

BioMed Insider

College of Veterinary Medicine
Research Newsletter

cvmresearch@oregonstate.edu



Artwork by Sally Byrd, M.D.

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Letter from Dean Susan J. Tornquist, DVM, MS, PhD, Dipl ACVP

Research and discovery are crucial elements of the College of Veterinary Medicine's mission and we are fortunate to be in a position where we can contribute to the health and well-being of animals, public health, and the environment, in both basic and applied or translational ways. It is also very important that we provide training and insight regarding the methods and role of research to our students, so they become lifelong members of a well-informed scientific community. We train comparative scientists at the CVM and this gives them the tools to study disease in one species and to use that knowledge to improve the health of other species.

We are proud of the work done by CVM faculty, staff and students in a variety of areas including cancer, infectious disease, metabolism and nutrition, aging, neurosciences, food safety, diseases of wildlife, and surgical techniques, among others. We have been receiving more and more national and often international attention for our work. This is important, as scientific knowledge must be disseminated to maximize its impact on animal and human health. This is achieved through publications and presentations to both fellow scientists and non-scientists.

Our research program depends heavily on collaboration with those in other departments within the College, with other colleges at the University and with colleagues at other universities, companies and institutions around the world. This is an excellent way to expand our resources and to expand our thinking to incorporate great ideas wherever we can find them. We have also recently greatly expanded our participation in clinical trials, which is a naturally great fit for our program as we can test and evaluate the efficacy and risks of potential new treatments for animals.

Research at the CVM fits well with the One Health framework and its impact is felt globally.

***Dean Susan J. Tornquist, DVM, MS, PhD, Dipl ACVP
College of Veterinary Medicine
Oregon State University***

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The Scope

“How does Chlamydia persist in the body for many years?”

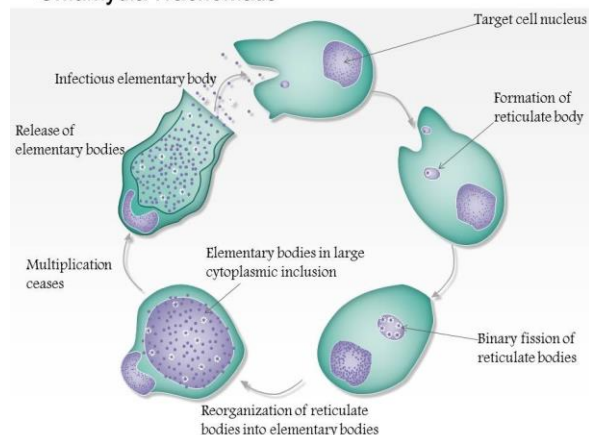
The biology of recurrent or long-term infection by Chlamydia organisms is not well known. Dr. Rockey's laboratory has been interested in answering the question of stability of the Chlamydia in patients with chronic infection.

It is an important question, because in many examples the pathogen can survive in the host body for long time by changing the molecules in their surface. So, when the host comes up with specific defense, the Chlamydia has changed and those defense strategies do not work any longer.

Dr. Rockey's group, in collaboration with researchers in the University of Washington used isolates from the same patient, followed for 5 to 7 years. They sequenced the whole genome of the isolates and asked the question: Does latent isolates from the patient differ from the initial ones? What they discovered is that Chlamydia, in contrast with many other pathogens, does not change overtime. Even against antibiotic treatment.

So, the conclusion is the Chlamydia evolved a different strategy to be able to persist in the host that is not associated with antigen change. The paper was published in the April issue of the Journal of Infectious Diseases.

Chlamydia Trachomatis

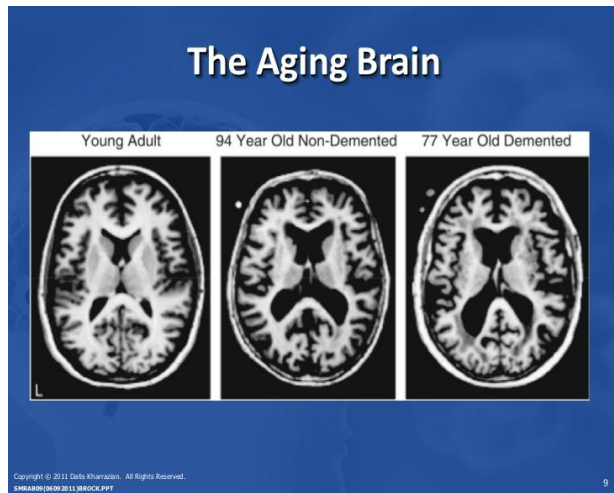


“How Safe It is to Use?”



Johnathan Denherder, a resident of the Laboratory Animal Medicine program at OSU always questioned the common practice of using stored analgesic compounds at small doses in rodents. Buprenorphine is a partial μ -opioid agonist used for analgesia in people and animals. Buprenorphine HCl is often diluted with sterile water or saline for use in smaller species, such as laboratory rodents. Protocols for storage and beyond-use dating of diluted buprenorphine vary by institution and there is little information available on the stability of these preparations. Dr. Denherder and his colleagues decided to determine the chemical and microbiological stability of buprenorphine diluted 1:10 with sterile bacteriostatic saline and stored for a maximum of 180 days. Diluted samples were stored in clear glass vials or plastic syringes, protected from light, and maintained at refrigerated or room temperature. We found that all samples remained sterile (by culturing them in aerobic and anaerobic conditions) for as long as 180 days. According to the analysis, samples stored in glass vials remained above 90% initial concentration regardless of storage temperature. The concentration of buprenorphine stored in plastic syringes however, declined to less than 20% initial concentration at room temperature and 72% initial concentration in the refrigerator after 180 days. Their data showed that carefully prepared dilutions of buprenorphine remain above 90% initial concentration and microbiologically stable when stored in glass vials for up to 180 days. Based on the rapid drop in concentration in our study, the authors do not recommend storage of diluted buprenorphine in syringes for any length of time.

