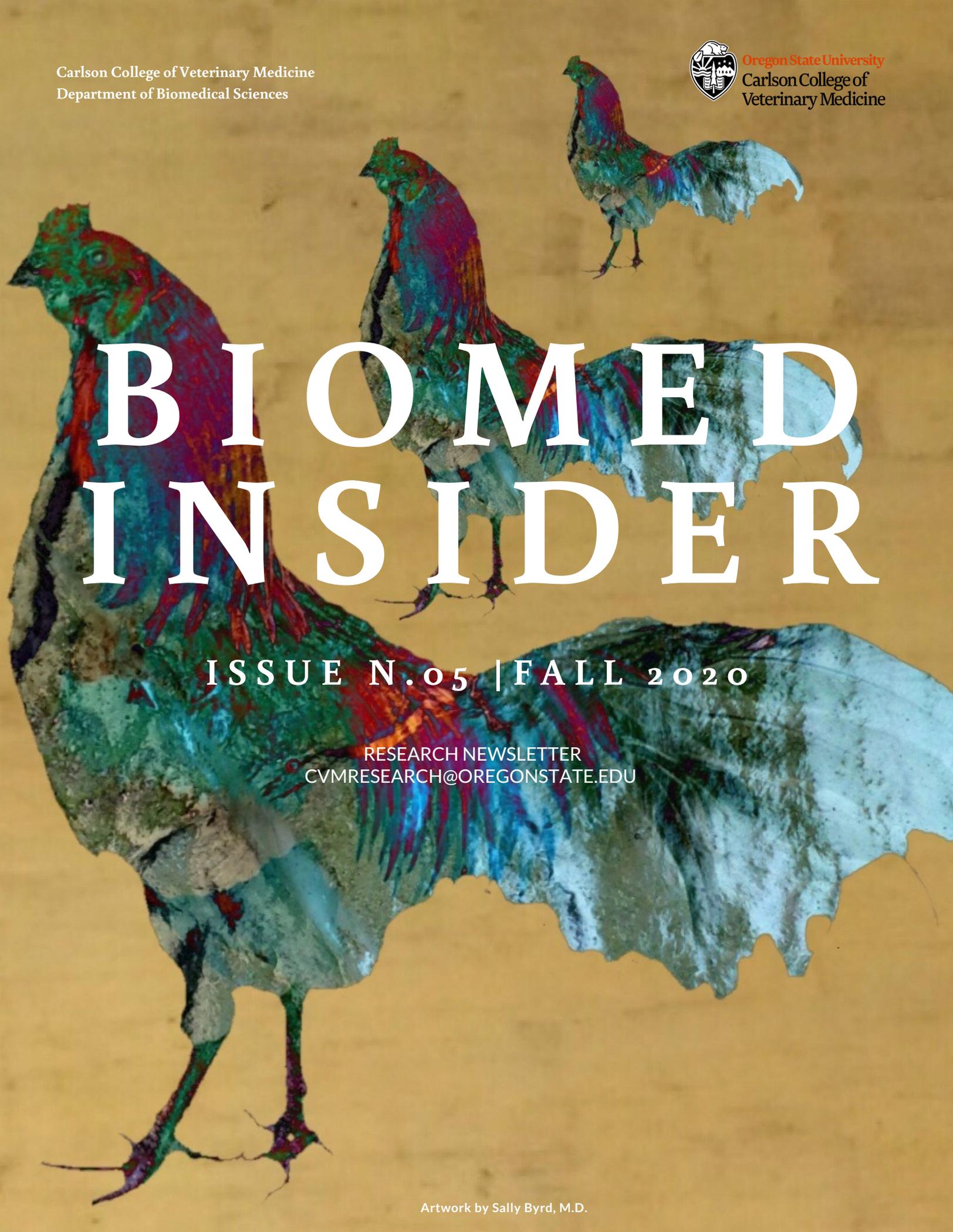


Carlson College of Veterinary Medicine  
Department of Biomedical Sciences



Oregon State University  
Carlson College of  
Veterinary Medicine

The background of the cover features three roosters of various breeds, including a large blue rooster in the foreground and two smaller ones behind it. The roosters are rendered in a vibrant, multi-colored style with shades of blue, green, red, and purple. The text is overlaid on the roosters.

# BIOMED INSIDER

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RESEARCH NEWSLETTER  
[CVMRESEARCH@OREGONSTATE.EDU](mailto:CVMRESEARCH@OREGONSTATE.EDU)

Artwork by Sally Byrd, M.D.

# LETTER *from Dr. Luiz Bermudez*

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

The Carlson College of Veterinary Medicine is built to educate future professionals, to serve the community. For the last fifteen years, the college has embarked on a mission for innovation in medicine. Although the faculty is small in number, its commitment to participate in the progress of medicine for the benefits of animals and humans is enormous.

As evidenced in this current issue of the Biomed Insider, the search for knowledge is broad and deep. There is work involving the COVID-19 pandemic, addressing major animal and human pathogens, the search in ways to derived chemotherapy for animals with fewer side effects, developing new treatments for type 2 diabetes in animals and humans by manipulating the microbiome, as well as working in neuroscience with wide implications for both animals and humans.

