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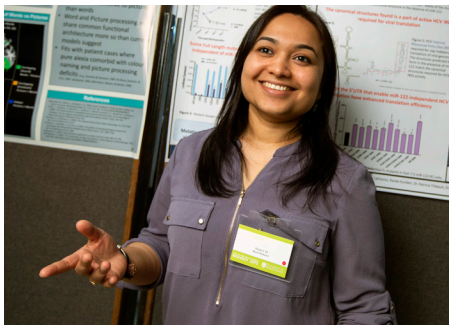
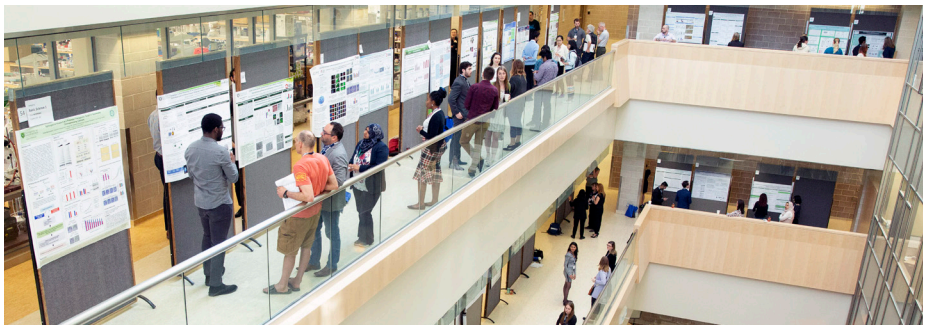
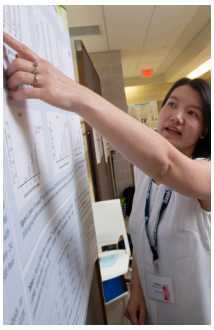
THE 30TH ANNUAL LIFE & HEALTH SCIENCES

RESEARCH EXPO






ACKNOWLEDGING EXEMPLARY RESEARCH AND LEARNING AT THE UNIVERSITY OF SASKATCHEWAN

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Agenda

09:30 – 11:30 a.m.	**STUDENT REGISTRATION*** D-Wing, outside Room 1B21, Health Sciences Building ***Registration and poster setup begin 30 minutes prior to each presentation session.***
10:00 – 11:30 a.m.	**MORNING POSTER SESSION #1** D-Wing Atrium, 2nd floor, Health Sciences Building • Undergraduate Research 1 • Basic Science 1 • Basic Science 2 • Social & Population Health 1
11:00 a.m. – 12:30 p.m.	**AFTERNOON POSTER SESSION #1** D-Wing Atrium, 3rd floor, Health Sciences Building • Basic Science 3 • Basic Science 4 • Translational, Clinical, or Applied Science 1 • Translational, Clinical, or Applied Science 2
12:00 – 1:30 p.m.	**AFTERNOON POSTER SESSION #2** D-Wing Atrium, 2nd floor, Health Sciences Building • Social & Population Health 2 • Undergraduate Research 2 • Basic Science 5 • Translational, Clinical, or Applied Science 3
1:30 – 2:30 p.m.	**NETWORKING BREAK (COFFEE & SNACKS PROVIDED)** B-Wing Atrium, Health Sciences Building
2:30 – 3:30 p.m.	**AWARDS CEREMONY AND SPECIAL PRESENTATIONS** HLTH GB03, Health Sciences Building

2023 Best Supervisor Award

Congratulations to the winner of the
2023 Life and Health Sciences Research Expo
Best Supervisor Award

Dr. Hassan Vatanparast, MD, PhD
Professor
College of Pharmacy and Nutrition /
School of Public Health

Nominated by Mojtaba Shafiee

2023 Best Paper Awards

BEST PAPER – BASIC SCIENCE

Poonam Dhindwal
Western College of Veterinary Medicine

A neglected and emerging antimicrobial resistance gene encodes for a serine-dependent macrolide esterase

Dhindwal P, Thompson C, Kos D, Planedin K, Jain R, Jelinski M, Ruzzini A. A neglected and emerging antimicrobial resistance gene encodes for a serine-dependent macrolide esterase. Proc Natl Acad Sci U S A. 2023 Feb 21;120(8):e2219827120. https://doi.org/10.1073/pnas.221982712

BEST PAPER – TRANSLATIONAL, CLINICAL, OR APPLIED SCIENCE

Saurav Rout
College of Medicine

Distinct effects of treatment with two different interferon-alpha subtypes on HIV-1-associated T cell activation and dysfunction in humanized mice

Rout, Saurav S.a; Di, Yunyuna; Dittmer, Ulfb; Sutter, Kathrinb; Lavender, Kerry J.a. Distinct effects of treatment with two different interferon-alpha subtypes on HIV-1-associated T-cell activation and dysfunction in humanized mice. AIDS 36(3):p 325-336, March 1, 2022.
https://pubmed.ncbi.nlm.nih.gov/35084382/

BEST PAPER – SOCIAL & POPULATION HEALTH

Masud Rana
College of Medicine

Modeling Obesity Rate with Spatial Auto-correlation: A Case Study

https://link.springer.com/chapter/10.1007/978-3-030-86133-9_3

Undergraduate Research 1

1. EVIDENCE OF MITIGATING STRATEGIES IN PERFORMANCE OF THE ODOUR SPAN TASK: IMPLICATIONS FOR ODOUR-BASED REWARD SEEKING TASKS

Presenter:	**Aiden Glass**
College:	**College of Arts and Science**
Collaborators:	Timothy J. Onofrychuk, College of Medicine
	Quentin Greba, College of Medicine
Supervisor:	John G. Howland

Background:
Reinforcing correct behavioural responses with a food reward is central to rodent studies of learning and memory. One example of this is the Odour Span Task (OST) which evaluates non-spatial working memory capacity by challenging rodents to remember an incrementally greater number of distinct odours to obtain a food reward. Many odour discrimination-based tasks include a small probe experiment to prove that rodents reliably respond to novelty cues in the absence of a food reward. However, these tests typically consist of only a few trials, and do not address the possibility of rodents detecting the food reward itself to guide behaviour.

Methods:
In the present study, we sought to expand on existing probe experiments by more rigorously evaluating whether rats respond to the scent of a food reward within the OST. Within a single one-hour testing session, rats were evaluated in their responses to: 1) a food reward hidden by a novel scented lid; 2) a novel scented lid alone; 3) a hidden food reward alone; 4) simultaneous presentation of the food reward and a novel scented lid at spatially distinct locations. Responses were evaluated in the presence or absence of scent-masking sand.

Results:
When presented with sand, rats accurately respond to novelty and the food scent alone but are significantly more likely to choose the novel stimulus when presented with both cues simultaneously. Without the use of sand, rats consistently respond to novelty cues alone, but also reliably respond to the scent of the food reward alone and perform at chance when presented with both cues simultaneously.

Conclusion:
Collectively, these results demonstrate the need for rigorous probe experiments in odour discrimination-based behavioural tasks to ensure the internal validity of experimental variables.

2. A NOVEL MODEL OF NEURONAL HNRNP A1 DYSFUNCTION USING CRISPR/CAS9

Presenter: **Nicole Gerber**
College: **College of Arts and Science**
Supervisor: Michael Levin
Co-supervisor(s): Hannah Salapa

Background:
Heterogeneous nuclear ribonucleoprotein A1 (A1) is an RNA-binding protein that plays a critical role in RNA metabolism in neurons. Neuronal A1 dysfunction is a characteristic of multiple sclerosis (MS) brains; however, the consequences of A1 dysfunction are not completely understood. Thus, we sought to develop a novel model of A1 dysfunction in neuronal cells using the clustered regularly interspaced short palindromic repeats (CRISPR)/cas9 system to better comprehend the consequences of A1 dysfunction in MS. We hypothesized that A1 knockout via CRISPR/cas9 would induce neuronal cell death.

Methods:
Neuro2A cells, a mouse neuroblastoma cell line, were transfected with a plasmid containing the cas9 endonuclease, a puromycin resistance marker for positive selection of cells that receive the plasmid, and a single-guide RNA targeting A1 (sgA1). Cells were harvested at different time points post-transfection (24, 48, 74, 96, 120, and 144 hours) to evaluate A1 knockout efficiency over time by western blots. The XTT cell toxicity assay was used to monitor neuronal cell viability.

Results:
A1 knockdown was evident as early as 72 hours post-transfection; however, 144-hours post-transfection showed the most significant knockout with greater than an 80% decrease in A1 expression (unpaired t-test, n=3; ****$p<0.05$). Additionally, cell viability decreased with time post-transfection where there was greater A1 knockout. This suggests that A1 knockout resulted in neuronal cell death.

Conclusion:
We established a model of A1 dysfunction using the CRISPR/cas9 system and observed significant knockout after transfection with sgA1. The cell viability assay demonstrated that loss of A1 induces neuronal cell death illustrating the importance for proper A1 functioning in neurons. Considering the critical role that A1 plays in MS neuronal homeostasis, these data support the hypothesis that A1 dysfunction contributes to the pathogenesis of neurodegeneration in MS.

3. PRODROMAL RNA BINDING PROTEIN DYSFUNCTION CONTRIBUTES TO NEURODEGENERATION IN IN VIVO MODELS OF MULTIPLE SCLEROSIS

Presenter:	**Cole Libner**
College:	**College of Medicine**
Collaborators:	Hannah E. Salapa, College of Medicine
	Michael C. Levin, College of Medicine
Supervisor:	Michael C. Levin

Background:
Multiple sclerosis (MS) is an autoimmune and neurodegenerative disease of the central nervous system affecting millions world-wide. Recent evidence indicates a prodromal period in MS, consisting of early signs/symptoms occurring before the clinical onset of disease, in which increased medical visits, lowered cognitive performance, and neuronal injury are observed. We have previously demonstrated the dysfunction of the RNA binding protein (RBP) heterogenous nuclear ribonucleoprotein A1 (A1) contributes to neuronal injury in later stages of MS and its models. The contribution of A1 dysfunction to the early disease processes of the MS prodrome is unknown.

Methods:
Using the experimental autoimmune encephalomyelitis (EAE) model of MS, spinal cord neurons were analyzed by immunohistochemistry for markers of RBP dysfunction and neurodegeneration prior to disease onset at 5-, 8-, and 11-days post immunization (DPI) (mimicking the MS prodrome), and at the onset of clinical signs.