“Altered synaptic localization of C-terminal splice variants of the GluN1 subunit of the NMDA receptor in the hippocampus of old mice with impaired spatial memory”

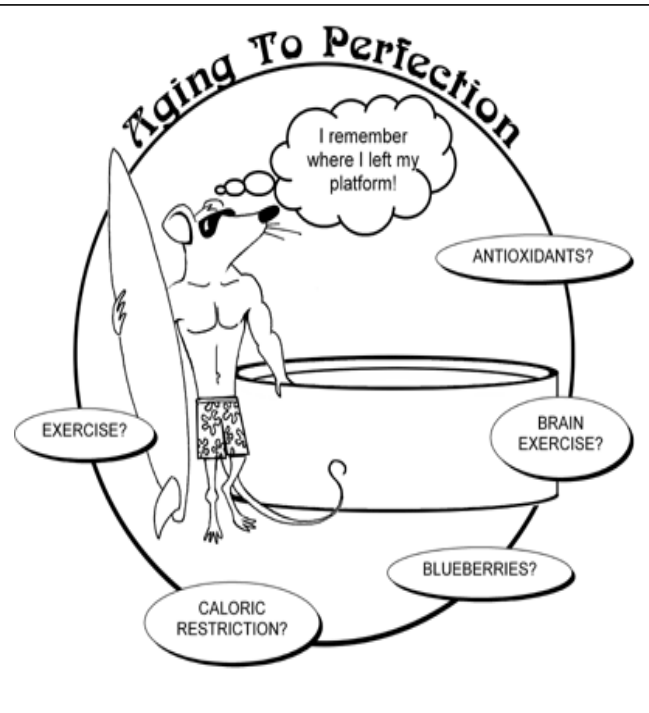


The N-methyl-D-aspartate receptor (NMDAR) is important for memory formation and is particularly vulnerable to the effects of aging. The GluN1 subunit of the NMDAR has 8 splice variants. There are two C-terminal splice cassettes, C1 and C2. When C2 is spliced out, there is a new terminal sequence, C2'. Age-related alterations of splice cassette expression in the frontal cortex are associated with spatial memory impairments.

In this study Dr. Magnusson and her group explored whether changes in C-terminal splice cassettes in the synaptic and extrasynaptic membranes of the hippocampus were related to age-related spatial memory declines. Two ages of mice, 3 and 24 months, were behaviorally tested in the Morris water maze. The old mice were divided into two categories (good and poor learners), based on reference memory performance of young in place trials (threshold = mean + 2SD). The hippocampus from each mouse was subjected to differential centrifugation, followed by solubilization in Triton X-100. Proteins from Triton insoluble membranes (synaptic membranes), Triton soluble membranes (extrasynaptic membranes), and intracellular membranes/cytosol were examined by Western blot.

Although old mice designated as good learners performed worse than young, the old mice assessed as poor learners were significantly worse than both the young and good old learners in place learning. The old good learners showed impairments in reversal trials, suggesting that improved memory in older individuals comes at the cost of reduced flexibility. The significant changes in the GluN1 splice cassettes were confined to the old poorer learners.

The protein expression of the C2 cassette was significantly higher in the old poor performers than the old good learners in the synaptic membrane of the hippocampus, but was reduced from young levels in the extrasynaptic membrane. In contrast, the C2' cassette was more prevalent in the extrasynaptic membrane in the old poor performers than both young and old good learners. There were no significant effects of aging or learning ability on the C1 cassette or GluN2B or GluN2A subunit expression patterns in the hippocampus. These results suggest that there are alterations in the trafficking of NMDA receptors in a subset of old mice that show the most impairment in spatial memory. These changes could impact synapse stability and NMDA receptor potentiation.



The Scope

“Rodent models to intervene in changes initiated by status epilepticus”

A seizure is characterized by excessive abnormal and hyper-synchronous neuronal discharges from the brain that manifests in physical symptoms. The area of the brain affected determines the type of seizure symptoms. Hence, many types seizures exist depending on the localization of seizure foci (in one or both hemispheres of brain) and depending on the sensory or motor regions of the brain, which can manifest as non-convulsive seizures (absence seizures), convulsive seizures (generalized tonic-clonic seizures) and so on. During a seizure episode, there is a clear demarcation in time such as a start and later, an end of seizure activity. However, during an extreme form seizure referred to as *status epilepticus* (SE), seizures occur in an uncontrolled manner, for a prolonged period, leading to a life threatening medical emergency. SE is regarded as an extreme form of seizure, occurs for a sufficient length or repeated seizures without recovery in between episodes. International League Against Epilepsy (ILAE) classifies SE in two scenarios, a continuous seizure activity without recovery for 5min or more or a continuous seizure activity for 30min or more with some recovery in between episodes. There are diverse causes such as head injury, chemical toxicities (pesticide poisoning), tumors, genetic causes can trigger an event such as SE. The effects of SE can be devastating producing immediate and long-term irreversible damages to the brain resulting in cognitive impairment, along with an increased risk of developing epilepsy (a disease characterized by unprovoked recurrent seizures).

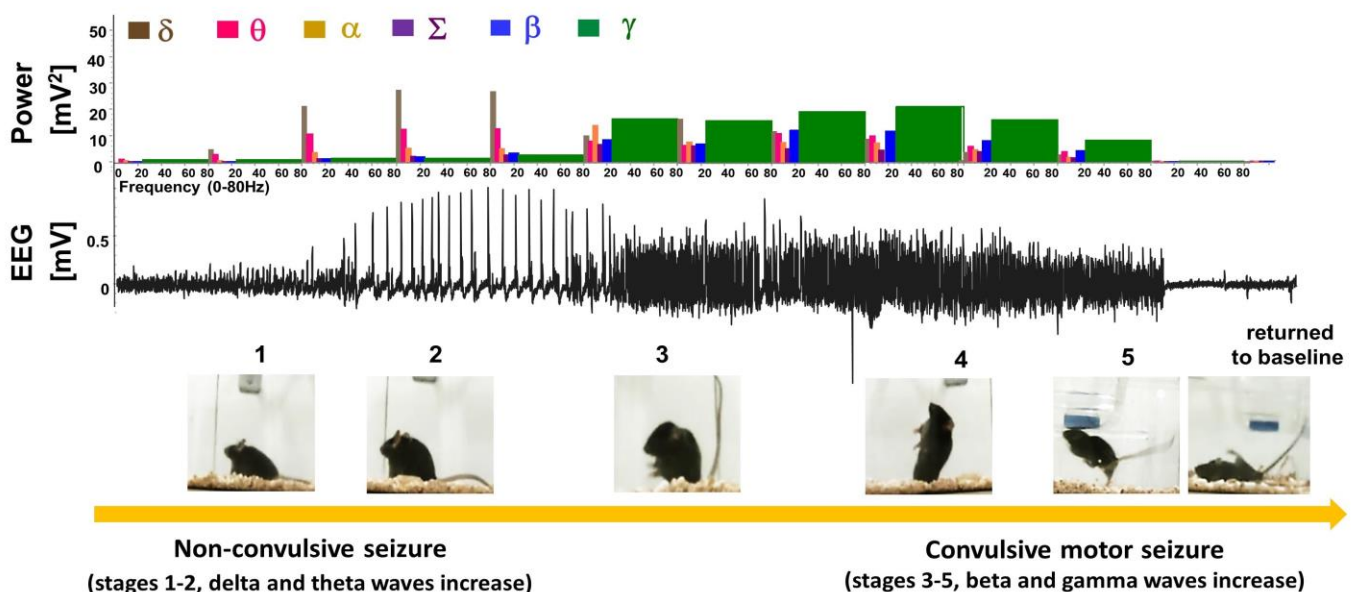
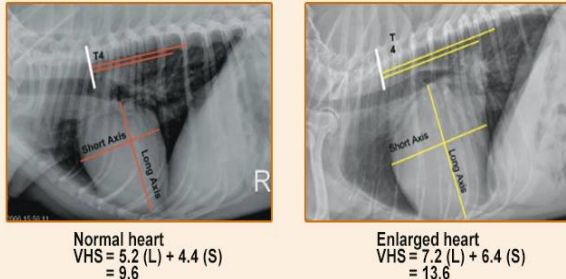


Fig.1. A single seizure episode showing increase in severity of behavioral seizure stages during a kainic acid induced SE. Stages 1 (freezing) and 2 (hunched rocking) are non-convulsive seizure stages. The convulsive seizure include stages 3 (forelimb clonus with rigid tail), 4 (rearing and falling) and 5 (generalized tonic clonic convulsions) shown in photographs (Tse et al 2013).