Veterinarian student participation in the college is a very important component of their education. This interest is captured by the large number of applications faculty receive every year, demonstrating interest in research and their application in the clinical setting. Many students dedicate so much of their time to research, and often end up being part of published findings.

I look forward to working very closely with faculty to use their talents in the development of very important pursue.

A handwritten signature in black ink, appearing to read 'Luiz Bermudez', is positioned below the text. The signature is stylized and cursive.

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# INACTIVATING COVID-19 VIRUS WITH RHAMNOLIPIDS

There is an urgent need to develop interventions to reduce the spread of new respiratory viruses, like the SARS-CoV-2, as vaccines are not readily available. Wearing masks and social distancing can slow transmission, but are not 100% effective in preventing the spread of respiratory viruses.

According to a recent study conducted by Dr. Ling Jin and colleagues on the application of rhamnolipids against Bovine Coronavirus (BCoV) and Herpes Simplex virus 1 (HSV-1), rhamnolipids may be able to reduce or prevent the spread of enveloped viruses.

To find out if rhamnolipids can inactivate enveloped viruses, BCoV and HSV-1, Dr. Jin and colleagues investigated two different rhamnolipids; 222A is a 10% aqueous solution with di-rhamnolipids and 222B is a 10% aqueous solution with mono-rhamnolipids.

Since viral envelopes derive from cell membranes, they checked the toxicity of rhamnolipids to uninfected Vero cells. Concentrations of rhamnolipids at or above 0.05% were found to be toxic. Although, cells with rhamnolipids at or below 0.005% had little to no viability loss, making it a biologically safe concentration.

To determine if rhamnolipids have antiviral effects specific to enveloped viruses, they tested rhamnolipids on HSV-1 and BCoV infected wells. The results showed 222B had better antiviral activity than 222A, suggesting mono-rhamnolipids

are better anti-enveloped virus agents. They decided to use 222B for the remaining of the study.

The mechanism of action of rhamnolipids against enveloped viruses was found specific to envelopes. The 222B rhamnolipids were able to directly act on the surface of the HSV-1 viral envelopes to have them lose viral proteins within five minutes. The anti-enveloped virus activity of the rhamnolipids seems to be dose-dependent.

Developing an antiviral mask using rhamnolipids could help prevent or reduce the spread of the virus. Dr. Jin and colleagues explored coating surfaces with rhamnolipids to see if antiviral masks could be useful. Since 222B at 0.005% is biologically safe, they coated plastic and fabric surfaces with that concentration and tested the anti-enveloped virus activity with HSV-1. Surprisingly, 222B at 0.005% on both surfaces were capable of killing at least  $1 \times 10^5$  PFU HSV-1/cm<sup>2</sup> within five minutes. If an infected person were to wear a mask coated with 222B, any droplet sizes typical of coughing and a runny nose would be inactivated inside the mask. Consequently, this would reduce the amount of viral droplets that can get casted on surfaces and environments.

Rhamnolipids may be able to play a role in future developments of interventions to enveloped viruses. Applying rhamnolipids 222B on masks to produce antiviral masks could not only help reduce or prevent enveloped viruses, but could help end the current pandemic of COVID-19.

# SHORT-CHAIN FATTY ACIDS PROMOTE GROWTH AND INFLUENCE ANTIBIOTIC SUSCEPTIBILITY IN *MYCOBACTERIUM AVIUM SUBSP. HOMINISSUIS* BIOFILM

*Mycobacterium avium subsp. hominissuis* (MAH) typically infects individuals whose respiratory and immune systems are affected. Even so, there are healthy individuals that can get MAH infection too. In the host, MAH survives and multiplies within immune cells however, this organism can also survive outside of cells such as patients' lung airways where bacteria form extracellular structures called biofilm. The biofilm helps MAH to overcome the hostile environment and allows for a more impermeable barrier against antibiotics, making MAH therapy less effective.

Recent studies have discovered a direct link between bacterial metabolic state in biofilms and antibiotic susceptibility and found that improved antibiotic efficacy and effective bacterial killing was associated with MAH that had active metabolism.

Luiz Bermudez, Lia Danelishvili, and Rajoana Rojony of Oregon State University and Carlos Adriano M. Silva of University of Sao Paulo, Brazil, tested around 200 exogenous carbon source-dependent metabolites

and discovered that MAH can consume certain short-chain fatty acids (SCFA) in different physiological states of biofilm formation.

The study identified butyric, caproic and propionic acids as SCFA metabolites that increased MAH metabolic activity during nutrient-limited conditions in planktonic and sessile states along with static and established biofilms. They also noticed a significant reduction in bacterial growth in vitro and in cultured macrophages when they assessed the effect of SCFA metabolites during MAH treatment with clinically used antibiotics.

These findings are evidence that the identified SCFA metabolites are important carbon sources that promote metabolism and growth of MAH and, subsequently, making bacteria more sensitive to antimicrobials. This study also emphasizes the need to research a link between changes in gut and lung microbiota during MAH infection; SCFA are the most abundant metabolites of microbiota and has many important roles in microbiome-host signaling. The study published in *Pathogens* provides a thrilling therapeutic opportunity to improve antibiotic effectiveness against drug tolerant forms of MAH using SCFA metabolites.