Results:
Compared to naïve mice, spinal cord neurons from mice 11DPI demonstrated increased A1 nuclear-cytoplasmic mislocalization (*$p < .01$), a feature of its dysfunction, which remained elevated at symptom onset. Similarly, immunoreactivity for SMI-32, a marker of neuronal injury, was elevated in mice 11DPI (*$p < .05$), which persisted to symptom onset. There was a trend of decreasing neuronal cell bodies leading up to, and a significant increase in the expression of necroptosis markers (P-MLKL), a cell death pathway (*$p < .001$) at, the onset of clinical symptoms.

Conclusion:
In conclusion, A1 dysfunction is present in the early stages of EAE (mimicking the MS prodrome) and may contribute to prodromal neuronal injury in MS and its models. Inhibiting A1 dysfunction could be targeted to prevent permanent neurological decline in persons living with MS.

4. A1 ANTIBODIES DISRUPT AUTOPHAGIC AND LYSOSOMAL PATHWAYS IN A NEURONAL MODEL OF MULTIPLE SCLEROSIS

Presenter: **Kaitland Fior**
College: **College of Arts and Science**
Collaborators: Hannah Salapa
Supervisor: Michael Levin

Background:
Multiple Sclerosis (MS) is a neurodegenerative disease resulting in the damage and death of neurons. Previous research shows that MS, along with several other neurodegenerative diseases, display abnormal RNA binding protein (RBP) pathology. As an autoimmune disease, patients with MS have been shown to make antibodies to the RBP heterogeneous nuclear ribonucleoprotein A1 (A1). Primary mouse neurons treated with A1 antibodies results in endogenous A1 dysfunction, including the mislocalization of A1 from its homeostatic nuclear location to the cytoplasm and an increase in select programmed cell death pathways, which is followed by neurite loss, indicative of neurodegeneration. The mechanisms mediating A1 antibody induced neurodegeneration are poorly understood. We hypothesize that A1 antibody-mediated neurodegeneration is dependent upon changes in autophagy, an intracellular degradation pathway.

Methods:
Primary mouse neurons were treated with A1 antibodies or IgG control antibodies to determine changes in autophagy, an intracellular degradation pathway, and lysosomal uptake of antibodies. Following antibody addition, primary neurons were analyzed by immunocytochemistry using fluorescence microscopy to identify changes in these markers, or pathways, due to A1 antibodies.

Results:
Primary mouse neurons treated with A1 antibodies, in contrast to control antibodies, showed increased formation and accumulation of autophagic vesicles at 12 and 24 hours between conditions (Two-way ANOVA (*$p<0.05$)), indicating alterations in the autophagy pathway. Additionally, in contrast to IgG control antibodies, A1 antibodies did not colocalize with lysosomes, suggesting A1 antibodies remain within the neuron for a greater amount of time, and negatively impact neuronal health.

Conclusion:
These data indicate that A1 antibodies disrupt normal autophagic processes and may be unable to be readily cleared by the neuron, thus negatively affecting neuronal health. Alterations in autophagy and lysosomal processing of antibodies may be one of several mechanisms that underlie A1 antibody-mediated neurodegeneration in MS.

5. THE EFFECT OF INHIBITING CELL STRESS IN AN IN VITRO MODEL OF MULTIPLE SCLEROSIS

Presenter: **Jacob Pilon**
College: **College of Arts and Science**
Collaborators: Joseph-Patrick Clarke
Supervisor: Michael Levin

Background:
Multiple sclerosis (MS) is an autoimmune disease of the central nervous system (CNS) characterized by axonal demyelination and neurodegeneration. Current studies suggest multiple mechanisms that underlie neurodegeneration, one of which is the dysfunction (cytoplasmic mislocalization and aggregation) of the RNA binding protein heterogeneous nuclear ribonucleoprotein A1 (A1). Evidence from our lab suggests that these aggregates initiate cell stress signalling pathways leading to neuronal death. Preliminary data from our lab suggests that the integrated stress response (ISR) pathway is activated, which is mediated upstream by the activation of protein kinase RNA-like ER kinase (PERK). In this study, we examined whether mitigation of the ISR, via PERK inhibition, may lead to reduced neurite loss in cells with A1 dysfunction.

Methods:
Differentiated, neuron-like Neuro-2A cells were transfected with OptoA1 (a vector containing A1 linked to the Cryptochrome 2 optogene and a mCherry fluorescent reporter gene). OptoA1 transfected cells were stimulated by blue light (BL) for 180 minutes to induce A1 mislocalization and clustering. This methodology allows for the controlled formation of pathogenic A1 clusters, like those observed in neurons of MS patients. In fixed cell imaging, OptoA1 cells were pre-treated with or without the PERK inhibitor GSK2606414 for 60 minutes, and subsequently BL stimulated to form clusters. Phosphorylation of eIF2□ was then measured in fixed cell imaging to determine the activation of the ISR. Additionally, live cell imaging was performed to observe A1 dysfunction in real-time and used to measure neurite loss over time with and without GSK2606414 treatment.

Results:
BL stimulated OptoA1 cells with A1 dysfunction had an 86% reduction in neurite length (N = 4). Pre-treatment with GSK2606414 showed a dose response (0.1-10uM) reduction of A1 dependent neurite loss, which was greatest, 35% ($p<0.001$, N=4), at 180 minutes. Further analysis of these cells showed a 33% reduction in the activity of phosphorylated eIF2a with GSK2606414 pre-treatment, as measured by the fluorescence expression of phosphorylated eIF2a (N=3) trending towards statistical significance.

Conclusion:
In this study, we show that A1 dysfunction leads to the cell stress activation of the ISR pathway through the activation of the PERK protein. Furthermore, our data show this response exacerbates neurite loss, a measure of neurodegeneration. Finally, we demonstrate that inhibiting PERK activity with GSK2606414 attenuates neurite loss, thus providing a target to reduce neurodegeneration and improve the lives of persons living with MS.

6. MANAGEMENT PERSPECTIVES ON BURNOUT AND WORK ENGAGEMENT IN COMMUNITY PHARMACY: A QUALITATIVE STUDY OF EXPERIENCES, CHALLENGES, AND OPPORTUNITIES

Presenter: **Stefanie Kiriazopoulos**
College: **College of Pharmacy and Nutrition**
Supervisor: Jason Perepelkin

Background:
There is a growing body of literature surrounding burnout and engagement among health care professionals, but community pharmacy practice remains underrepresented. With increasing demands on pharmacy teams, particularly since the start of the COVID-19 pandemic, burnout poses a significant risk to these care providers and their patients. Consequently, effective interventions to minimize burnout and enhance work engagement need to be identified for this practice context. The present study aims to characterize the issue of burnout from the perspective of community pharmacy management and identify their current approaches to managing burnout and work engagement, or the lack thereof.

Methods:
Semi-structured interviews were conducted with 16 participants involved in community pharmacy management—including pharmacy managers, district managers, owners, and executives. Inductive thematic analysis of transcripts was then used to identify emerging themes and subthemes in consultation with researcher memos.

Results:
To date, 8 of the 16 interviews have been fully analyzed. Initial analysis reveals a marked increase in the levels of perceived burnout and a related loss of engagement among staff in the community pharmacy setting, which has persisted since the start of the COVID-19 pandemic. Emerging themes surrounding disconnected pharmacy decisionmakers, overwhelming work demands, apprehensive views of expanding the pharmacy scope of practice, the appropriateness of existing resources, the importance of employee recognition, and the need for collaborative burnout solutions have been identified thus far.

Conclusion:
While analysis is ongoing, the supplied management insights reveal opportunities to address burnout and work engagement among community pharmacy staff members. The results of this study are intended to guide future research and implementation of specific, community pharmacy-oriented burnout interventions.

Self-declaration of research alignment with additional themes (optional):
COVID-19 Pandemic Research, Response, and/or Outreach

7. INTEGRATIVE MULTI-OMIC ANALYSIS TO IDENTIFY SPACEFLIGHT NEURONAL HEALTH BIOMARKERS

Presenter: **Anurag Sakharkar**
College: **College of Medicine**
Supervisor: Changiz Taghibiglou
Co-supervisor(s): Jian Yang

Background:
Space exploration has captured the imagination of humanity for generations. From the first steps on the moon to the recent Mars rover missions and Artemis lunar exploration missions taking humans back to the moon for the first time in 50 years, space travel has always been an ambitious goal for humanity. However, as we venture further into space and prepare for long-term missions to other planets, the physiological and health risks associated with prolonged space travel are becoming more apparent. One such risk is the potential impact of spaceflight on the human brain and its functioning. The human brain is a complex and delicate organ, and the effects of microgravity, radiation, and isolation on brain health during long-duration spaceflight are still not fully understood.

Methods:
This study employed a novel method of combining microarray and RNA-seq datasets to increase statistical significance and improve the reliability of our findings. Combining these different types of datasets, allowed for a more comprehensive understanding of the molecular mechanisms that underlie neuronal health during spaceflight. Microarray and RNA-seq datasets capture different aspects of gene expression, and by combining them, we were able to overcome some of the limitations of each method and obtain a more complete picture of gene expression in astronauts. This approach has the potential to be useful in other areas of space biology research, as well as in other fields of molecular biology research. By combining datasets, researchers can obtain a more comprehensive understanding of biological processes and improve the statistical power of their analyses, ultimately leading to more robust and reliable findings. Differential expression analysis enabled us to identify genes that were differentially expressed in astronauts compared to control preflight datasets, while genome correlation analysis allowed us to identify co-expressed genes that were potentially involved in similar pathways. Network analysis identified groups of genes that were functionally related, while functional analysis allowed us to identify the biological functions associated with these genes. Interconnectivity analysis allowed us to identify genes that were important in connecting different functional pathways. Finally, principal component analysis allowed us to identify patterns of gene expression that were associated with astronaut status.