Rodent models have played a fundamental role in modelling SE to understand its pathology. In our past research, we have used chemoconvulsants such as kainic acid and organophosphates such as diisopropylfluorophosphate (DFP) to model SE. We are focused on identifying behavioral and electrographic characteristics of SE (Fig.1) in different species, strains of mice, and chemoconvulsants to understand commonalities in the process of epileptogenesis that leads to epilepsy (abstract submitted to Frontiers in Neurology, June 2017). Our research helps to understand the immediate and long-term impacts of SE in order to identify novel drug targets for intervention.

The Scope

“Reader Performance of Radiographic Left Atrial Enlargement In Dogs”



Accurate assessment of the left atrial (LA) size (cavity of the heart) is integral to the evaluation of left-sided cardiac disease in dogs and provides risk stratification for the interpretation of cardiac disease. Thoracic radiographs are routinely used to subjectively assess the presence and severity of left atrial enlargement (LAE). Dr. LeBlanc and her team at OSU's College of Veterinary Medicine have decided to investigate the interreader agreement of the image in the radiographs.

This retrospective study identified dogs that underwent thoracic radiographs and an echocardiogram on the same day at the Oregon State University Veterinary Teaching Hospital between 2012 and 2014. All thoracic radiographic views were anonymized and reviewed by 2 board-certified cardiologists, 2 board-certified radiologists, and 2 small animal rotating interns.

Echocardiographic LA size was evaluated objectively by the left atrial-to-aortic root ratio (LA:Ao) and LA volume (LAV) via the monoplane modified Simpson's method of discs (MOD). LAV was indexed to body weight as mL/kg. Objective measures of LA size were stratified into categories of normal ($LA:Ao < 1.5$; $LAV < 1.3$), mild ($1.5 \leq LA:Ao < 1.7$; $1.3 \leq LAV < 2.35$), moderate ($1.7 \leq LA:Ao < 2$; $2.35 \leq LAV < 3.4$), or severely enlarged ($LA:Ao \geq 2$; $LAV \geq 3.4$). Interreader agreement of radiographic LAE evaluation was assessed by linearly weighted kappa (κ) and intraclass correlation (ICC). Sensitivity (Se) and specificity (Sp) of identifying varying degrees of echocardiographic LAE on radiographs was assessed using both LA:Ao and LAV.

101 dogs were included in the study. Agreement for the presence or absence of radiographic LAE was excellent between all readers. The interreader agreement for the degree of radiographic LAE ranged from moderate to substantial dependent on the combination of readers. For the seven distinct subjective LAE criteria, the interreader agreement ranged from fair to good.

The results of this study confirm the hypothesis that interreader agreement for radiographic classification of LAE is strong, even across specialty disciplines and levels of experience.

“Evaluating a Common Medical Treatment in Horses”

The administration of commercial equine (horse) plasma is a mainstay treatment for severe gastrointestinal disorders in horses that create hypoproteinemia, or low blood protein concentration. Loss of the protein albumin from diseased bowel is associated with a decline in plasma colloid osmotic pressure (COP) which can result in decreased organ perfusion, tissue edema, and other complications. This is where plasma comes in. A natural colloid substance, plasma not only supports blood albumin concentrations, but also provides globulins to assist in fighting infection.

However, treatment with plasma is not cheap and adult horses require high volumes for impact. Dr. Erica McKenzie of the College of Veterinary Medicine is collaborating with Dr. Jennifer Johns to study the impact and duration of plasma transfusion in horses, compared with an alternative synthetic colloid, Hetastarch. Dr. McKenzie's goal is to determine the benefits and limitations of colloid transfusion by studying specific variables in clinical cases in the hospital. In addition to working as a medicine specialist in the large animal clinic, and teaching second through fourth year students, Dr. McKenzie's primary focus is to benefit animal health and those involved. Contributing viable evidence towards efficient treatment practices for severe gastrointestinal disease is just another way she hopes to assist the veterinary community.

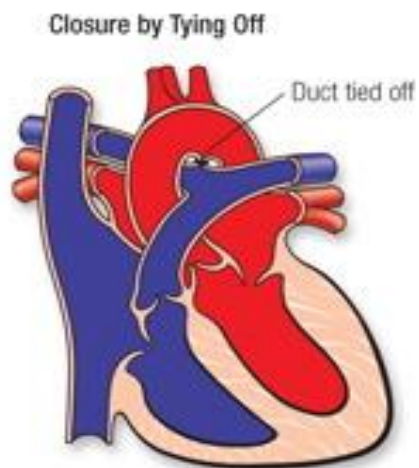
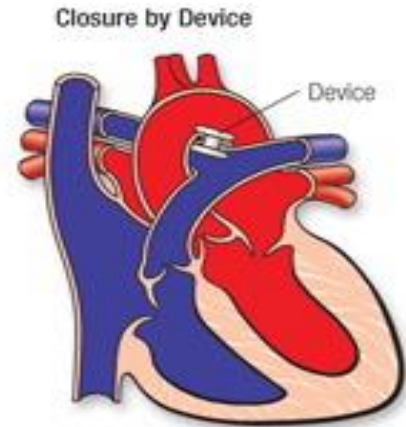
The Scope

“Heart Structured Shunts”

Left to right shunting patent ductus arteriosus (PDA) is one of the most commonly recognized congenital diseases in dogs. Significant shunting can potentially lead to congestive heart failure, with 64% of affected dogs developing heart failure within 1 year of age. The treatment involves permanent attenuation of ductal flow, either through surgical ligation or minimally invasive transcatheter occlusion with devices like an Amplatz Canine Ductal Occluder (ACDO).

At many referral institutions, ACDO is the preferred modality of PDA closure when feasible due to its minimally invasive nature, infrequent complication rate, and efficacy. The primary limitation of ACDO deployment is patient size due to the required delivery sheath. Therefore patients <3 kg are routinely referred for surgical ligation. A direct comparison between ACDO and surgical ligation outcomes is absent in the peer-reviewed veterinary literature. Therefore we sought to compare the perioperative complications and survival to discharge between the 2 methods via a retrospective interdepartmental study between the cardiology and surgery services at Oregon State University.