Reference: Silva CAME, Rojony R, Bermudez LE, Danelishvili L. Short-Chain Fatty Acids Promote *Mycobacterium avium subsp. hominissuis* Growth in Nutrient-Limited Environments and Influence Susceptibility to Antibiotics. *Pathogens*. 2020 Aug 26;9(9):700. doi: 10.3390/pathogens9090700. PMID: 32859077. PMCID: PMC7559849.

# NEONATAL LAMB MODEL UPDATES IMPROVES THE ABILITY TO TEST VACCINE EFFICACY AND OTHER FORMS OF THERPAY AGAINST RSV INFECTION, A SERIOUS INFECTION OF INFANTS AND ELDERLY

Dr. Mark Ackermann of Oregon State University, Dr. Panchan Sitticharoenchai of Iowa State University, and Dr. Sarhad Alnajjar of University of Baghdad have published a review manuscript that features updates to the model of the human respiratory syncytial virus (RSV) infection of human infants in neonatal lambs.

Newly developed antiviral drugs against RSV have been used for preclinical efficacy testing on the RSV lamb model. Positive results showed with JNJ-53718678 and JNJ-49214698 fusion inhibitors. Administrating JNJ-49214698 in the lamb model also exhibited reduced lesions, viral load, and clinical signs.

Among the ways to prevent viral infection in lungs, some methods may reduce RSV infection. In two different studies, the use of VEGF in lambs reduced RSV disease severity. Although there are limitations and side effects of VEGF delivery, it demonstrated anti-RSV activity when delivered prophylactically. Another study in lambs reduced RSV disease severity by prophylactic administration of potassium iodide in replacement of cyanide in the Duox-lactoperoxidase system. The application of antibodies can also have anti-RSV activity through passive immunity.

Prophylactically and therapeutically delivering nanobody ALX-0171 was effective against RSV in lambs at various doses and nebulization times.

Previous studies on lambs coinfecting with RSV and *Streptococcus pneumoniae* have demonstrated similar enhanced disease activity severity similar to human combined RSV-*Streptococcus pneumoniae* infection. Establishing viral bacterial coinfection in neonatal lambs proves the potential of the model to be used for RSV studies.

A unique feature of lambs is that some lack colostrum and have zero maternal antibodies, resulting in no antibodies directed explicitly to RSV. This helps to eliminate inquiring about passive antibody inhibition of RSV in stages at infection, replication, and release. Studies on the effect of RSV maternal immunity in lambs showed those born to vaccinated ewes have notable reduction in disease pathology and viral titer and increase in viral neutralizing compared to others born to non-vaccinated ewes.

Newborn lambs have also been used for studies in other types of viruses. The lamb model has provided progression in RSV studies and can expand the knowledge on RSV that will one day help develop preventive measures and treatment options.

Dr. Ackermann and Dr. Alnajjar have also submitted an abstract, Respiratory Syncytial Virus (RSV) Tropism in Bronchiolar Epithelium, to the 2020 annual meeting of the American College of Veterinary Pathologist. Their work includes Dr. Pun Sriboonyapirat, who is completing a PhD degree and is a resident of Veterinary Pathology at the Carlson College of Veterinary Medicine.



# UNDERTANDING THE GROSS AND HISTOLOGICAL PROGRESSION OF BOVINE TUBERCULOSIS IN AFRICAN BUFFALO

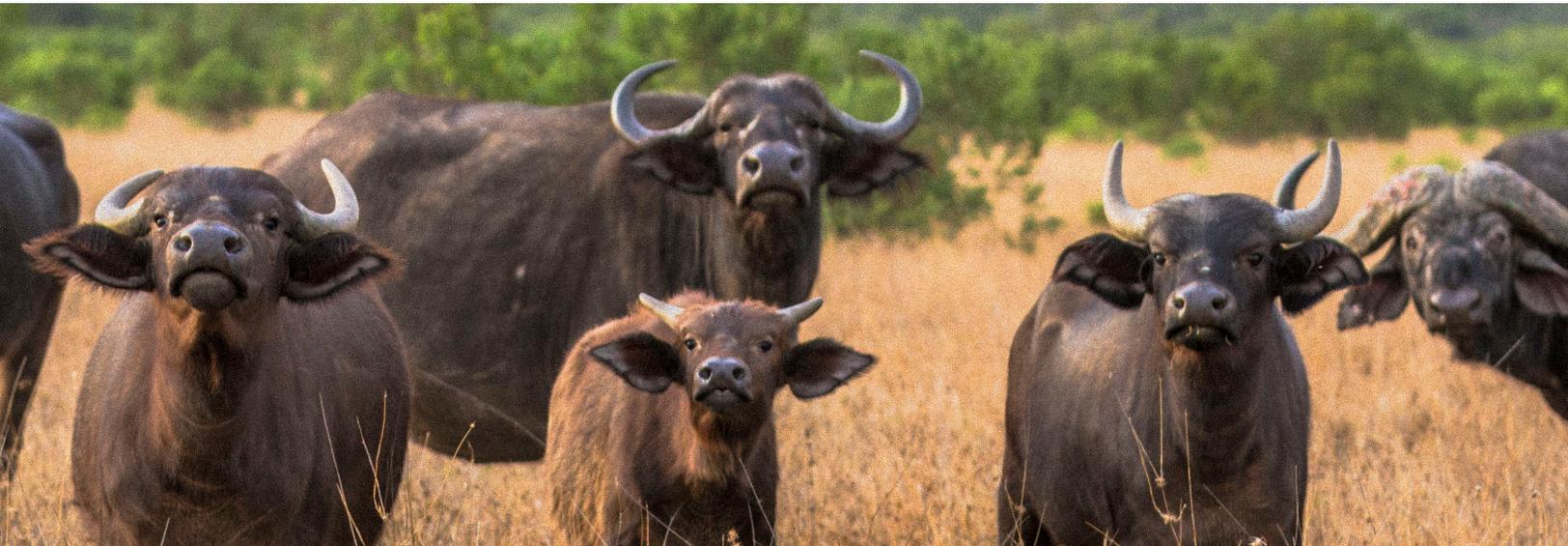
Bovine tuberculosis (bTB) is an infectious disease in cattle caused by the bacteria *Mycobacterium bovis*. Animals with bTB will often not show signs or symptoms until later stages of the infection, which is a challenge in understanding the development of the disease.