Results:
Using these different methodologies, we identified 10 genes that are potential biomarkers for neuronal health in astronauts and can be characterized for up to 81% of the difference in astronaut gene expression caused by space travel. These genes included SNORA81, PKP1, NR4A2, RPPH1, MGST1, ST14, SFN, POSTN, SCARNA2, and AKR1C1. These genes have been previously associated with neuronal health, including synaptic plasticity, cell survival, and neuroprotection. The identification of these genes as putative biomarkers for neurodysfunction in astronauts suggests that these genes may be involved in neurodysfunction during spaceflight. The identification of these genes as potential biomarkers for neuronal health in astronauts has important implications for space exploration and

human health. The long-term effects of spaceflight on the human brain are not well understood, and identifying biomarkers for neuronal health will be critical for understanding these effects. Furthermore, the characterization of these genes as treatment opportunities for maintaining and treating neuronal health during spaceflight could improve the safety and well-being of astronauts during long-term space travel.

Conclusion:

This study consisted of the use of a comprehensive, integrative multi-omic analysis pipeline to identify 10 potential biomarkers of neuronal health in astronauts. These genes are proposed to serve as hub genes and act as extremely important regulators of neurodysfunction while in spaceflight. Therefore, they could be used as diagnosis, monitoring, and treatment opportunities for astronaut health while in space. The identification of these genes as potential biomarkers and treatment opportunities highlights the importance of further research in this area and the impact of these findings on space exploration and human health. Future studies in space conditions, such as on the ISS, are proposed to serve as optimization and validation platforms for this geneset. The identification of these biomarkers could allow for a better understanding of the molecular mechanisms that underlie and control neuronal health during spaceflight. Furthermore, the identification of treatment opportunities for maintaining and treating neuronal health during spaceflight could improve the safety and well-being of astronauts during the long-term space missions that are undoubtedly a large part of humanity's future.

Self-declaration of research alignment with additional themes (optional):

Interdisciplinary/Interprofessional Collaboration

8. A SCOPING REVIEW OF PEDIATRIC TRANSPLANT EDUCATION

Presenter:	**Ashley Burghall**
College:	**College of Pharmacy and Nutrition**
Collaborators:	Michelle Ruhl, College of Medicine
	Brianna Groot, College of Pharmacy and Nutrition
	Nicola Rosaasen
Supervisor:	Holly Mansell

Background:
Education is crucial for pediatric patients and families throughout the transplant continuum, yet data is lacking around which interventions are effective and in what circumstances.

Methods:
We undertook a scoping review with the objectives of a) describing the types, effects, and outcomes of patient-focused educational interventions before and after pediatric transplant and b) understanding the educational experiences of patients and caregivers. Five scientific databases were explored for relevant literature using the JBI methodology. Educational interventions published in English, targeting pediatric solid-organ transplant patients (0-25 years) and their caregivers were included. Relevant data from eligible articles (n=27) were extracted and summarized.

Results:
Eighteen articles describing seventeen educational interventions were identified for objective A, and nine articles qualitatively assessing patient or parental learning needs were identified for objective B. Most interventions were directed toward teenage patients and their caregivers post kidney transplant, primarily focusing on medication self-management and adherence, or providing general information on transplant using multi-component delivery formats. Most interventions achieved statistically significant improvements in knowledge (n=8/9) and expressed satisfaction with the intervention (n=7/7) but health-related outcomes such as adherence (n= 2/5) or behaviour (n=2/4) change rarely achieved statistically significant results. In objective B, patients and caregivers described the transplant process as overwhelming, but indicated that social supports and education helped them cope. Participants consistently wanted more information than they received.

Conclusion:
Caregivers and pediatric patients value transplant education, but high-quality studies are limited. Since education is a fundamental part of the transplant process future research in this area should be prioritized.

Self-declaration of research alignment with additional themes (optional):
Interdisciplinary/Interprofessional Collaboration

Undergraduate Research 2

9. EXAMINING KNOWLEDGE, BELIEFS, AND PERSPECTIVES ON DRUG DECRIMINALIZATION IN SASKATCHEWAN

Presenter: **Christine Balderama**
College: **College of Arts and Science**
Supervisor: Barbara Fornssler

Background:
Canada's stance on substance use and addictions has evolved throughout the years. Current drug policies in the country continue to be punitive rather than supportive of a humane approach to persons who use drugs (PWUDs). Furthermore, marginalized community members are disproportionately targeted under these existing policies while experiencing additional barriers to health services and access, housing, and employment. More importantly, despite these punitive policies, drug-related harms continue to prevail in Canadian society. Of particular significance to this issue is the recent pilot program launched by the British Columbia provincial government to decriminalize personal possession of specific illicit substances to reduce the harms experienced by PWUDs and address the rising mortalities of the country's opioid crisis.

Methods:
The present research study sought to explore the knowledge, beliefs, and perspectives of service providers in Saskatoon, Saskatchewan, on drug decriminalization. A total of four service providers (n=4) participated and were recruited using non-probability purposive sampling. A pragmatic approach that incorporated knowledge translation theory was utilized in this study. Data collection employed a semi-structured interviewing style consisting of five questions and was analyzed using an inductive thematic analysis developed by Braun and Clarke (2006).

Results:
Three major themes were generated from the responses of the participants of this research study (n=4): (a) shifting to a public health approach, (b) establishing a safer supply, and (c) providing as well as expanding access to both harm reduction and health services. Based on these conversations, participants' responses were unique but had some overlaps in some areas of this study, notably on the theme of access to harm reduction and health services.

Conclusion:
This study generated old and new insights on drug decriminalization. Substance use is a societal issue that touches both public health and criminal justice systems. We have identified in this study that decriminalization is not the only solution to the drug crisis in North America - it is a part of the bigger picture. It requires other initiatives such as implementing safer supply programs, safe consumption sites, needle programs and addressing other health-related needs (e.g. mental health illness) for drug reform to be successful on a municipal, provincial and federal level. Overall, what we have gained in

this research study is a deeper insight into how stakeholders are impacted by policy and how multifactorial elements like belief systems shape policy.

Self-declaration of research alignment with additional themes (optional):
Interdisciplinary/Interprofessional Collaboration

10. MEDICAL STUDENTS' MENTAL HEALTH: BURNOUT AND ITS CORRELATIONS WITH ANXIETY, DEPRESSION, AND LONELINESS

Presenter: **Sapanpreet Saran**
College: **College of Arts and Science**
Supervisor: Adam Stacey

Background:

Burnout, Anxiety, Depression, and Loneliness have all been tied to factors regarding the mental health of medical students. A great deal of research has been done with this cohort focused on aspects of mental health. Research has found correlations between Burnout, Anxiety, and Depression, and more recent literature has demonstrated the influence of Loneliness on medical students' mental health. The present study aimed to survey Canadian medical students across all four years of medical education at the University of Saskatchewan to examine correlations between Burnout, Anxiety, Depression, and Loneliness. It is hypothesized that increased levels of Burnout among medical students correlates with increased levels of Anxiety, Depression, and Loneliness.

Methods:

An anonymous online survey was completed by medical students at the University of Saskatchewan. This survey measured Burnout using the Maslach Burnout Inventory. It measured Anxiety using the State-Trait Anxiety Inventory. Depression was measured using the Beck Depression Inventory-2. Lastly, Loneliness was measured using the UCLA Loneliness Scale. The survey was made available to students in all four years of medical study at the university. The survey was conducted using SurveyMonkey, to which the link was provided through email, class presentations, and social media posts. Only fully completed surveys were used for analysis, incomplete surveys were deemed withdrawals from the study.

Results:

A total of 41 medical student responses were collected, of which 37 were fully complete and used for data analysis (31 participants were needed to meet statistical power). Data was exported from SurveyMonkey and analyzed using SPSS Statistical Software to determine correlations between the variables being analyzed. This study resulted in various statistically significant correlations. Depression was positively correlated with Burnout on the Exhaustion and Cynicism subscales. Depression was also found to be negatively correlated with Burnout on the Professional Efficacy subscale. Furthermore, increased rates of Loneliness were positively correlated with both State and Trait Anxiety. Positive correlations were also found within the Anxiety measure itself between the State and Trait Anxiety subscales.

Conclusion:

The results of this study identified strong correlations between Depression and Burnout as well as strong correlations between Loneliness and Anxiety. These findings add to the previously existing literature on the mental health of medical students and aims to guide future research while pushing for more positive changes toward medical students' wellness. These results also aim to decrease the stigma that exists surrounding the discussion of mental health. Some of the limitations of the present study include its sample only being from the University of Saskatchewan, the small sample size, a majority of respondents being female, self-report, and the purely correlational nature of this research. Future research should look at taking a longitudinal approach to this topic to find directional and causal relationships between Burnout, Anxiety, Depression, and Loneliness.

11. CHARACTERIZATION OF PATIENTS DEVELOPING VENOUS THROMBOEMBOLISM WHILE ADMITTED TO THE DUBÉ CENTRE FOR MENTAL HEALTH

Presenter: **Ryan Chan**
College: **College of Pharmacy and Nutrition**
Supervisor: Katelyn Halpape
Co-supervisor(s): Emma Hamid, Thuy Le, Mariam Alaverdashvili, Annabelle Wanson

Background:
Venous thromboembolism (VTE) is a source of preventable morbidity and mortality among hospitalized patients. The incidence of VTE in psychiatric inpatients ranges between 2-25%. Within the past 15 years, the Dubé Centre for Mental Health (DCMH) clinical team identified an ongoing trend of inpatients developing VTEs. This study aimed to identify risk factors associated with VTE development in psychiatric inpatients.

Methods:
Retrospective case-control chart review of patients admitted to the DCMH from January 2007 to December 2021. Cases were identified through the inpatient pharmacy software by screening for individuals aged 18 and older who received anticoagulation for VTE treatment. Controls were randomly selected from patients with a discharge diagnosis other than VTE. Case-to-control ratio was 1:4. Data was extracted, coded, and comprehensively analyzed using descriptive analysis, univariate, followed by multivariable logistic regression analysis to identify risk factors associated with VTE diagnosis.

Results:
A total of 32 cases and 159 controls were included. Case mean age was 52 years, 21 patients (65.6%) were female, and 27 patients (84.4%) were Caucasian. Chronic comorbidities including cancer, cardiovascular, and hematological-related diseases, insomnia, psychiatric-specific interventions such as electroconvulsive therapy and mechanical restraints, acute medical diagnoses, and catatonia were independently associated with VTE development (OR>1, p-values<0.05). Substance use was significantly associated with decreased odds of VTE diagnosis (OR=0.14, p<0.001). Scheduled use of lorazepam, clozapine, and olanzapine intramuscular were significantly associated with VTE diagnosis.