Drs. LeBlanc and Scollan studied 120 client-owned dogs eligible for study inclusion. The surgical ligation group had 62 dogs and ACDO group had 58 dogs. Dogs in the surgery group were significantly younger and had lower body weights compared to the ACDO group (median 4 vs. 8 months and 2.8 vs. 10.3 kg, respectively). Six of 62 surgical ligation dogs (10%) experienced intra-operative complications. Three of these 6 cases had hemorrhage from a ductal tear necessitating blood transfusion and leading to cardiopulmonary arrest, with 2 dogs being successfully resuscitated and surviving to discharge while the remaining dog died. The other 3 surgical cases experienced complications related to technical errors: inadvertent lung laceration during chest tube insertion and failure to tighten the PDA ligature adequately, which required a revision surgery. None of the dogs in the ACDO group experienced major intra-operative complications, although these dogs had longer anesthetic and procedure durations.



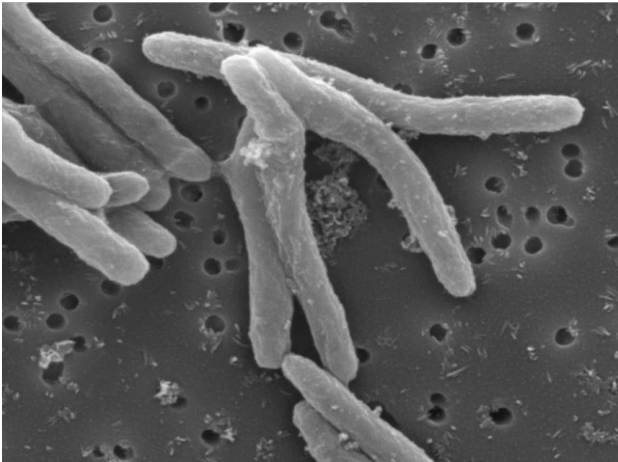
Ultimately 119/120 (99%) of dogs survived to discharge, with no difference in peri-operative mortality between groups.

Our findings suggest that while surgical ligation has a higher complication rate than ACDO, successful outcomes can be expected with either treatment modality. For more details, the full text of this study has been accepted for publication in an upcoming issue of JAVMA.



The Scope

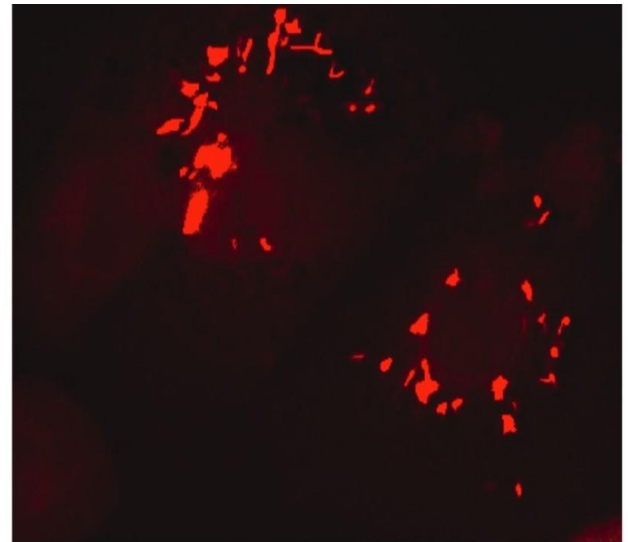
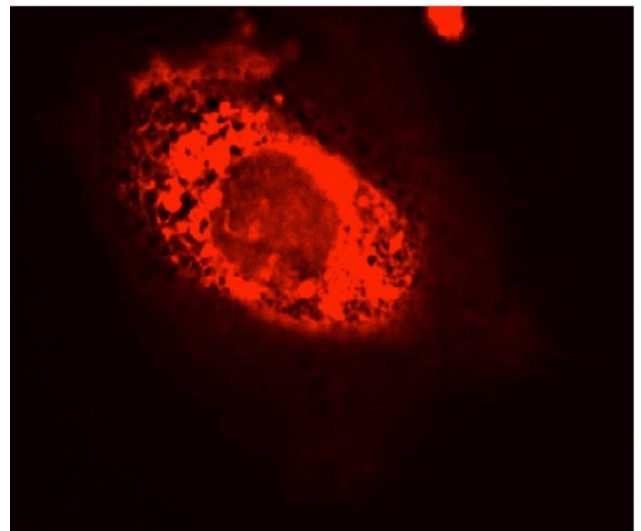
“Bacteria Uses Host Cell Machinery to Transport Products into Phagocytic Cells’ Cytoplasm”



Some bacteria, once inside phagocytic cells, export their own proteins using a “syringe like” apparatus that penetrates the vacuole membrane and transports the bacteria’s proteins to the phagocytic cell cytoplasm. This is not the strategy though for mycobacteria. Dr. Danelishvili and Dr. Bermudez have recently unveiled the mechanism involved mycobacteria’s ability to export proteins into the macrophage cytoplasm. *Mycobacterium avium* subsp. *hominissuis* is associated with infection of immunocompromised individuals as well as patients with chronic lung disease.

Despite the significant progress made in the past decade, it is still unknown how mycobacteria transports effectors/proteins through the membrane-bound phagosome and deliver the molecules into the cytosol of the host cell. Since intracellular mycobacterium is found juxtaposed to the phagosome membrane, the goal of this study was to identify possible phagosomal proteins that are employed by *M. avium* to export virulence factors into the cytosol of host cells. *M. avium* needs to attach to the internal surface of the vacuole before releasing secreted molecules. The voltage dependent anion channels (VDAC) were identified as components of *M. avium* vacuoles in macrophages. *M. avium* mmpL4 proteins were found to bind to VDAC-1 protein.

The inactivation of VDAC-1 function by pharmacological means or by mutating the RNA lead to significant decrease of *M. avium* survival. This study demonstrated that these VDAC channels are associated with the export of bacterial cell wall lipids outside of vacuole. Understanding the molecular basis of phagosome channels, its regulation and activation mechanism most likely will have a crucial importance for designing new therapeutic tools against mycobacterial diseases.



Macrophages with bacterial lipids in the cytoplasm (top) and with bacterial lipids in the vacuoles due to the inhibition of the transport through VDAC channels.

