of patients. The lack of response to therapy is due to the bacterium being able to enter a non-replicating persistent state that makes it tolerant to antibiotics. To combat this issue, Dr. Luiz Bermudez and his colleagues sought to find answers that could lead to new treatment methods. Their study aimed to find global proteomic remodeling of MAH with various environmental conditions of the host with and without treatment. They wanted to establish and target important also

bacterial pathways linked to MAH tolerant/persistent phenotype. They tested a widely accepted hypothesis in which suggests: "to overcome unfavorable environmental conditions and killing effect of antimicrobials, bacteria require expression and synthesis of specific subsets of proteins that play important role in adaptation within new conditions and because of that, contributing to a long-term survival of a pathogen".

Dr. Bermudez and his colleagues tested that hypothesis. They conducted a proteomic study under various conditions to identify the database of proteins in MAH with and without exposure to antimicrobials. Along with that, they wanted to find metabolic changes promoting MAH tolerance in environments that the pathogen encounters within host cells. They found that under certain conditions in the lung along with a rich environment of substrates, MAH can activate several alternative pathways to support its metabolic activities. Several novel targets were identified with the potential to assist in killing of MAH at more rapid pace. The study conducted brought significant information on MAH response to various host environments and MAH response to therapy.

#### WHAT IS IN THE HOSPITAL?

Bermudez, student Hanna Dr. Shoen. and their colleagues conducted a study to better characterize the epidemiology of Staphylococcal infections in a teaching hospital, where people can increase possible transmission of infectious bacteria in the hospital setting. They found prevalence of Staphylococcal infections in the OSU Veterinary hospital. It highlights the importance of these pathogens as 'hospital acquired

infections' in veterinary hospitals. They used bacterial submission records from the hospital to the diagnostic laboratory in 2010 to 2015. Swabs were taken from the small animal ICU and the "anesthesia prep room."

Of the 998 clinical isolates submissions, around 23% identified as *Staphylococcus* species. Antibiotic resistance and susceptibility profiles were examined. The study showed possible trans-mission of genetic components of the pathogens. Antibiotic resistance can spread from animals to humans but vice versa as well. This could suggest that hospital personnel are potential carriers of infectious pathogens. It is important to understand the issues in antibiotic resistance and cleaning protocols. Future additional studies should investigate water sources in the surgical room as a potential cause of infection.



### HERBS, SPICES, AND HEALTH SURPRISES



Aeromonas salmonicida is a pathogenic bacterium that severely impacts salmon populations and similar species. Such bacterial infectious diseases are a threat to farmed sablefish, resulting in death. Treatment options are still not fully developed. Although antibiotic supplement feeds exists, those could cause the creation of pathogens being resistant to antibiotics. To look for more effective and sustainable treatment options, Dr. Carla Schubiger, an assistant professor over at the Hatfield Marine Science Center. looked into this problem.

Her team came up with a project to explore how herbal supplements and marine probiotics can strengthen the sablefish's innate immune response to improve their survival during infection and/or disease outbreaks. They wanted to evaluate herbal supplements and dietary probiotics, combined and alone, to trigger the immune system and improve growth. Their research focused on developing an eco-friendly treatment for sustainable sablefish production. They chose purple coneflower and turmeric for this project.

Purple coneflower is said to boost the immune system and turmeric, commonly used in cooking, has proven health benefits. It has the potential to prevent heart disease and cancer. It is also potent in anti-inflammatory and antioxidant. Dr. Schubiger and her colleagues worked on developing their custom formulas for feeding in Seattle. The custom feed included one formula with 33% turmeric and another with 6% purple coneflower. After feeding, they began an infection trial and infected one fish from each feeding group with *Aeromonas salmonicida*.

They found that fish fed with the purple coneflower formula showed significant improved survival rates compared to all of the feeding groups. The fish fed with turmeric did not rank has high within the other groups. The results could be due to the low percentage of turmeric in the formula as compared to the percentage of purple coneflower in the other formula. The project is still ongoing and they are excited to try new diets in the future to find a more ecofriendly treatment for farmed sablefish.

### LETTER from Courtney Wilson

Director of Development Carlson College of Veterinary Medicine Oregon State University

It has been a wonderful year since arriving in Corvallis and at the Oregon State University Foundation in 2019. I am originally from the East Coast, and before moving to Oregon, I was at the Virginia-Maryland College of Veterinary Medicine as the Associate Director of Development. I have had the pleasure of working within veterinary medicine for almost four years, and cannot even touch all of the things I have learned and continue to learn.

One of the biggest impacts faculty have is through research. Their research produces groundbreaking discoveries and gives hope to continue solving the world's most challenging animal, human, and environmental health issues. Over the past year, I have had the opportunity to meet with faculty members of the department and learn about current research, why the research matters, and how I can connect the right donors to their projects. I will continue these meetings as they have helped me learn more about the scientific world and grow as a fundraiser.

Donors give because they are passionate about a specific cause, issue, disease, etc. When I know more about the work our researchers and faculty are involved in, I can better connect our community and excite them about what makes Carlson College of Veterinary Medicine a leader in research and veterinary medicine.

The moment donors give to our college, they are part of a bigger mission. They can help our researches and scientists solve and understand a multitude of topics: the biological behavior of canine sarcomas, creating fibrocartilage-like tissue, probiotics and their influence on the immune response and the gut of the microbiome, and how certain drugs affect epileptogenesis and their influence on the disease progression.

I am only a small part of the college. Working together, we can make a vast difference for animals and humans alike. As I continue to learn about research and projects, I am excited to see the change we can make through a great partnership.

Contrue M. Wilm

Editor: Amy Ma

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