Conclusion:
Psychiatric inpatients have unique risk factors which increase the likelihood of VTE. Resources targeted at VTE prophylaxis for at risk patients, including staff education and clinical practice tools, could help to optimize inpatient psychiatric care.

Self-declaration of research alignment with additional themes (optional):
Interdisciplinary/Interprofessional Collaboration

12. CAN WE IMPROVE ON FREEZE IN JANUARY, AMPUTATE IN JULY: EVALUATION OF ILOPROST TREATMENT FOR FROSTBITE IN SASKATCHEWAN

Presenter:	**Emma Yanko**
College:	**College of Medicine**
Collaborators:	Breanne Paul, College of Medicine Michael Verdirame, College of Medicine Chris Thomson, College of Medicine
Supervisor:	James Stempien
Co-supervisor(s):	Tracy Wilson

Background:

Amputation after frostbite injury is thought to be related to small blood clots forming during the rewarming process, leading to tissue ischemia and infarction. Iloprost is a medication that has been used to reduce injury secondary to frostbite and has been shown to reduce amputation rates in retrospective observational research studies. This retrospective chart review will compare outcome data in patients diagnosed with grade 2-4 frostbite in Regina and Saskatoon. A formal frostbite treatment protocol using iloprost was implemented in Saskatchewan in the fall of 2022. This current study aims to determine the difference in amputation rates between patients diagnosed with frostbite and treated with iloprost compared to standard treatment.

Methods:

This retrospective observational study will examine charts of all patients diagnosed with grade 2-4 frostbite injuries, treated in Saskatoon and Regina Hospitals between July 1, 2017 and February 15, 2023. Charts were identified by triage complaints of “frostbite/cold injury”, “hypothermia” or diagnosis of “frostbite”, “cold injury”, “hypothermia” and/or ICD-10 discharge diagnoses of “superficial frostbite (T33)”, “frostbite with tissue necrosis (T34)” or “hypothermia (T68)”. These charts are currently under review by members of the research team. Descriptive statistics and qualitative data will be calculated from the information recorded from patient charts. At this time, data has been collected for all patients presenting to the Royal University Hospital. This initial data collection has allowed for a preliminary analysis of our data, as this patient population is generalizable to the other four intended hospitals.

Results:

We identified that frostbite treatment in Saskatoon presents unique challenges due to multiple patient and system factors, distinguishing it from the treatment landscapes of other Canadian institutions that have previously protocolized the use of iloprost for frostbite management. While our chart review and descriptive analysis is ongoing, preliminary qualitative data is available for the Royal University Hospital. We identified multiple unique factors impacting patients receiving care for frostbite, including homelessness, substance use, and psychiatric disorders.

Conclusion:

During the Fall of 2022, the emergency departments in Saskatoon established a protocol for treating frostbite using iloprost. Prior to this, very few patients with grade 2-4 frostbite received iloprost. We

are conducting a retrospective chart review to assess the use of iloprost and amputation rates before and after protocolization. While our chart review is ongoing, we have identified that frostbite treatment in Saskatoon presents unique challenges due to multiple patient and systemic factors. While our primary descriptive results are pending, we recommend harm reduction strategies to optimize the treatment approach for patients with frostbite injuries.

Self-declaration of research alignment with additional themes (optional):
Interdisciplinary/Interprofessional Collaboration

13. INVESTIGATING THE POTENTIAL HOMEOSTATIC ROLE OF HIF-1C IN C. ELEGANS NEURONS

Presenter:	**Kate Andrea Locsin**
College:	**College of Arts and Science**
Collaborators:	Amir Sabeti, College of Arts and Science Carlos Carvalho, College of Arts and Science
Supervisor:	Carlos Carvalho

Background:
Evolving literature underpins that acute or chronic shortage of physiological oxygen is an underlying threat to fetal development. Most problems that arise from hypoxic insults are neurological, which may be apparent as early as after birth or later in life. Upon the discovery of the hypoxia-inducible factor (HIF-1), a master heterodimeric transcription regulator conserved in higher eukaryotes, there is now a better understanding of how oxygen is critical for many creatures to sustain life. The observed prolonged stability of HIF-1a proteins found in all tissues in C. elegans during hypoxia was revealed to be problematic, suggesting to be the reason behind the adverse effects of hypoxic insults in humans.

Methods:
This research is based on Dr. Carvalho's laboratory preliminary data, where the relatively unexplored shortest isoform of HIF-1 proteins, HIF-1c, is found exclusively in neurons and is uniquely expressed as a product of an internal promoter transcriptional machinery in C. elegans. These preliminary results suggest a protective and unique homeostatic role of HIF-1c in neurons linked to hypoxic challenges. Using a mixed-methods approach, this study will examine the spatial and temporal expression of hif-1c during neurogenesis in C. elegans' developing nervous system and functionally characterizing HIF-1c in the context of hypoxia response.

Results:
N/A

Conclusion:
Findings from this study will contribute to the understanding of a neuronal-specific HIF-1c isoform, which may be the initial step in discovering a novel preventative or therapeutic for human hypoxia-related damage.

Self-declaration of research alignment with additional themes (optional):
Sustainable Progress

14. USING INTRAORAL SCANNERS TO STUDY ENDODONTIC ACCESS OPENINGS: A HYBRID-METHODS INVESTIGATION

Presenter: **Shiva Farrahi**
College: **College of Dentistry**
Supervisor: Renata Grazziotin
Co-supervisor(s): Diego Ardenghi

Background:
Intraoral scanners have not been used to study procedural errors in endodontic access. This research investigated the dental students` perceptions (qualitative), and the accuracy (quantitative) of different digital tools originating from the scanning of pulp chambers in depicting optimal and suboptimal (containing procedural errors) access openings.

Methods:
Human-extracted teeth were used to perform optimal and suboptimal access openings. Accessed teeth were digitally scanned with an intraoral scanner, originating three different tools for each tooth: i) an STL File, ii) a movie, and iii) a set of pictures. The 'real tooth' was used as a fourth tool, for comparison. Dental students answered questions about access and the presence of procedural errors (according to the tools). Participants also answered questions on perceptions and feedback.

Results:
Of the students invited, 51.5% participated. The overall accuracy of each tool (ability to depict appropriate or inappropriate access) was real tooth 71.55% > movie 60.29% > STL File 57.35% > pictures 56.86%. The best tool to depict appropriate access was the 'real tooth'; and the best tool to depict inappropriate access was the set of pictures. Overextended access, underextended access, and damage of the pulpal floor/canal entrances were some of the procedural errors accurately detected. Two themes originated from the qualitative analysis, containing critics and ideas on the use of STL Files to study endodontic access openings.

Conclusion:
Human-extracted teeth were the most accurate and preferred by students for learning endodontic access cavities. STL Files were considered satisfactory - if used as a supplemental material.

15. DO SEX AND AGE INFLUENCE SCAPULAR AND THORACOHUMERAL KINEMATICS DURING A FUNCTIONAL TASK PROTOCOL?

Presenter:	**Alexander Waslen**
College:	**College of Medicine**
Collaborators:	Kenzie B. Friesen
	Angelica E. Lang, College of Medicine
Supervisor:	Angelica E. Lang

Background:
There is mixed evidence on the role that biological sex plays in shoulder biomechanics despite known differences in musculoskeletal disorder (MSD) prevalence between males and females. Additionally, advancing age may contribute to shoulder kinematic changes. The purpose of this study was to determine if sex and age influenced scapular and thoracohumeral kinematics during a range of functional tasks.

Methods:
Sixty healthy participants aged 19 to 63 years (30 males; 30 females) completed a functional task protocol while their upper limb motion was recorded. Scapular and humeral angles were calculated and compared with multiple linear regressions to assess the interaction effects of sex and age.

Results:
Shoulder kinematics were not different between sex and age groups for many of the functional tasks. However, females had lower humeral external rotation in the Overhead Lift task (15°, $p < 0.001$), and less scapular anterior tilt angles in the Forward Transfer task (6°, $p < 0.001$) than males. Age was positively associated with humeral elevation ($R2 = .330$, $p < .001$) and scapular rotation ($R2 = .299$, $p<.001$) in the Wash Axilla task.

Conclusion:
There exist some kinematic differences between sex and with advancing age for select functional tasks, which should be considered for MSD development.

16. THE IN VIVO INTOXICATION EQUIVALENCY OF CANNABIGEROL AND Δ9- TETRAHYDROCANNABIPHOROL COMPARED TO Δ9- TETRAHYDROCANNABINOL

Presenter:	**Kenzie Halter**
College:	**College of Arts and Science**
Collaborators:	Ayat Zagzoog, College of Pharmacy and Nutrition Robert B. Laprairie, College of Pharmacy and Nutrition Ashley Cabecinha
Supervisor:	Robert B. Laprairie

Background:
Since the introduction of the Cannabis Act in 2018, overall cannabis use has increased among the general Canadian population, extending a trend that existed before legalization. However, cannabis use among youth during this time has not increased. Cannabis holds therapeutic potential via it's agonism at cannabinoid receptor type 1 (CB1R) and type 2 (CB2R); however, activation of CB1R via Δ9-tetrahydrocannabinol (THC) is also responsible for the characteristic "high" induced by cannabis. The plant-derived (phyto) cannabinoid, THC has a well-established pharmacology and is known to be a CB1R partial agonist. The pharmacology of the less-abundant phytocannabinoids – such as cannabigerol (CBG) and Δ9-tetrahydrocannabiphorol (THCP) – are less established.

Methods:
Using THC as a reference compound, we have worked to determine the acute intoxication equivalency index of CBG in C56BL/6 mice, with THCP assays ongoing. THC, CBG, and THCP are being assessed for their pharmacokinetic profiles, as well as cataleptic, hypothermic, and anti-nociceptive effects following oral (p.o.), intraperitoneal (i.p.), and intravenous (i.v.) administration in mice.

Results:
Our data thus far reveal in vivo responses associated with CB1R activation following THC administration, but not CBG. We expect further experiments will show that CBG is not intoxicating, even when compared at the same routes of administration and plasma concentrations, whereas THCP will be intoxicating.