The Scope

“Use of disposable ventilated cages to maintain germfree/gnotobiotic mice without contamination”



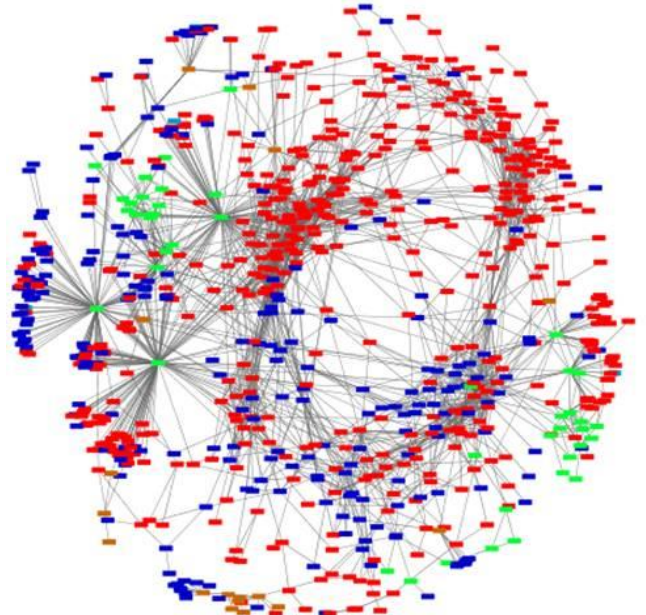
The use of germ-free animals allows for a specific microbial environment to be studied in a condition or disease state. Germ-free mice are also routinely used to study single microorganism interactions with a host after colonization with a microbe of interest. The limitation is that isolators housing colonized mice must be completely cleaned and re-sterilized before use. This can be labor intensive and costly as animals must be kept in separate isolators if different organisms are of interest. The re-sterilization process can be time consuming and has a long turnaround time before another study can take place. Individually ventilated cages (IVCs) are an alternative to avoid contamination of an entire isolator. There is however a greater chance of contamination when germ-free mice are taken out of the isolator unit. In our Out-of-Isolator study, germfree mice were housed in sealed disposable IVCs with dual HEPA filters at the cage and rack level. Cages were handled with sterilant and changed under a biosafety cabinet every 2 weeks and stool pellets were collected during that time. Cages were kept on positive pressure with 50 air changes per hour (ACH) to allow for proper ventilation. Stephany Vasquez-Perez, and Katherine Norris of Dr. Shulzhenko and Dr. Morgan's group have become experts in keeping the environment sterile. This is quite

important if research team wants to draw key conclusions relating to the role of individual microbes.

To validate sterility, stool DNA was isolated and amplified by PCR using universal bacterial primers. We observed that cages remained germ-free for 12 weeks with the use of IVCs, irradiated bedding supplies and strict aseptic protocol. We concluded that following strict aseptic technique and maintaining supplies sterile allows for the use of germ free mice in IVCs with any microbial context and complete bio exclusion.



Network image Modelling Host-Microbiome Interactions as Networks



The Scope

“The Future of Echocardiography?”

Pericardial effusion (PE) is a condition in which the pericardial sac fills with an abnormal amount of serous fluid or blood. Accumulation of fluid between the pericardium and the walls of the heart can lead to increased pressure, causing decreased cardiac filling/output and even death if remained untreated. Causes of PE include congenital disorders such as trauma or infection, but is most commonly associated with idiopathic pericarditis and cardiac neoplasia. Potentially the most problematic being neoplastic related incidences. Neoplasms that develop near the heart have a relatively high rate of metastasis, which makes prognosis crucial. Cardiologist Dr. Katherine Scollan of The College of Veterinary Medicine regularly encounters PE cases in domestic canines. Beyond treating the immediate issue of pericardial pressure, Dr. Scollan is developing a method of efficiently diagnosing neoplastic origins.

The current prognostic method for pericardial effusion is via magnetic resonance imaging and echocardiography. Though these techniques are effective, both have room for improvement. In a study conducted by Dr. Scollan and her colleagues contrasting various methods of PE prognosis, the researchers concluded that echocardiographic examinations performed by board certified cardiologists ranged from '17 to 82%' when detecting cardiac mass lesions. In comparison, MRI has a higher rate of accuracy in detecting malignancies, but is a lengthy process that requires frequent doses of anesthetics and subsequently prolonged periods of exposure to the MRI.

In an effort to develop a more efficient prognostic method, Dr. Scollan has recently started a project that involves the use of 3D echocardiography in assessing cardiac neoplasms. Echocardiography is a widely used practice when assessing ventricular size and function, but the inconsistencies arise from the 1D and 2D images being used to evaluate 3D volumetric variations. In her most recent studies, Dr. Scollan has been comprising large quantities of 3D canine cardiac regions as a standard referencing tool for providing accurate measurements. Dr. Scollan and her colleagues have developed a 3D map of the left ventricle and left atrium as well as an evaluation of cardiac output in a range of breeds and sizes.

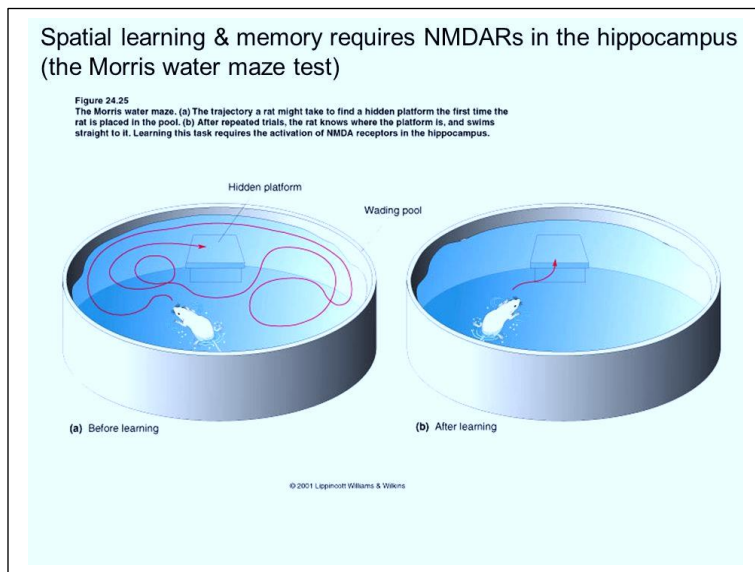
Each canine presents individual dimensions, but creating a large sample size makes referencing more efficient than 'geometric assumptions'. The right atrium and ventricle are all that remain on the to-do list for Dr. Scollan and her colleagues. Gathering a considerable sample size takes time, but Dr. Scollan hopes that these efforts will lead to an implementable method of evaluating cardiac neoplasms as well as other diseases that are assessable via the use of echocardiography.



Sample of QLAB reservoir phase cast. QLAB image displaying reservoir phase endocardial image cast

Paws for the News

“Quality in Aging”



We have all seen a movie or two about future medical and technological advances that lead to everlasting life. It has possibly been a staple of aspiration since the beginning of human existence. Undeniably, the overall health of society has improved over the centuries, but reverting the process of aging still seems far out of reach. Dr. Kathy Magnusson is professor in microanatomy that specializes in aging neuroscience who is interested in shifting the approach towards quality of life, rather than years extended. Dr. Magnusson has built a career's worth of research on the characterization of neuronal aging. Her primary research aim is to prevent the cognitive repercussions of aging such as memory loss and learning. Improving the ability to retain and process information can go a long way towards the quality of life an individual leads, which Dr. Magnusson believes is a more achievable goal in the near future.