Dr. Anna Jolles and Dr. Brianna Beechler served as mentors for Hilary Ann Lakin, who investigated the gross and histologic development of bTB. She used a four-year cohort study on African Buffalo completed in Kruger National Park, South Africa. She used the gross pathology and histological data from the study to explore bTB progression in the wild model.

The percent affected in disease progression and the length of infection had a weak positive correlation with variations in gross and histological data.

A majority of the buffalo with bTB had the retropharyngeal lymph nodes infected first, followed by tracheobronchial lymph nodes. This supports the theory that it is an inhalation infection, descending down the airways with inhalation.

A previous researcher used the same dataset and identified two regions of DNA using single nucleotide polymorphisms (SNP). Hilary Ann used the SNP data to see if it changes the speed of progression once



She tracked the infection progression across different variables using the dataset. She observed high correlation between percent of lung histologically affected, percent lung necrotic, and stage severity scoring. Additionally, there was a high correlation between the amount of lesions and total of lobes affected. This suggests the infection spreads through the lungs, with more lung lobes affected with bigger lesions as opposed to one lobe remaining infected with large lesions.

the buffalo is infected. She noted a trend of faster progression in infected buffalo with SNP 3195 risk allele, however continued research will be conducted to find the significance of this trend.

Hilary Ann would like to further examine SNP 3195, investigate the severity of each lobe as infection progresses and map out lymph node spread. The exploration can provide more insight to bTB, as well as understanding the development of the disease.

# BIGHORN SHEEP GUT MICROBIOMES MAY DIFFER DEPENDING ON THE ENVIRONMENTAL CHARACTERISTICS



A group of desert bighorn sheep in the Mojave National Preserve in Southern California

Claire Couch, a former graduate student of Dr. Brianna Beechler and Dr. Anna Jolles, analyzed microbiome variation across a metapopulation of wild bighorn sheep. Findings reveal that microbiome composition may differ based on the landscape-scale host population and environmental characteristics.

Claire and colleagues identified bacterial lineages conserved across the metapopulation, in spite of varying microbiome composition between populations. The differences in microbiome communities associated with genetic heterozygosity at the population level suggests that intrinsic host factors are associated with microbial composition even at a metapopulation scale. The data at the individual level indicates that home range overlap mediates microbial exposure, although host genetics mediates selection of different microbial lineage.

Overrepresented microbial clades across the metapopulation were also identified. The largest numbers of conserved clades belonged to gene *Christensenellaceae* R7 group, family

*Lachnospiraceae*, and family *Ruminococcaceae*. The inferred relationship with ruminant nutrition and host heritability suggests these bacterial taxa could be conserved across bighorn populations because of the adaptive relationship with their host,

A positive link between shared absence/presence of microbial amplicon sequence variants observed in the study supports the theory that close distance between hosts mediates exposures to similar microbial sources, which lets the indirect transfer of microbes between animals.

Their findings provide knowledge of the key variation of host-associated microbiomes at the metapopulation scale and gut microbiome communities of a bighorn sheep. Future studies ought to explore metapopulation-scale effects of host selection on the microbiome, environmental differences, and microbial distribution.



A team of OSU researchers in the field. From left to right: Brianna Beechler, Anna Jolles, Marci Witzczak, Claire Couch, Clinton Epps, Rachel Crowhurst.

A golden retriever is sitting on a dirt path in a lush green forest. The dog is looking upwards and to the right with its mouth slightly open. The background is filled with dense green foliage and trees. A semi-transparent dark grey banner is overlaid across the middle of the image, containing white text.

# FASTING MAY REDUCE SIDE EFFECTS IN DOGS RECEIVING VINCRISTINE

Maintaining the quality of life for dogs with cancer is of utmost importance, and can be difficult especially when considering treatment options such as chemotherapy.

A study published in *Veterinary and Comparative Oncology* suggests fasting may be a suitable treatment to consider for constitutional and gastrointestinal adverse events for large breed dogs receiving the chemotherapeutic vincristine. The study was conducted by Kaitlin Curran, Margaret Duckett, Haley Leeper, and Carl Ruby of Oregon State University and Shay Bracha of Texas A&M University (previously of Oregon State University).

The study involved a prospective, crossover clinical trial to evaluate the effects of fasting in tumor-bearing dogs receiving vincristine.