Conclusion:
These experiments will provide valuable data on how phytocannabinoid pharmacology differs from THC and whether this difference is of medicinal importance or significant in the context of harms reduction. Acknowledgment: This work was supported by Research Chair funds from the College of Pharmacy and Nutrition, University of Saskatchewan and a Health Canada Research Contract awarded to RBL. AZ is supported by a graduate student scholarship from the College of Pharmacy and Nutrition.

Basic Science 1

17. THE EPHB4 RECEPTOR REGULATES CANINE AND HUMAN OSTEOSARCOMA INVASIVENESS AND TUMORSPHERE FORMATION

Presenter:	**Jessica Sharpe**
College:	**Western College of Veterinary Medicine**
Collaborators:	Evelyn Harris, Western College of Veterinary Medicine
	Tim Strozen, Western College of Veterinary Medicine
	Behzad Toosi, Western College of Veterinary Medicine
Supervisor:	Behzad Toosi

Background:

Osteosarcoma is a highly aggressive bone cancer in both canines and humans with a high rate of metastasis and corresponding poor prognosis. Advances in treatment options are limited, highlighting the need for more effective therapeutic approaches. The Eph receptors are the largest group of receptor tyrosine kinases, regulating many cellular activities including proliferation, survival, migration, and invasion. Effects of altered expression of Eph receptors on tumor aggressiveness have been characterized in multiple human malignancies, making these receptors attractive targets for therapeutic intervention. Recent evidence suggests that the EphB4 receptor is involved in the regulation of invasion and metastasis of various human cancers. However, the role of the EphB4 receptor in the fitness of human and canine osteosarcoma has been poorly evaluated. Due to the physiological and cellular similarity between canine and human osteosarcoma, we are using a comparative approach to investigate the role of the EphB4 receptor in promoting osteosarcoma. We initially showed that EphB4 inhibition reduced OSA cell proliferation. Subsequently, we investigated EphB4 effects on other osteosarcoma aggressive traits including cell migration, invasion, and propagation of tumor-initiating cells (TICs).

Methods:

EphB4 expression was evaluated by western blotting in multiple canine and human osteosarcoma cell lines and compared to normal osteoblasts. EphB4 expression was silenced using specific shRNAs and stable cell lines were created. We investigated the effects of EphB4 on canine and human osteosarcoma cell migration and invasion using transwell assays and on propagation of tumor-initiating cells (TICs) using the tumorsphere formation assay. Human osteosarcoma tumorsphere cells were stained with Ki-67 proliferation marker and evaluated by flow cytometry.

Results:

We found upregulated expression of the EphB4 receptor in canine and human osteosarcoma cells when compared to normal osteoblasts. Both canine and human EphB4-silenced osteosarcoma cells had reduced migration and invasion. EphB4 knockdown enhanced TIC proliferation in both canine and human osteosarcoma.

Conclusion:
Our findings demonstrate that the EphB4 receptor is overexpressed in canine and human osteosarcoma and promotes cellular migration and invasion which are important processes in the development and invasiveness of osteosarcoma. Tumor-initiating cells represent a slow-proliferating and drug resistant sub-population of cancer cells. EphB4-silencing enhanced proliferation of TICs formed by both canine and human OSA cells. Interestingly, this original finding suggests that EphB4 inhibition could conceptually make these cells more sensitive to DNA-damaging drugs. Given that similar results were observed in both species, these results emphasize the benefit of using a comparative oncological approach. Currently, an assessment of EphB4 function on tumor development and invasiveness in mouse xenograft models is in progress.

Self-declaration of research alignment with additional themes (optional):
Interdisciplinary/Interprofessional Collaboration

18. EFFECTS OF EXERCISE ON CEREBRAL PERFUSION AND NEUROGENESIS IN A RODENT MODEL OF HEART FAILURE

Presenter:	**Cameron Morse**
College:	**Western College of Veterinary Medicine**
Collaborators:	Boyes, N. G., College of Kinesiology Luchkanych, A. M. S., College of Kinesiology Turnbull, K. Y., Western College of Veterinary Medicine
Supervisor:	Olver, T. D.
Co-supervisor(s):	Tomczak, C. R.

Background:
Almost half of heart failure (HF) patients will develop cardiogenic dementia, but the underlying pathophysiology remains unclear. Cerebral hypoperfusion may contribute to reduced neurogenesis and represents a potential mechanistic link between cardiac and neurologic dysfunction. Exercise training (EX) has been shown to improve neurologic and cardiac function in healthy individuals, but whether it alters the pathophysiology of cardiogenic dementia is unknown. This study tested the following hypotheses: 1) EX would increase cerebral perfusion evidenced by increased internal carotid artery blood flow (iCBF) in a rodent model of HF; 2) EX would increase neurogenesis in the sub ventricular zone (SVZ) and dentate gyrus (DG) in a rodent model of HF.

Methods:
Male Sprague Dawley rats underwent left anterior descending coronary artery ligation (experimental model of HF; n=10). HF rats were subdivided into sedentary (HF-SED, n=5) or EX (HF-EX; n=5) groups. EX began 1.5 weeks post-ligation and consisted of progressive treadmill running three days per week. At 10 weeks post-ligation rats were injected with the thymine analogue 5-ethynyl-2'-deoxyuridine (EdU; 50ug/kg/day for three days) to assess neurogenesis. At 12 weeks post-ligation, iCBF (perivascular flow probe) and mean arterial pressure (MAP; arterial catheter line) were measured.

Results:
iCBF and vascular conductance were greater in HF-EX rats (p=0.01). The number of EdU+ cells was not different between groups in either the DG (p=0.21) or the SVZ (p=0.70).

Conclusion:
These preliminary findings indicate that EX improves cerebral perfusion, but not neurogenesis at 10-12 weeks post-ligation in HF rats.

Self-declaration of research alignment with additional themes (optional):
Interdisciplinary/Interprofessional Collaboration

19. ENHANCED VACCINE EFFECTIVENESS BY DELIVERING CPG-ODN IN BROILER CHICKENS

Presenter: **Iresha Subhasinghe**
College: **Western College of Veterinary Medicine**
Supervisor: Susantha Gomis

Background:

Bacterial diseases including necrotic enteritis cause significant economic losses in the poultry industry. Oligodeoxynucleotides containing cytosine phosphodiester guanine motifs (CpG-ODNs) act as a pathogen-associated molecular pattern (PAMP) that can stimulate immune cells and promote antimicrobial immunity in chickens. Activated immune cells require profound metabolic changes to meet cellular signaling processes. Increased mitochondrial respiration is a metabolic phenotype of memory cells while active cells prefer increased glycolysis pathway.

Methods:

In this study, we hypothesized that CpG-ODNs promote profound metabolic programming to facilitate the antimicrobial activities of immune cells. CpG-ODN or saline was injected into broiler chickens via the intramuscular route/ in-ovo route. Immunometabolic responses were evaluated as a longitudinal study. Immune cells in the peripheral blood mononuclear cell (PBMC) layer were isolated and processed to measure cellular mitochondrial activity [oxygen consumption rate (OCR)] and glycolysis rate [extracellular acidification rate (ECAR)] in real-time using newly optimized Seahorse XFp Extracellular Flux Analyzer. To identify whether T cells or B cells were responsible for CpG-ODN mediated immunometabolic activities, PBMCs were incubated with Pokeweed mitogen (PWM)- mitogenic for both T and B cells and Phytohaemagglutinin (PHA-P)- mitogenic for T cells, separately and ECAR and OCR were quantified. In order to measure the effect of CpG-ODN on vaccine delivery, OCR were measured following oral delivery of a live C. perfringens (CP) vaccine in broiler chickens following in ovo delivery of CpG-ODN and compared with CP vaccine with no CpG-ODN. Immune cell profiles were measured by flow cytometry.

Results:

CpG-ODN injected group showed higher ($P<0.001$) mitochondrial respiration (MR) and glycolytic capacity than the saline group, but cells had higher (MR) for a prolonged period with the highest, 48 hours. Further, PWM-stimulated cells showed increased ($P<0.001$) OCR than PHA-P stimulated cells in the CpG-ODN group, indicating a substantial elevation of immune cell metabolism with increased B cell activity. The group exposed to CpG-ODN prior to CP vaccination showed higher OCR and changing B cell phenotype to increase IgM production than CP vaccine-only group.

Conclusion:

Results of this study demonstrated that CpG-ODN increased metabolic activities of immune cells following CP vaccine delivery to meet energy demand. Further CpG-ODN was able to develop a nonspecific immune memory response in chickens. This study demonstrated the utility of mitochondrial respiration and cellular glycolysis pathways to study immuno-metabolic interactions leading to immune responses in broiler chickens.

Self-declaration of research alignment with additional themes (optional):

Interdisciplinary/Interprofessional Collaboration

20. INVESTIGATION OF THE ROLE OF THE EPHA2 RECEPTOR IN HUMAN AND CANINE MELANOMA

Presenter:	**Shabnam Abdi**
College:	**Western College of Veterinary Medicine**
Collaborators:	Shabnam Abdi, Western College of Veterinary Medicine Behzad Toosi, Western College of Veterinary Medicine
Supervisor:	Behzad Toosi

Background:

: Melanoma is the most lethal type of malignant skin cancer in humans and dogs since it spreads rapidly throughout the body. Despite significant advances in treatment, cancer at an advanced stage has a poor prognosis. Hence, more effective treatments are needed to enhance outcomes with fewer side effects. Erythropoietin-producing hepatocellular receptors are the largest family of receptor tyrosine kinases and are divided into two subfamilies, EphA and EphB, both of which play a significant role in disease especially cancer. Due to their association with proliferation and invasion in many aggressive types of cancer, Eph receptor tyrosine kinases (Eph RTKs) are promising cancer therapy molecules. Because these receptors have not been studied in canine melanoma, we investigated how EphA2 influences survival and tumorigenicity of melanoma cells.

Methods:

Expression of EphA2 protein in canine melanoma cell lines (Parks and Jones) and human melanoma cell line (A375) was evaluated by Western blot. Melanoma cells were transduced with lentiviral particles encoding Eph-targeting shRNAs or non-silencing shRNAs (control) for silencing the expression of an Eph receptor and silencing was confirmed by Western blotting and immunofluorescence. The effect of siRNA treatment on cellular proliferation, colony formation, invasion was analyzed by Resazurin assay, Matrigel invasion assay respectively.