Many of Dr. Magnusson's studies involve the NMDA receptor and its relationship with the declines associated with aging. N-methyl-D-aspartate (NMDA) is a glutamate receptor that is in high density in certain areas of the brain involved in memory and cognition, such as the hippocampus and cerebral cortex. As aging increases, both binding and transmission of glutamate in the NMDA receptor decreases. A published study from 2009 by Dr. Magnusson and her associates found that in both the frontal cortex and hippocampus, decreased protein expression of the synaptic membrane was evident.

In any science, molecular evidence can be difficult to correlate to a cause. It can be even more difficult in the case

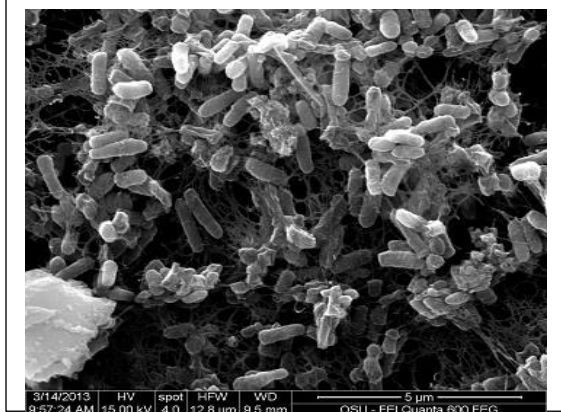
of neuroscience due to the complexity of gaging treatments. Often times, neuronal research is associated with behavioral analysis in order to measure the outcome of any given experiment. To analyze the effect of NMDA antagonists on learning and memory, Dr. Magnusson used a Morris water maze to quantify the spatial awareness of mice. Aged and youthful mice are individually placed in the water maze with repetition and continuous adjustment in order to examine their ability to spatially remember both short and long term. It's a common practice used in the study of neurodegenerative diseases such as Alzheimer's and ALS. Dr. Magnusson found that there was a significant difference between the age groups and their ability to complete the behavioral tests. As expected, the younger mice showed greater signs of short and long term spatial referencing than that of aged mice.

Dr. Magnusson and her colleagues formulated a study in that was published in 2013 on rehabilitating the function of the NMDA receptors and its effect on memory and cognition. By increasing the expression of a particular subunit of the NMDA receptor in aged mice, Dr. Magnusson first was able to analyze the ability of the synaptic membrane to transmit and receive glutamate. After finding that synaptic transmission could be significantly restored, Dr. Magnusson used the Morris water maze to analyze retention. Aged mice showed significant improvements in memory and behavioral tasks.

With a compound of evidence and published work, Dr. Magnusson's standing goal is to focus on furthering the treatment of memory and neuroplasticity declines as well as translating these treatments to humans. As she continues to advance her studies, Dr. Magnusson hopes to change the mantra to quality of life. Improving or maintaining her patient's ability to learn, memorize and be fully cognitive individuals throughout the process of aging is maybe the greatest contribution Dr. Magnusson can hope for.

Paws for the News

"Monsters in the Shower"



This is not an attempt to deter people from daily hygiene, in fact please don't, but there may be an unexpected invader residing in your household. *Mycobacterium avium* complex (MAC) is a multispecies pathogen that typically infects patients suffering from immunodeficiencies. This pathogen has a keen interest towards potable water systems, such as your shower, pool, hot-tub and sink. As a suitable environment, warm, moist and unimpeded, potable water systems have become a common environment for the formation of MAC biofilms. Studies have shown that these biofilms have multiple biological functions, including a mechanism that protects the bacteria from the host immune system. Researchers at OSU are studying the mechanisms of these biofilms in an attempt to better understand their ability to resist the host immune response.

Bacterial biofilms are adherent clusters that attach to the membrane of adjoining cells. Bacteria have the ability to form an extracellular coating, typically a polymeric matrix that also facilitates the binding and infiltration of the host. MAC follows this pattern in the epithelial tissue of its host, mainly in bronchial sites. The reason they have the affinity for tissue in the lungs remains unknown, but it's apparent that immune system has the inability to defend the host once infected. This has become the main focus of Dr. Luiz Bermudez and Dr. Sasha Rose of the College of Veterinary Medicine. During an early investigation, they noted that macrophages were readily undergoing apoptosis while fighting *M. hominissuis*. Later in the study, they

determined that the biofilms of these microorganisms were one of the main sources of protection against the innate immune response. Phagocytes, when in contact with the biofilms, could not engulf the bacteria which onset the death of the cell. With the inability of the host immunity to rid the bacteria, *M. avium* readily establishes the infection.

Studies have shown that biofilms are comprised of multiple components, primarily polysaccharides and extracellular DNA (eDNA). Once established, eDNA is involved in recruitment, nutrient absorption and colonization of MAC. This level of dependence provides evidence that eDNA is vital to the general the survival of the species. In order to better comprehend the development of the biofilms, Dr. Bermudez and Dr. Rose examined the role eDNA plays in *M. avium* biofilms in-vitro. Their study concluded that eDNA is essential in the structural integrity and antimicrobial tolerance of biofilms, including resistance to antibiotics. In a method using a DNase enzyme, they found that *M. avium* biofilm formation was significantly reduced in the presence of DNase. It also aided in the breakdown of presently established *in-vitro* biofilms and when co-treated with DNase, antibiotics Moxifloxacin and Clarithromycin significantly reduced bacterial viability compared to typical treatments. Observing the implications of eDNA in biofilm formation led the research towards the understanding the mechanisms of recruitment and transport of eDNA in the host. Contrary to prior evidence, it was observed that massive cell lysis was not involved in the development of MAC biofilms, but rather a genomic factor aids in the recruitment of eDNA with the help of bicarbonate ions.

Independent of pH, the researchers determined that bicarbonate ions facilitate the export of eDNA. This was the first genetic evidence that bacterial biofilms implement and active form of eDNA transport, as well as the first report showing that mycobacteria recognize bicarbonate for biological regulation. To detect the genes involved with the eDNA export and potentially bicarbonate sensing, the researchers made a transposon library of eDNA deficient bacterial mutants. This led to the result that multiple proteins in close proximity to the biofilms aid in the recruitment of eDNA, suggesting an involvement in the assisting of bacterial biofilms in terms of bicarbonate recognition. Presently, the research aim has focused towards the immune response. Looking at different stages of macrophage infection to see how eDNA/bicarbonate genes are regulated. The current hypothesis is that the bacteria invest a significant amount of energy into sensing bicarbonate while in biofilms. Studying the established genes of interest intracellularly may provide new evidence towards this essential process which is still insufficiently understood. The research continues to narrow, leading to the underlying biology of this vastly evolving species; ultimately contributing to the goal of novel treatment for immunodeficient patients.