Each dog was its own control with a crossover design of when to fast. The dogs were randomly chosen to fast before their first chemotherapy treatment and then fed a regular diet before the second treatment, or fed a regular diet before the first treatment and then fast before the second treatment.

The results showed fasting a day before the vincristine treatment greatly reduced the incidence of adverse events. There was a considerable decrease in nausea and reduced serum insulin levels in fasted dogs.

Anorexia and lethargy was noted more in dogs when fed than when fasted. Dogs had mild constitutional and gastro-intestinal adverse events throughout both treatments. Majority of the dogs experienced improvement in at least one category of adverse events when fasted before vincristine administration.

On the other hand, fasting before the treatment did not particularly impact IGF-1 concentration, neutrophil count, serum glucose, or vomiting. Adverse events of diarrhea were not as different between fasted vs fed.

The fasting and treatment order did not significantly impact neutrophil count. Serum glucose remained within normal limits for fasted or fed time points.

Limitations to the study included dog size, lower dose of vincristine, medications dogs took before and during the study period, and the bias of owner reports on constitutional and gastrointestinal adverse events. The dogs were also unequally distributed into the two groups by coin flip.

More studies have demonstrated the potential benefits of fasting before chemotherapy treatment, but further research is needed to expand knowledge and protocols in this area. Veterinarians will continue to work on finding effective therapeutic options to maintain high quality of life for our furry friends.

# AMOEBAS AS AN INITIAL SCREENING TOOL FOR VIRULENCE-ASSOCIATED GENES IN *MYCOBACTERIUM AVIUM* SUBSPECIES *PARATUBERCULOSIS*

Protozoan hosts such as amoeba have become a valuable model organism for studying pathogenicity mechanisms of different pathogens including mycobacterium in recent years. A study conducted by Dr. Luiz Bermudez and colleagues demonstrates the use of *Acanthamoeba castellanii* as a quick initial screening tool to find genetic factors that may contribute to virulence and pathogenicity of *Mycobacterium avium* subspecies *para-tuberculosis* (MAP).

They determined that MAP infection has an influence on the metabolic activity of *A. castellanii*. During the early stages of MAP infection, *A. castellanii* metabolism is highly stimulated due to ingesting live MAP in a dose-dependent and viability-dependent way. Later stages of infection show a direct relationship with the intracellular burden of MAP and the metabolic changes in *A. castellanii*.

The researchers further analyzed the possibility of bacterial virulence determinants that may be contributing factors for metabolic stimulation of *A. castellanii*. Using a transposon library of gene knockout mutants, they identified two bacterial subgroups that could either inhibit or promote metabolic activity in *A. castellanii*.

Follow-up tests on mutants with murine macrophages showed that most MAP mutants that highly stimulate *A. castellanii* metabolism were negatively affected during macrophage infection.

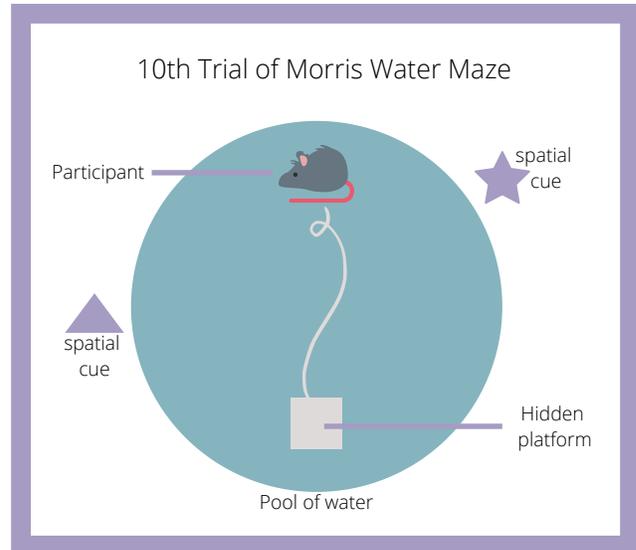
However, most MAP mutants with low stimulatory effect on *A. castellanii* metabolism were enhanced in macrophages. Follow-up tests on mutants with murine macrophages showed that most MAP mutants that highly stimulate *A. castellanii* metabolism were negatively affected during macrophage infection. However, the MAP growth and bacterial survival rate of most MAP mutants with low stimulatory effect on *A. castellanii* metabolism were enhanced in macrophages.

To see if the changes resulting in low MAP survival rate were directly correlated to genes knocked out by the transposon, they generated complemented clones for four macrophages attenuated MAP mutants. The selected MAP mutants include 3D3 and 15G2 of high metabolic activity as well as 7C1 and 18F6 of low metabolic activity in *A. castellanii*.

The functional repair of high metabolism genes MAP\_3634 and MAP\_3893c along with low metabolism genes MAP\_0949 and MAP\_2291 fully restored the attenuation phenotype in MAP mutants. It was also noted that the complemented clones revealed intracellular growth much like the wild-type MAP.

From these mutants, Dr. Bermudez and colleagues identified virulence factors associated with mycobacterial survival and detected new genetic factors that may contribute to MAP pathogenicity. Their findings through this in vivo MAP infection model using *A. castellanii* validates the use of the amoeba model system and aids in the study of MAP.