Results:

Expression of EphA2 was detected in canine and human melanoma cell lines. Moreover, stably silencing EphA2 by specific shRNAs significantly and consistently decreased the expression of EphA2 protein in both human and canine melanoma cells. Proliferation, colony formation, and invasion of melanoma cells were significantly decreased in EphA2 siRNA-treated cells compared to control.

Conclusion:

Our data provide the first functional evidence that the EphA2 receptor plays a critical role in the malignant cellular behavior of melanoma.

Self-declaration of research alignment with additional themes (optional):

Interdisciplinary/Interprofessional Collaboration

21. TRANSGENERATIONAL EFFECTS OF ANCESTRAL ARSENIC EXPOSURE ON COGNITIVE PERFORMANCE AND DOPAMINE SIGNALING PATHWAY IN THE BRAIN OF ZEBRAFISH

Presenter: **Mahesh Rachamalla**
College: **College of Arts and Science**
Supervisor: Som Niyogi

Background:
Arsenic is a neurotoxicant and its adverse effects on the functions of the brain and central nervous system (CNS) are well characterized in mammals including humans. The neurotoxicity of As has been suggested to be mediated by its capacity to induce reactive oxygen species (ROS) production leading to oxidative stress and its high affinity to thiol groups causing protein dysfunctions in the brain and CNS. Chronic As exposure has been reported to cause dopamine imbalance and cognitive deficits in mammals2. However, very little is known about the neuro-behavioural toxicity of As in fish. The main objectives of the present study were to: (i) investigate the effects of environmentally relevant chronic exposure on the cognitive performance of fish, (ii) evaluate how chronic As exposure influences dopamine signalling and the antioxidative status of the fish brain, and (iii) to examine the transgenerational inheritance of these neuro-behavioural effects in fish. We used zebrafish (Danio rerio) as a model species for this study.

Methods:
Adult zebrafish (6 months old; F0 generation) were exposed to 4 different concentrations of arsenic via diet [0 (control), 30, 60 and 100 As µg/g dry weight; as arsenite] for 60 days. The cognitive performance of fish was then examined using a latent learning paradigm in a complex maze, which is composed of a start chamber and a reward chamber, which are connected by the right and left tunnels. Fish were first trained in groups for 16 days to explore the maze from the start chamber and find the reward chamber either via the right or the left tunnel. Subsequently, the probing trials were conducted where an individual fish were given 10 minutes to find the reward chamber with both right and left tunnels kept open. During probing, 6 zebrafish were placed in the reward chamber as a reward or motivation. The cognitive performance was evaluated by analyzing the video footage of fish behaviour in the maze and quantifying the following parameters: (i) the latency to leave the start chamber, (ii) the time spent in the correct tunnel, (iii) difference between time spent in the correct vs. incorrect tunnel, (iv) the latency to enter the reward chamber, and (v) the time spent in the reward chamber. Following behavioural assessments, As-exposed females from each treatment were crossed with control males to produce F1 generations of fish that were maternally exposed (ME) to As. Similarly, F1 generations of fish that were paternally exposed (PE) to As were also produced by crossing As-exposed males with control females. Subsequently, adult males and females from each F1 generation ME and PE groups were bred again to produce F2 generations of the same ancestral As treatment groups. F1 and F2 generations were raised to adulthood (6 months) in clean water and diet when their cognitive performance was evaluated using the same latent learning paradigm described above. This experimental design allowed us to examine whether the transgenerational inheritance of As-induced neuro-behavioural effects occurs via maternal and/or paternal lineage in zebrafish. Fish brains were collected from each treatment group across 3 generations (F0, F1 and F2) and analyzed for arsenic accumulation (F0 only), oxidative stress (reduced to oxidized glutathione ratio and lipid

peroxidation), dopamine level, and expression of genes involved in dopamine signaling as well as memory functions

Results:

1. Transgenerational Cognitive Effects Chronic dietary exposure to As induced a dose-dependent adverse effect on the cognitive performance of in F0 generation. For example, As-exposed fish showed a significantly higher latency to leave the start chamber, and also to enter the reward chamber, and spent significantly less time in the correct tunnel (i.e., the left or right tunnel they were trained in) relative to the control. Moreover, a similar trend in cognitive impairment was also observed in F1 and F2 generations of fish that were ancestrally exposed to As via maternal lineage. F1 and F2 behavioural data of the maternal lineage does not include 100 As µg As/g treatment group because of the high mortality in the F1 generation of that treatment. In contrast, in the F1 and F2 generations from the paternal lineage of ancestral As exposure, cognitive impairment was recorded only in the highest As treatment group (100 As µg/g). 2.Elevated Oxidative Stress and Altered Gene Expression As accumulation, dopamine level and oxidative increased in the brain in a dose-dependent manner in the F0 generation following chronic As exposure. Interestingly, elevated oxidative stress in the brain was also recorded in both F1 and F2 generations following ancestral exposure to As via both maternal and paternal lineage, although the magnitude of the effect was relatively lower in the latter group. Moreover, the expression of genes that regulate dopaminergic pathway [e.g., dopamine receptors (Drd1, Drd2), and monoamine oxidase (MAO)] and memory functions [e.g., BDNF, endopeptidases such as entpd2_mg and entpd_mq)] were consistently downregulated across 3 generations (F0-F2), most notably following ancestral As exposure via maternal lineage.

Conclusion:

Overall, our findings suggest that chronic exposure to As impairs the cognitive performance of zebrafish by inducing oxidative stress and dysregulation of the dopaminergic pathway in the brain. Moreover, we also demonstrated transgenerational inheritance of ancestral neuro-behavioural effects of As in zebrafish via both maternal and paternal lineages, indicating the involvement of epigenetic alterations.

Self-declaration of research alignment with additional themes (optional):

Sustainable Progress

22. THE RELIABILITY OF AN IMU-BASED MOTION CAPTURE SYSTEM FOR DYNAMIC MARGINS OF STABILITY AND RANGES OF TOTAL BODY ANGULAR MOMENTUM: EFFECTS OF WALKWAY LENGTH AND NUMBER OF STRIDES

Presenter:	**Jackson Lordall**
College:	**College of Kinesiology**
Collaborators:	Atabak Mehrabani, College of Engineering Sunny Bui, College of Kinesiology
Supervisor:	Joel Lanovaz
Co-supervisor(s):	Alison Oates

Background:

Portable inertial measurement unit- (IMU) based motion capture systems enable evaluation of walking outside of the lab. The reliability of IMU motion capture systems for assessing balance measures like ranges of total body angular momentum (H) and average margins of stability (MOS) during walking have not been established. Research suggests that including more steady-state strides in an analysis may improve reliability outcomes. The number of steady-state strides captured per trial may differ between walkway lengths. We investigated the test-retest reliability of an IMU-based motion capture system for estimating ranges of H and average MOS during overground walking with different walkway lengths and total number of steady state strides.

Methods:

Participants completed two study visits (>48 hours apart) consisting of walking trials in a lab (walkway length: 8m) and a hallway (walkway length: 20m). A full-body IMU-based kinematic data collection system computed the ranges of anterior-posterior (AP) and medial-lateral (ML) H and average ML MOS over a stride. Relative reliability was assessed using intraclass correlations (ICC) (3, k) which were interpreted as poor (<0.5), moderate (0.5-0.75), good (0.75-0.9), and excellent (>0.9) (Shrout & Fleiss, 1979). Absolute reliability was assessed using minimal detectable change at a 95% confidence level (MDC95) expressed as a percentage of the mean. Separate ICC and MDC95 values were calculated across a range of stride counts.

Results:

28 young adults (12 male, 16 female, age=24□4yrs, height=171□9.5cm, mass=76□21kg) participated. The ICCs indicated good to excellent reliability for H ML ranges; moderate to excellent reliability for H AP ranges in the lab and hallway; and, moderate to excellent reliability for average ML MOS in the hallway. The hallway and lab had similar ICCs for H ranges after 10 strides were completed. Ranges of H and average MOS ML MDC95 values were <2% of the mean values and were not largely affected by the number of strides included in the analysis or the walkway length.

Conclusion:

The high relative and absolute reliability for walking balance measures using small numbers of strides, regardless of collection environment (i.e. walkway length), indicates that IMU systems may be valuable tools for walking balance research. This is encouraging for studies that are conducted outside of lab environments in an effort to improve ecological validity.

Self-declaration of research alignment with additional themes (optional):

Sustainable Progress

23. IS REPRODUCTIVE HISTOPATHOLOGY OF HONEY BEE DRONES USEFUL FOR ECOTOXICOLOGICAL RISK ASSESSMENT?

Presenter: **Marina Carla Bezerra da Silva**
College: **Western College of Veterinary Medicine**
Supervisor: Sarah C. Wood
Co-supervisor(s): Elemir Simko

Background:
The widespread use of pesticides has increased the exposure of agrochemicals to the reproductive health of honey bees. Histopathology is the gold standard method used in animals to assess the gonadotoxic risk of new compounds; however, histopathology is not used in ecotoxicological risk assessment for honey bees. Therefore, the objective of this study is to evaluate the effect of in vitro pupae exposure to the compound Amitraz (gonadotoxic) on honey bee drone testicular development using histopathology.

Methods:
We divided six honey bee drones' age-synchronized frames into six groups, three of which were controls (environmental, procedure, and solvent-only controls), and three were exposed to Amitraz at different concentrations. Treatments were repeated daily for seven days, and three drones were collected daily for histopathological evaluation.

Results:
A dose-response was observed following drone pupae exposure to control, low, medium, and high doses of amitraz, demonstrating 95%, 79%, 28%, and 10% survival to adulthood, respectively. The preliminary histological evaluation suggests a decrease in sperm density with central vacuolation in the seminiferous tubule epithelium of the drone's testis in a dose-dependent manner.

Conclusion:
We successfully developed a model for in vitro drone pupae exposure to gonadotoxic compounds and demonstrated dose-response histopathologic changes in the developing testis of exposed drones. Future work will correlate reproductive histopathologic changes in honey bee drones with functional effects on sperm quantity and viability as part of a standardized protocol to evaluate the safety of new agrochemicals.