Paws for the News

“Potential Biomarker for Canine Osteosarcoma”



Osteosarcoma (OSA) is the most commonly diagnosed bone cancer in the canine species, generally effecting adolescent and elderly patients. Typically originating in an appendage, Canine OSA aggressively metastasizes to the lungs and other osteoblastic tissues. If diagnosed prior to infiltration of the lungs, treatment involves amputation of the affected limb, followed by periodic chemotherapy to prevent metastasis. Patients who have received timely treatment can live active and healthy lives for up to a year or more. Once the tumor does metastasize though, patients often have a 30-60day mortality rate even with chemotherapy. This makes identifying the malignancy prior to metastasis critical. The current method of diagnosing OSA involves radiological examination via coherence tomography (CT). The difficulty with this method is characterization of the tumor, which results in surgical tissue sampling to accurately determine if it is benign or malignant; this procedure can be invasive and costly. Oncologist Dr. Shay Bracha of OSU's College of Veterinary Medicine has recently been working on the characterization of canine OSA in-order to implement more effective screening and treatment strategies.

Prior to metastasis, cancer compromises the hosts immune system, providing optimal environmental conditions for infection. One recently studied method of impacting the hosts immune system is via the secretion of microvesicles called exosomes. Exosomes are a common microvesicle, found in most eukaryotic fluids. These vesicles contain high concentrations of proteins, lipids and carbohydrates. The role of exosomes has been cumulatively theorized to contain specialized functions that impact coagulation and intercellular signaling. With the knowledge that malignant cell derived exosomes induce immunosuppression and evasion, Dr. Bracha and his colleagues sought out to characterize the

exosomes secreted via malignant canine osteosarcomal cells. Dr. Bracha exposed healthy T-cell lymphocytes to healthy and malignant osteoblastic exosomes and examined their impact. The study concluded that OSA derived exosomes inhibited the function of cytotoxic T-cells as well as induced apoptosis. The study also found that healthy osteoblastic exosomes had a similar result, but to a lesser extent. The most interesting determination was that malignant exosomes had a major regulatory effect; something that healthy exosomes did not. The T-cells incubated with malignant derived exosomes induced reduced proliferation as well as an alternate phenotype.

Proteomic analysis was used to create a profile of the exosomes involved in the study. Exosomes from both healthy and malignant osteoblasts shared a number of common proteins. As expected though, there was found to be a relatively high concentration of unique proteins in the malignant exosomes. Many of these immune related proteins were determined to be proteosomal. The evidence suggests that these exosomes not only inhibit innate immune function, but also act as a target molecule for metastasis due to its specific cargo. The unique proteomic signature of malignant derived canine OSA exosomes has created an opportunity. Dr. Bracha is now able shifted his focus towards the use of these exosomes as effective biological marker when screening for malignant canine OSA. Rather than involving invasive surgical procedures, Dr. Bracha endeavors to be able to diagnose canine OSA via a simple blood test. If the presence of malignant OSA exosomes are found then immediate action can be taken towards treatment. Dr. Bracha believes that this method will allow DVM's to delay and prevent lung metastasis at a much higher rate as well as characterize OSA with more efficiency.

In his current study, Dr. Bracha is looking at the characteristics of exosomes derived from malignant cells post-chemotherapy. By examining the unique protein signature exhibited by exosomes after being treated with Cisplatin and Doxorubicin (common chemotherapy treatments), Dr. Bracha hopes to create a profile that will allow him to more efficiently administer treatment methods. "Quality of life" says Dr. Bracha, "it's the focus of all DVM's". Extending the life of a patient is wonderful, but extending it with a relatively clean bill of health is the optimal goal. The research on malignant OSA exosomes will hopefully result in the implantation of an effective alternative to preliminary procedural methods while leading to timely diagnostics. Ultimately resulting in patients being able to indulge in the joys of daily activities, living happier, healthier and more fulfilled lives.

Paws for the News

“Symbiotic Microbial Relationships; Importance to Diabetes and Glucose Tolerance”

Advancements in the field of biomedical sciences has led research to new perceptions about mammalian and microorganismic interactions. Certain species of microorganisms have been known to have a commensalistic (beneficial for one organism, but limited or no effect on the other) or even parasitic relationship with a host system. However, it has long been known that some bacteria have mutualistic relationship with its host. In the last few decades research has led to the discovery of a multitude of symbiotic relationships between the mammalian and microorganismic species. For example, certain microbes play a key role in the development of mammalian systems and maintain general health of the host. With this knowledge, studies have aimed towards determining which of these microorganisms make up the microbiota (the collection of microorganisms that reside within a host) and what are their benefits are to the mammalian system. These influential species have been found to improve immune, intestinal, neurological and many other key functions of many organisms and if better understood can potentially be used for treatment purposes.

The combination of mammals and microorganisms seem to comprise a complex superorganism (termed holobiont). Professor Natalia Shulzhenko and her group at OSU's College of Veterinary Medicine has focused her efforts towards discovering the effects of the microbiota on mammalian organisms. She is mainly interested in host microbiotic interactions, primarily focusing on immunological conditions and diabetes. In such a short time frame, relatively minimal knowledge has been uncovered about how probiotics benefit human health. Without a map differentiating which bacterial species might be harmful and which are potentially beneficial, it's difficult to isolate any individual strains impact, let alone examine phenotypic variations. It is not feasible to examine all bacterial species in each biological system, but general insight can be provided about specific organisms in relation to a certain aspect of human health. Using a mouse model, Dr. Shulzhenko and her colleagues studied the effects of the microbiota on the intestinal system. A method of examining if the microbiota facilitates

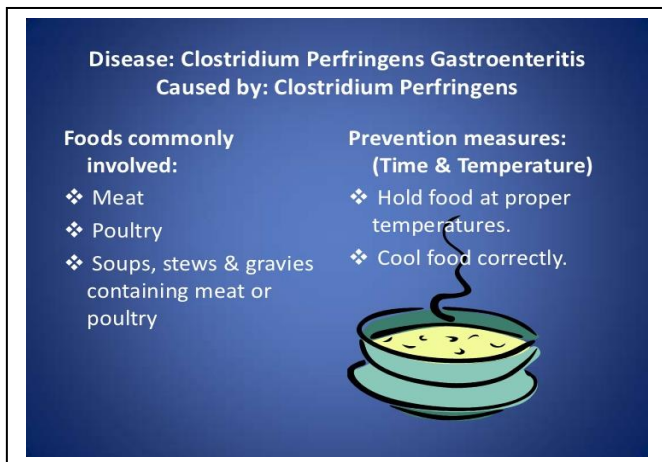
any phenotypic variation is by removing it from the host. Dr. Shulzhenko monitored the epithelial alterations after implementing a “cocktail” of antibiotics to eliminate nearly all the subject's microbes. The researchers found that variation in the mouse intestine can generally be attributed to three key factors; primarily depletion of the microbiota, but also direct effects of antibiotics and the effects of antibiotic resistant strains. Downregulation of the microbiota correlated to a downregulation of immune function. The antibiotics led an inhibition of mitochondrial functions, resulting in epithelial apoptosis in the mouse intestine. These results provide direct evidence towards a mutualistic relationship.