# VIRTUAL MORRIS WATER MAZE AND HUMAN COGNITIVE TESTING



The Morris water maze is a widely used task in behavioral neuroscience to study spatial learning and memory when given spatial cues. As a neuroscientist interested in preventing or repairing the decline of learning and memory ability during aging, Dr. Kathy Magnusson has been working on screening intervention in mice. She recently transitioned to working with human participants.

Dr. Magnusson and colleagues conducted two studies using a virtual Morris water maze (vMWM) they developed for human participants.

One study analyzes the age-related differences between female and male participants, young and old, and the effect of non-combat military service in older men in spatial memory and cognitive flexibility. Of the various memory tasks performed, younger men performed better than older men overall. They performed better on Logical Memory I and on tasks that depend upon past learning experiences. Between the older men, veterans performed worse than civilian participants on the same tasks.

Veterans overall performed worse compared to civilian participants. Experiences with military support services may not have a positive impact lasting into old age according to the results.

Another study analyzes the association between the NIH Toolbox Cognitive battery and cognitive functions tested in the vMWM across aging. Participants performed a long-term memory task, reversal trials, working memory trials, and immediate trials. The trials performed in the vMWM identified similarly with the tests and measurements of the NIH Toolbox Cognitive battery. This suggests that the vMWM tasks are applicable for human cognitive testing.

References: K.R. Magnusson, N. Reynolds, A. Mollusky, and J.Y. Zhong (2020) Relationships between memory and flexibility performances in virtual Morris Water Maze and NIH Toolbox Cognitive Battery. Proceedings of virtual International Society for Behavioral Neurosciences.

N. Reynolds, A. Mollusky, J.Y. Zhong, and K.R. Magnusson (2020) Non-combat military service in older men impaired memory and cognitive flexibility in a virtual Morris water maze task. Proceedings of virtual International Society for Behavioral Neurosciences.

# PROTEOMIC ANALYSIS SUGGEST THAT THE RESPONSE OF MYCOBACTERIUM ABSCESSUS TO ANTIBIOTICS MAY DEPEND ON THE ENVIRONMENTS IN THE HOST

A study published in *Microorganisms* establishes global changes in enzyme synthesis of *Mycobacterium abscessus* sub-species *abscessus* (MAB) that promote metabolic shift and increases resistance to antibiotics within different environments. Dr. Luiz Bermudez and colleagues explored MAB proteome response under aerobic, anaerobic, and biofilm conditions, as well as during exposure to antibiotics.

They identified various synthesized enzymes of metabolic pathways regulated by MAB. Certain changes to these enzymes are suggested to support bacterial survival. Exposure to antibiotics, amikacin and linezolid, under the same conditions showed enhanced glycerophospholipid metabolism and oxidative phosphorylation in MAB.

Anaerobic and biofilm phenotypes of MAB seemed to prompt a metabolic shift that focuses on up-regulating metabolic pathways that plays a role to the pathogen survival. These metabolic pathways have shown to give carbon sources and help provide levels of requirements in other pathogens for survival purposes in certain conditions. This implies that MAB upregulates those pathways in anaerobic and biofilm conditions for similar reasons.

Enzymes of nitrogen metabolic pathways and the pyruvate metabolism pathway in MAB were highly upregulated in anaerobic and biofilm conditions. *Mycobacterium tuberculosis* and *Pseudomonas aeruginosa* are known to use nitrites to reduce ammonium and use for respiration in lack of oxygen, respectively.

This could indicate that nitrogen metabolism is an important component for MAB to survive in those conditions. Previous studies have shown that *Pseudomonas aeruginosa* and *Staphylococcus aureus* enhances pyruvate metabolisms in anaerobic and biofilm conditions. Dr. Bermudez and colleagues speculate that the pathogen uses enzymes to condensate molecules of pyruvate to 1-acetoacetate.

The proteomic profiles of MAB and *Mycobacterium avium* subspecies *hominissuis* (MAH) under the different conditions were also compared to find common pathways that mycobacteria undergo within similar environments. They identified metabolic pathways of nitrogen metabolism, pantothenate and CoA biosynthesis, and peptidoglycan biosynthesis in both MAB and MAH within both anaerobic and biofilm conditions.

*Mycobacterium smegmatis* protein overexpression clones were used to examine the bactericidal and drug activity of MAB and MAH. It was observed that *M. Smegmatis* endured the presence of antibiotics and extended bacterial survival during exposure to antibiotic treatment. Inhibition of those metabolic pathways could affect the conditions of MAB and MAH.

The findings of this study show some of the survival tactics MAB uses in the various conditions. It highlights the common pathways used in MAH as well as among other pathogenic mycobacteria. The information provides insight on possibly new targets for treatment options to fight against MAB and nontuberculosis mycobacteria.



# PROTEOLYTIC ACTIVATION OF SARS-CoV-2 WITH FURIN AND TMPRSS2

Studies on coronaviruses provide knowledge and information to further research therapeutic treatments and vaccines for humans and animals. With the COVID-19 pandemic taking over 2020, understanding these viruses is crucial. To further the search for knowledge and treatment options, Dr. Hong Moulton and colleagues investigated the proteolytic activation of the virus that caused COVID-19, SARS-CoV-2.