Self-declaration of research alignment with additional themes (optional):
Sustainable Progress

24. THE CONSEQUENCES OF LOSS OF PITX2 IN CARDIAC METABOLISM AND PREDISPOSITION TO ATRIAL FIBRILLATION

Presenter:	**Mitra Sabetghadam Moghadam**
College:	**College of Medicine**
Collaborators:	Eli Wiens, College of Arts and Science Ramaswami Sammynaiken
Supervisor:	Michelle M Collins

Background:

Background and Aims: Atrial fibrillation (AF) is the most frequent cardiac rhythm disorder. Several studies have shown that PITX2, a transcription factor playing a critical role in several cardiac processes, such as left-right asymmetry and left-sided pacemaker identity during development and the regulation of genes involved in atrial function, including ion channels and cardiac metabolism. Using a pitx2c deficient zebrafish model which display cardiac phenotypes with significant similarities to the pathologies seen in AF patients, we found changes to metabolic function in cardiomyocytes. The pitx2c −/− zebrafish larvae produced more reactive oxygen species (ROS), and antioxidant therapy reduced the severity and duration of cardiac arrhythmia. Based on these existing data, we hypothesize that the loss of pitx2c compromises cardiac metabolism changes and elevates ROS levels in larvae, juvenile and adult zebrafish, which could contribute to developing cardiac arrhythmia. To test this hypothesis, we aim to measure metabolite content and ROS level in the zebrafish heart at different stages. Furthermore, we generate transgenic lines, including Pitx2c overexpression and lines to activate endogenous antioxidant genes to test if rescuing Pitx2c expression or over activating antioxidants ameliorate the metabolic phenotypes and cardiac arrhythmia.

Methods:

Methods: We characterized cardiac energetics of all pitx2c genotypes at 4 days postfertilization after heat shock using fluorometric assays. Notably, we established the electron paramagnetic resonance assay using a CMH (1-hydroxy-3-methoxycarbonyl-2,2,5,5-tetramethylpyrrolidine) spin probe to measure ROS level in the dissected zebrafish heart from adult animals. We also generated different transgenic zebrafish with overexpression of an antioxidant enzyme, catalase, and pitx2c.

Results:

Results: No significant differences in glucose, lactate, and ATP concentrations were observed at this stage. Ongoing work will examine levels at later stages. Our pilot experiments suggest that this technique is able to detect ROS in the adult zebrafish atria and ventricles.

Conclusion:

Conclusion and Future Directions: While a growing body of evidence suggests that changed metabolic activity affects heart electrophysiology, more research is needed to determine how an altered metabolic state affects membrane excitability in AF. We did not find any changes in the metabolite amounts in various pitx2c genotypes of the larvae stage. Future experiments will focus on analyzing cardiac energetics and comparing ROS levels in dissected hearts for different genotypes and sexes at juvenile and adult stages. In addition, we will use the transgenic lines to overexpress antioxidants and pitx2c to activate genes encoding proteins that regulate cellular redox to see how they affect cellular energetics, ROS level, and finally cardiac arrhythmia.

Basic Science 2

25. ELUTION OF AEP PROTEINS FROM COLLECTION STRIPS BY SONICATION

Presenter: **Ahmed Eldib**
College: **College of Dentistry**
Supervisor: Walter Siqueira
Co-supervisor(s): Lina Marin

Background:
The aim of this study was to develop a protocol for eluting proteins from paper strips that are used to collect acquired enamel pellicle (AEP) proteins. The eluted proteins can then be quantified, analyzed using mass spectrometry, and characterized.

Methods:
Methods: In this study, standard collection strips were loaded with known amounts of a standard protein, Bovine Serum Albumin (BSA), ranging from 10 to 100 ug. The strips were then treated with various elution solutions and purification protocols to recover the proteins. The elution solutions included 100% Acetonitrile (ACN), 80% ACN + 0.01% formic acid, and distilled water. Filtration with a 100-kDa molecular weight cutoff (MWCO) membrane filter was used in some cases. The resulting protein extracts were air-dried using a vacufuge, and the protein concentration was determined using the micro-bicinchoninic acid assay. The protocol that gave the best results with BSA was then tested with different amounts of collection strips containing AEP proteins

Results:
Results: The amount of protein eluted from each group was measured and recorded. The results showed that the protocol using distilled water and filtration with a 100-kDa MWCO membrane filter allowed for the maximum recovery of proteins from the collection strips. The amount of proteins eluted from the AEP collection strips using this protocol ranged from 0.55 to 16.17 ug protein/ml, depending on the number of strips used.

Conclusion:
Conclusion: Based on the results, it can be concluded that the use of distilled water combined with filtration using a 100-kDa MWCO membrane filter is the reliable protocol for eluting proteins from paper strips used to collect AEP proteins.

26. APOBEC3B INTERFERES WITH THE TUMOR SUPPRESSOR ROLE OF PFDN5 AND CAUSES INCREASED LEVELS OF THE ONCOGENE CMYC

Presenter: **Arzhang Shayeganmehr**
College: **College of Medicine**
Collaborators: Gwendolyn M. Jang, Arun Kumar Annan Sudarsan, Robyn M. Kaake
Supervisor: Linda Chelico

Background:
The human APOBEC3 cytidine deaminases (APOBEC3A-H, excluding E) single-stranded DNA cytidine deaminases restrict the replication of viruses and retroelements. By deaminating cytosine to promutagenic uracil, APOBEC3 enzymes cause mutations or induce degradation of viral genomic or proviral DNA. The APOBEC3 enzymes that restrict DNA viruses have access to the nucleus and can also deaminate cytosine in host genomic DNA that contributes to cancer evolution. We hypothesized that the APOBEC3 enzymes that can access the nucleus have unique protein-protein interactions compared to the cytoplasmic enzymes and that these interactions may also contribute to the role of APOBEC3 enzymes in cancer.

Methods:
We used affinity purification and mass spectrometry to identify the protein interaction network of the APOBEC3 enzymes. Select interactions were validated by using co-immunoprecipitation and immunoblotting and cell based assays were used to determine functionality.

Results:
We found that APOBEC3B, APOBEC3D, and APOBEC3F interact with the six prefoldin (PFD) proteins. Notably, PFD5 plays a tumor suppressor role by inducing degradation of the oncogene cMyc. We found that APOBEC3B interfered with PFD5-mediated degradation of cMyc by blocking cMyc degradation through a competitive interaction with PFD5, likely at the site where cMyc also binds. This suggests that APOBEC3B may play a role in cancer that is not related to its deaminase activity.

Conclusion:
The data suggest that APOBEC3B may contribute to chromosomal instability in cancer through protein-protein interactions and deaminase activity.

27. 450 MILLION YEARS LATER, ARE ALL MODERN VERTEBRATES MAKING BONE OR BONE-LIKE TISSUES THE SAME WAY?

Presenter: **Oghenevwogaga Joseph Atake**
College: **College of Medicine**
Supervisor: Brian Eames

Background:
Bone is an evolutionary novelty of vertebrates, and most modern jawed vertebrates retained bone in their internal supporting skeleton (endoskeleton) 450 million years after evolving from ancestral jawless vertebrates. Cartilaginous fishes (sharks, skates, and relatives) or osteichthyans (bony fishes and tetrapods) are the two main clades to which all modern jawed vertebrates belong. The endoskeleton of modern cartilaginous fishes is routinely described as lacking bone. However, some sharks and skates have mineralized tissues in perichondral regions of neural arch and tesserae that show morphological, histological, and molecular features consistent with vertebrate perichondral bone. To test the hypothesis that "bone-like" tissues and perichondral bone are homologous, we assessed whether the developmental program of bone-like tissues corresponds to that of perichondral bone because homologous characters are defined by a shared developmental program.

Methods:
Development of bone-like tissues in the little skate was characterized using several histological assays including alcian blue, alizarin red, safranin O, fast green, pricrosirius red, and Col2 immuno stainings.

Results:
Cartilage cells (chondrocytes) in neural arches of the little skate exhibited hydration-induced swelling of lacunae, indicating hypertrophy. Bone-like tissue developed following chondrocyte hypertrophy. Histological staining patterns of bone-like tissue were consistent and continuous with the perichondrium, suggesting that bone-like tissues derived from the perichondrium.

Conclusion:
Perichondral bone derives from the perichondrium and its formation is driven by cartilage maturation. Our data showed that chondrocyte hypertrophy (a main histological feature of cartilage maturation) is conserved during development of bone-like tissue in neural arches in the little skate.

28. IDENTIFICATION OF ICP4-INTERACTING PROTEINS TO CHARACTERIZE THE MECHANISM OF ICP4 TO REGULATE TRANSCRIPTION AND HISTONE DYNAMICS.

Presenter: **Harsha Benny**
College: **Western College of Veterinary Medicine**
Supervisor: Kristen Conn

Background:
Herpes simplex virus 1 (HSV1) is a double-stranded DNA virus that causes lytic or latent infections. During lytic infection viral genes are expressed and infectious viral progeny are produced whereas in latent infection most viral genes are not expressed, and no progeny virions are produced. Latent infectious are life long and it cause lytic infection. During lytic infection, HSV1 has a temporal cascade of gene expression with genes categorized as Immediate Early (IE), Early (E), or Late (L) based on their expression kinetics. Infected cell polypeptide 4 (ICP4) is one of the five IE proteins. It is an essential viral transcription activator that interacts with cellular RNA polymerase II and general cellular transcription machinery to regulate viral gene expression. Gene expression requires access to DNA. Access to the DNA is regulated by chromatin. Chromatin is composed of regularly spaced nucleosomes that consist of two molecules each of core histone (H2A, H2B, H3, and H4), wrapped in 147bp of DNA. The linker histone H1 binds to DNA entry and exit sites of the nucleosome to stabilize and compact chromatin. DNA assembled in stable, compacted chromatin is generally not accessible to factors that require a DNA template, whereas DNA assembled in unstable “open” chromatin is accessible to such factors. Consequently, transcription requires that DNA is assembled in accessible, “open” chromatin. Chromatin compaction is affected by the assembly of histone variants into chromatin, post-translational modifications (PTMs) of histones, and the association of other chromatin proteins that bind to histone-DNA or histone-histone interactions. Such factors also influence the chromatin exchange of histones (histone dynamics). HSV1 genome enters the nucleus devoid of histones. During lytic infection, HSV1 genome is assembled in highly unstable nucleosomes. The unstable HSV1 chromatin is consistent with the observation that histone dynamics increased in HSV1-infected cells. The histone dynamics are higher in replication compartments where HSV1 genomes and ICP4 localize than in the cellular chromatin, consistently ICP4 is sufficient to enhance histone dynamics. Based on these data I propose a model in which ICP4-mediated histone exchange regulates HSV1 chromatin accessibility to regulate transcription. To test this model, I will first identify ICP4-interacting partners to characterize the mechanisms of ICP4 to regulate histone dynamics and test how this activity relates to HSV1 transcription and chromatin stability. Previous studies used harsh conditions to purify ICP4 interacting proteins that only identify very strong and direct protein interactions. However, these proteins are likely not involved in histone dynamics based on their known functions. Therefore, I hypothesis ICP4 interacts with more cellular proteins.