To determine specific microbial strains and the expressed genes that alter phenotypes, Dr. Shulzhenko and her colleagues formulated a figurative gene map. By mass sequencing the prevailing bacterial genomes, identification of general abundance and relationships both intraspecific and interspecific were established, creating what the researchers call a “transkingdom network”.

With the transkingdom network's ability to comprise applied analytics to elucidate specific microbes, Dr. Shulzhenko is now able to focus the research towards metabolic function. Her most recently published study involved the influence of a microbial species on glucose metabolism. Interferon gamma (IFN γ) is a cytokine that has been shown to negatively impact glucose tolerance in mice, but is mediated in the presence of a gut microbe, *Akkermansia muciniphila*. IFN γ also decreases the abundance of *A. muciniphila* in the mouse intestine which was found to be regulated by an IFN γ gene. Both factors effecting glucose metabolism are present in the human intestine as well, which could lead to a cross-link in novel treatment methods for metabolic diseases. The researchers hypothesize that this multispecies regulatory relationship may be due to an evolutionary adaptation. Dr. Shulzhenko's current study involves the common metabolic disease diabetes, specifically type 2. Almost half of the patients with type 2 diabetes still have uncontrolled symptoms which creates a need for a better understanding of the diseases pathology. Applying the transkingdom networks analysis, isolating certain microbes and their alterations in the host phenotype is the main priority. Dr. Shulzhenko and her colleagues have already identified a few microbes involved in the mouse model. The results of this experiment could potentially lead to novel probiotics used in targeted treatment of metabolic phenotypes.

Paws for the News

“Clostridium perfringens Spores: How it Pertains to Foodborne Illness and Gastrointestinal Disease”



As of 2016, the Center for Disease Control estimates an annual 48 million illnesses and 128,000 hospitalizations are due to foodborne pathogens. *Escherichia coli* and *Salmonella enterica* are among the most common household names, but there are many well documented food bugs that effect consumers today. An under-the-radar bug, *Clostridium perfringens*, a spore forming pathogenic bacterial species of the Clostridium genus, ranks higher in the CDC's foodborne pathogens than one would expect. Often found in raw meats such as poultry, *Clostridium perfringens* causes an estimated 1 million foodborne illnesses annually (CDC). A characteristic of *Clostridium p.* is that it thrives in oxygen deprived environments as it provides an optimal condition for rapid multiplication. The resulting effects on consumers consist of abdominal cramping, irregularity and severe dehydration. *Clostridium p.* is also involved in a number of gastrointestinal diseases such as traumatic gas gangrene. Its epidemic ranking is theorized to be due to their ability to rapidly sporulate. Dr. Mahfuzar Sarker, a professor of microbiology and biomedical sciences, focuses on bacterial pathogenesis and is currently working on a project involving the inhibition of *Clostridium p.* spore formulation to reduce foodborne illnesses.

Food processing goes through rigorous preparation before being shipped to public. From high pressure treatment to ionizing irradiation, the raw meats that

consumers receive are FDA approved and sterilized. In some cases though, certain colonies of bacteria persist. Especially in the case of fast growing bacterial species, a single colony can unknowingly germinate in the transition between production and consumption. *Clostridium perfringens* is a fast growing bacteria (can germinate in less than 20mins) that also has a method of protecting itself from the common FDA approved treatments. *Clostridium perfringens* has the ability to sporulate, which forms a multilayered external shield with various resistant properties. The outermost layer has been studied to contain a high concentration of proteins that are resemblant to other gram-positive bacteria in protecting against chemicals and lytic enzymes. The inner layers are key in providing nutrients as well as preventing dehydration and hydrolases. This spore formation allows *Clostridium p.* to lie dormant until optimal conditions rearise for germination as well as facilitates the release of the histotoxins associated with illness.

Dr. Sarker has conducted an immense amount of research on *Clostridium perfringens* and the mechanisms of spore formation. His initial studies revolved around treatments to eradicate *Clostridium p.* post-sporulation. Though the study resulted in significant success of inactivating vegetative cells, the treatments required high stress environments as well as excessive chemicals; impractical for consumer products. This led Dr. Sarker to search for a more innovative method of eliminating germinated spores. After taking a deeper look into the mechanisms of *Clostridium p.* sporulation, Dr. Sarker found a potential biomarker involved in latent germination. He and his colleagues were able to isolate amino acids as the activating signal for these spore formers. Containing most of the essential amino's, raw meats provide an ideal environment, preparing *Clostridium p.* to readily sporulate.

Dr. Sarker's laboratory now aims the research towards the prevention of germination via the inhibition of amino acid binding. This study is novel to *Clostridium p.* research and will hopefully lead to an effective method of inactivation. This combined with common stress treatments such as high pressure are being used to test the inactivation of spore formation as well. Dr. Sarker believes that there is much work to be done, but feels confident in the direction of the research. With the large number of *Clostridium perfringens* related foodborne illnesses and limited treatment due to the complexity of consumer products, Dr. Sarker's research becomes increasingly vital.

CVM CUTTING EDGE NEWS

ONE Health Program



Research is a crucial pillar of higher education. Integration of fields of study, and the development of a “mixing-up” environment in which students are exposed to different perspectives and methodologies can ultimately create innovative minds that will challenge the current understanding. The college is now embarking in a new and exciting experience with the “One Health” program. Spearheaded by Dr. Brianna Beechler, the program will involve undergraduate, graduate and veterinary students in research about the interface of social, environmental and public health. Antibiotic resistance and stewardship as well as vector-borne diseases will be the initial focus of the program. A practical, hands-on learning experience is been planned to Nicaragua. The students will be allowed to structure their own research interest within the broad “One Health” concept. The opportunity for students to learn on a close, discussion-based interaction with faculty, dealing with real problems and develop our inquisitive mind, capable of coming up with creative ideas to address clinical and/or research problems.

Editor: Janice S. Blouse
Assistant: Jordan K. Rice

COLLEGE OF VETERINARY MEDICINE Department of Biomedical Sciences
Oregon State University, 106 Dryden Hall, Corvallis, Oregon 97331-4802
T 541-737-6532 | F 541-737-2730 | oregonstate.edu/vetmed
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