The study, published in Life Science Alliance, focuses on the surface spike glycoprotein (S protein), a major surface protein in coronaviruses. The S protein initiates infection when activated by cellular proteases cleaving at two specific sites, S1/S2 and S2'. Among the proteases found to activate coronaviruses in vitro, the researchers show proteolytic activation of the S protein of SARS-CoV-2 with proprotein convertase furin and transmembrane serine protease 2 (TMPRSS2).

Tests conducted in the study show that the S protein can be cleaved by furin at S1/S2 site and TMPRSS2 at the S2' site. Preventing furin or TMPRSS2 activity

suppresses multicycle replication of SARS-CoV-2 in Calu-3 human airway cells. This suggests each protease cannot compensate for the lack of the other and both proteases are critical for S protein activation for virus entry and membrane fusion.

To detect if these proteases have effective antiviral activity, Dr. Moulton and colleagues tested a combination of furin and TMPRSS2 inhibitors together and alone to target the proteases. The data show combining furin and TMPRSS2 inhibitors produce more effective antiviral activity against SARS-CoV-2 in human airway cells and help reduce virus multiplication at lower doses than an equally same amount of each inhibitor alone.

Combining the two proteases show potential as drug targets for treatment of COVID-19. The study also highlights TMPRSS2 inhibitor, aprotinin, and the use of PPMO in reducing TMPRSS2 expression is worthy of further testing as possible therapeutic treatment for coronavirus infections. Although there is very little development of host protease inhibitors as methods for preventing and treating virus infections, the data in the study provides the potential of protease inhibitors as promising drugs for SARS-Cov-2.

Reference: Bestle, D., Heindl, M. R., Limburg, H., Van Lam van, T., Pilgram, O., Moulton, H., Stein, D. A., Harges, K., Eickmann, M., Dolnik, O., Rohde, C., Klenk, H. D., Garten, W., Steinmetzer, T., & Böttcher-Friebertshäuser, E. (2020). TMPRSS2 and furin are both essential for proteolytic activation of SARS-CoV-2 in human airway cells. *Life science alliance*, 3(9), e202000786. <https://doi.org/10.26508/lsa.202000786>

# ANTISENSE THERAPY SUPPRESSES GROWTH OF SARS-CoV-2

David Stein, a Faculty Research Assistant of the Moulton lab, and colleagues conducted a study on antisense agents, peptide-conjugated morpholino oligomers (PPMOs), on the ability to specifically and potently suppress the growth of severe acute respiratory syndrome SARS-CoV-2.

PPMOs are synthetic molecules that mimic DNA or RNA and can inhibit gene expression by binding to complementary sequences of cRNA. Researchers can create PPMOs to match RNA strands to a specific gene and inhibit the expression of the targeted gene.

Five PPMOs were designed to target sequences of genomic RNA in the five prime untranslated region of SARS-CoV-2. The PPMOs were synthesized and evaluated for their effect on the viability of

uninfected cells and potential to suppress the viral growth of SARS-CoV-2. They controlled non-specific effects of the PPMO chemistry by also synthesizing a negative control PPMO random sequence.

The results showed that four PPMOs targeting the transcription-regulation sequence leader region or the 5'terminal region were highly effective inhibitors of SARS-CoV-2 replication.

They highly reduced viral titers in a non-toxic and dose-dependent manner after 48-72 hours of infection. Although the PPMO targeting the polyprotein 1a/b AUG translation start region was not as effective in comparison to the other four PPMOs.

The study demonstrates that antisense agent PPMO targeted against SARS-CoV-2 can easily enter cells and inhibit viral replication in a non-toxic, sequence-specific, and dose-dependent manner.

Photo by Tatyana Nekrasova from StopTheSpread



References: Robertson, S. (2020, October 04). Anti-sense agents suppress SARS-CoV-2 virus. Retrieved October 10, 2020, from <https://www.news-medical.net/news/20201004/Anti-sense-agents-suppress-SARS-CoV-2-virus.aspx>

Stein D, et al. Inhibition of SARS-CoV-2 in Vero cell cultures by peptide-conjugated morpholino-oligomers. bioRxiv, 2020. doi: <https://doi.org/10.1101/2020.09.29.319731>

# HUMAN-ALTERED ENVIRONMENTS MAY PROMOTE DISEASE-SPREADING MOSQUITO SPECIES

A study conducted by Dr. Brianna Beechler and colleagues found that disease-spreading mosquitoes are more likely to inhabit areas altered by human activities than areas less affected. They worked in the Kruger National Park in South Africa and trapped over 3,000 female mosquitoes from 39 species both inside and outside the park. A large difference was found in the amount and species composition of mosquitoes inside versus outside the park.

Five “pressures” caused by human activity were analyzed to compare mosquitoes inside the national park versus populated areas outside. The five “pressures” were observed to be constantly higher outside than inside the national park.

They also tested for differences in mosquito abundance and found that mosquito abundance outside the national park was almost three times higher, in areas populated by humans, than inside the park. Species known to spread diseases were more common in the human-impacted areas outside the park than inside. This suggests disease-carrying mosquito species fare better in human-altered environments.



Students and a game guard set up a tent trap for mosquitoes in Kruger National Park, South Africa



A tent trap for mosquitoes is set up near a reservoir in Bushbuck Ridge, South Africa



Photo on the left shows local field technician Skumbuzza Mdletshe setting up a light and CO<sub>2</sub> baited mosquito trap in Kruger National Park, South Africa

Photo on the right shows Maarten Schrama, a lead researcher on the project from Leiden University setting up a mosquito trap in a community area outside Kruger National Park, South Africa.

References: Human activities are more likely to promote disease-spreading mosquitoes. (2020, September 15). Retrieved September 15, 2020, from <https://www.news-medical.net/news/20200915/Human-activities-may-be-more-likely-to-promote-disease-spreading-mosquitoes.aspx>

Gorsich, E. E., et al. (2019). A comparative assessment of adult mosquito trapping methods to estimate spatial patterns of abundance and community composition in southern Africa. *Parasites & vectors*, 12(1), 462. <https://doi.org/10.1186/s13071-019-3733-z>

# POTENTIAL PROBIOTIC STRAINS FOR TYPE 2 DIABETES

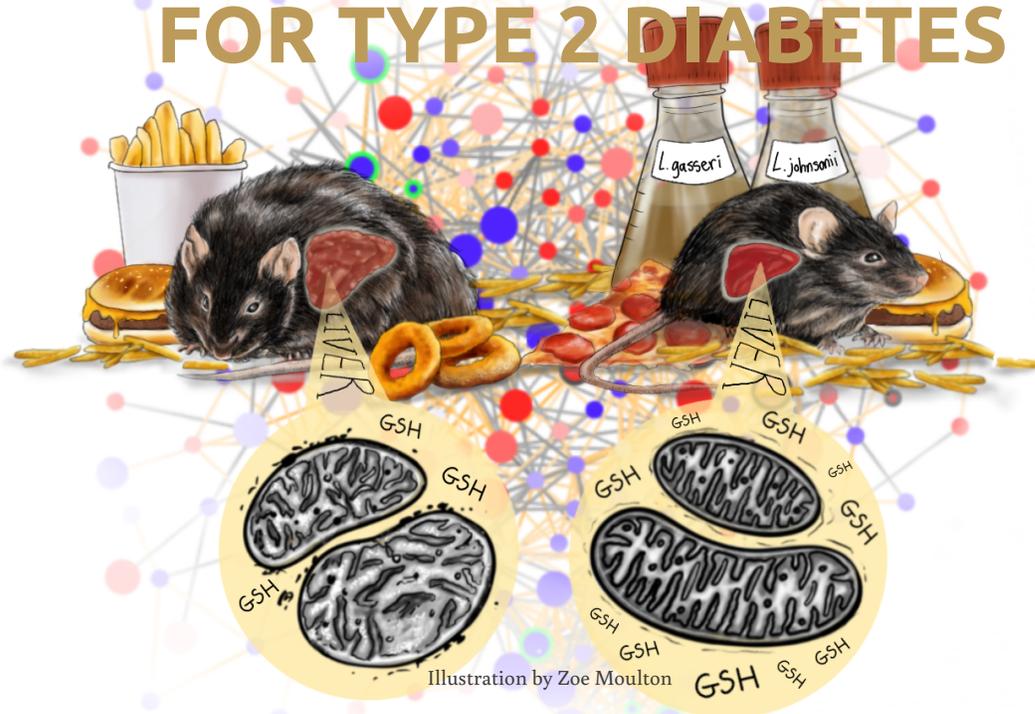


Illustration by Zoe Moulton

A study, published in *Nature Communications*, conducted by Dr. Natalia Shulzhenko and colleagues pinpoint potential probiotic strains for type 2 diabetes. The study focuses on examining if particular members of gut microbiota and/or their interactions play a role in changes to host metabolism under a western diet in a mice model.

Described as a high dietary intake of fats and sugars, the 'western diet' is a major factor to type 2 diabetes with the effects of diet being modulated by gut microbiota. Mice were split into two groups with one group fed a western diet and the other group fed a normal diet. Mice on western diet displayed metabolic disease similar to human type 2 diabetes.

They examined host-microbe interactions induced by a western diet using a Transkingdom Network analysis and identified four operational taxonomic units with high similarity to bacterial species of *Lactobacillus gasseri*, *Lactobacillus johnsonii*, *Rumboutsia ilealis*, and *Ruminococcus gnavus*. The *Lactobacilli* are considered potential 'improvers' to glucose metabolism,

*Rumboutsia ilealis* and *Ruminococcus gnavus* are considered potential 'worseners'. This suggests that microbes and/or their interactions could be important in type 2 diabetes.

Further analysis on 'improvers' showed improved glucose tolerance in mice fed a western diet and supplemented with *Lactobacilli*. It also reduced lipids and enhanced mitochondrial function in the liver. On the contrary, mice fed a western diet and supplemented with 'worseners' showed a decrease in glucose tolerance.

A human study conducted earlier in obese patients showed similar results with correlations between the amount of microbes and Body Mass Index (BMI). More 'improvers' associated with a healthier BMI, while more 'worseners' was linked to a less healthy BMI.

The study demonstrates potential probiotic strains that can be used for treating type 2 diabetes as well as obesity. Dr. Natalia and colleagues also provide notable insights of probiotic mechanisms and the potential to develop therapies for diabetes.

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