Methods:
BioID: I will use BioID (Proximity-dependent labeling) to identify ICP4-interacting partners, including those interacting partners with transient, temporal, weak, and indirect protein-protein interactions. PCR sequence of ICP4 amplified from HSV1 genome. Subcloned PCR amplified ICP4 expression vector is labeled with BirA biotin ligase. BiraA biotin ligase catalyzes the generation of reactive biotinyl-AMP from biotin and ATP. Biotin will be added to the cells to biotinylate the ICP4 interacting proteins within a 10 nm radius. Mass spectrometry will be used to identify the isolated proteins.

Results:

I expect to identify weak, transient, temporal, and indirect ICP4-interacting proteins. Previous studies used harsh protein purification conditions that only detect very strong direct protein-protein interactions. In my study, I will use the BioID method, which is never used for ICP4, to make possible the identification of transient, temporal, and indirect protein-protein interactions.

Conclusion:

Identification of chromatin proteins that interact with ICP4 will support the characterization and mechanism whereby ICP4 enhances histone dynamics.

29. ARMING T CELLS WITH TUMOUR-SPECIFIC ANTIBODY THROUGH METABOLIC GLYCOENGINEERING FOR TARGETED CELL KILLING

Presenter: **Ashley Sutherland**
College: **College of Medicine**
Supervisor: C. Ronald Geyer

Background:
Adoptive cell therapies aim to re-engineer the patient's own immune cells to mount an anti-cancer response. Engineering T cells to express chimeric antigen receptors (CAR Ts) has proved successful in treating some cancers; however, the genetic methods for cell surface engineering are laborious, expensive, and inefficient. Due to the genetic manipulation required, CAR Ts carry potential side effects such as insertional mutagenesis and toxicities when they over proliferate. Here, T cells are given target specificity through anchoring antibodies to the T cell surface in a two-step engineering reaction without genetic manipulation. Using metabolic glycoengineering, azide groups are introduced to the surface of T cells. Modified antibody is conjugated to the cell surface through azide-alkyne cycloaddition click reaction to create antibody-conjugated T cells (ACT cells) with new targeting abilities.

Methods:
T cells are glycoengineered with unnatural azido-containing monosaccharide, Ac4ManNAz through culturing for 24 hours. Antibody modified with DBCO is anchored to the T cell membrane by 'click' reaction via incubation in PBS for 2 hours, generating antibody conjugated T cells. T cell functionality and target cell killing is measured by flow cytometry.

Results:
Antibody conjugated T cells cultured with target cells form increased effector/target clusters, increase in cell surface activation marker, CD69 and granzyme/perforin content compared to IgG control T cells. ACT cells demonstrate an increase in target-specific lysis over control T cells but do not generate significant killing against antigen-negative cells.

Conclusion:
T cells can be given new target specificity through glycoengineering with Ac4ManNAz to display DBCO-labelled antibody on their surface. These antibody-conjugated T cells can become activated and bind to and specifically lyse target cells without affecting antigen-negative cells.

30. DEVELOPMENT OF SARS-CORONAVIRUS-2 REPLICATION TOOLS TO STUDY EMERGING VARIANTS AND FOR ANTIVIRAL SCREENS

Presenter:	**Megha Rohamare**
College:	**College of Medicine**
Collaborators:	Franco Vizeacoumar, College of Medicine
	Darryl Falzarano, College of Medicine
	Anil Kuma, College of Medicine
Supervisor:	Joyce Wilson

Background:
The global COVID-19 pandemic has led to 5.9 million deaths so far. Despite the availability of effective vaccines, emerging variants and endemicity of SARS-CoV-2 continue to cause hospitalizations and deaths. This study aims to develop novel tools including cell lines and SARS-CoV-2 molecular clones for the analysis of virus-host interactions, antiviral agents, and variants of concern.Currently there are very few lungs cell lines that support high levels of SARS-CoV-2 replication. As the first part of our study, we screened a panel of lungs cell lines transduced with human angiotensin converting enzyme 2 (ACE2) for high susceptibility and permissibility to SARS-CoV-2. While multiple cell lines supported sufficiently high replication of SARS-CoV-2, NCI-H23ACE2 cells exhibited over 95% cell death from SARS-CoV-2 infections, making them attractive targets for live-dead selection based genetic screens.

Methods:
To complement our efforts to develop better cell lines, we generated the molecular clones of multiple SARS-CoV-2 variants and reporter versions for high throughput screens. We were able to rescue the virus from both wild-type and nano luciferase (Nluc) reporter version of SARS-CoV-2 Wuhan-1 construct and both the wild type and reporter viruses showed similar replication kinetics to clinical isolates. We have used the Nluc reporter virus to develop a rapid screening platform to assess potential antiviral agents. We have also generated molecular clones and reporter viruses and successfuly rescued viruses for the SARS-CoV-2 delta and omicron variants of concern. These clones are currently being used to study the role of VOC specific mutations in increased virulence and transmissibility.

Results:
Using reverse genetics, we rescued SARS-CoV-2 Wuhan1 (D614G variant) wild type (WTFL) and reporter virus (NLucFL) using molecular BAC clones. The replication kinetics, plaque morphology and titers were comparable between rescued molecular clones and a clinical isolate (VIDO strain), thus providing confidence that the rescued viruses can be used as effective replication tools. Furthermore, the reporter SARS-CoV-2 NLucFL virus exhibited robust luciferase values over the time course of infection and was used to develop a rapid antiviral assay using remdesivir as proof-of-principle. In addition, as a tool to study lung-relevant virus-host interactions, we established novel human lung cell lines that support SARS-CoV-2 infection with high virus-induced cytopathology. Six lung cell lines (NCI-H23, A549, NCI-H1703, NCI-H520, NCI-H226, and HCC827) and HEK293T cells, were transduced to stably express ACE2 and tested for their ability to support virus infection. A549ACE2 B1 and HEK293TACE2 A2 cell lines exhibited more than 70% virus-induced cell death and a novel lung cell line NCI-H23ACE2 A3 showed about ~99% cell death post-infection.

Conclusion:
These SARS-CoV-2 tools will enrich COVID-19 research aimed at developing treatments and understand the progress of the global pandemic

Self-declaration of research alignment with additional themes (optional):
COVID-19 Pandemic Research, Response, and/or Outreach

31. STRUCTURAL MODELING OF MERS-COV SPIKE PROTEIN AND ITS RECEPTOR DPP4 PREDICT AN ALTERNATE ANIMAL RESERVOIR IN THE ORDER EULIPOTYPHLA

Presenter: **Robyn Ralph**
College: **Western College of Veterinary Medicine**
Collaborators: Jocelyne Lew, Vahid Rajabali Zadeh
Supervisor: Darryl Falzarano

Background:
A commonality between all of the highly pathogenic coronaviruses that have emerged in the past two decades is their zoonotic nature. In regards to MERS-CoV, only 54% of primary human cases are attributed to direct or indirect contact with camels. With no evidence of asymptomatic human-to-human transmission, there is no known source of infection for 46% of human cases, suggesting an alternate animal reservoir may exist.

Methods:
. Structural modeling/docking between the MERS-CoV spike protein and its receptor, DPP4, from different animal species was performed. This identified that shrews, namely Sorex araneus and Suncus etruscus, have a high level of conservation in the binding interface between spike and DPP4. This suggests that these species could be susceptible to MERS-CoV infection. Following transfection of human (hDPP4), shrew-adapted human (h/saDPP4, h/esDPP4), Sorex araneus DPP4 (saDPP4), Suncus etruscus DPP4 (esDPP4) or mouse DPP4 (mDDP4) into the non-permissive BHK cell line, cells were infected with MERS-CoV (EMC-2012) at an MOI of 0.1 and supernatant and cell lysate were collected.

Results:
Human, shrew-adapted, and both shrew DPP4s could facilitate MERS-CoV infection while mouse DPP4 could not.

Conclusion:
This functional data supports that certain species of shrews, as well as possibly other members of the Eulipotyphla order, may be capable of serving as vectors of MERS-CoV, and require further ecological investigation.

Self-declaration of research alignment with additional themes (optional):
COVID-19 Pandemic Research, Response, and/or Outreach

32. ASSESSMENT OF SARS-COV-2 SUSCEPTIBLE ANIMAL SPECIES

Presenter: **Arianna Hurtado-Monzón**
College: **Vaccine and Infectious Disease Organization (VIDO)**
Supervisor: Angela L. Rasmussen
Co-supervisor(s): Arinjay Banerjee

Background:
SARS-CoV-2 spike (S) protein mediates viral entry into host cells by binding to the cellular receptor angiotensin-converting enzyme 2 (ACE2). ACE2 from a diverse range of mammalian species, can act as a receptor for SARS-CoV-2, but the full range of susceptible hosts for SARS-CoV-2 infection remains unknown and underestimated. Recently, transmission of SARS-CoV-2 from humans to animals (zooanthroponotic transmission) has been reported in animals such as mink and white-tailed deer among others. Thus, there remains a significant risk of establishment of new animal reservoirs of SARS-CoV-2 and emergence of animal-adapted SARS-CoV-2 variants that can infect or re-infect humans and other mammals. Despite numerous reports of animals being susceptible to SARS-CoV-2 infection, no systematic approach has been applied to the surveillance and assessment of the zooanthroponotic risks of SARS-CoV-2 variants of concern (VOCs).

Methods:
As a first step, to discover animal species that are susceptible to SARS-CoV-2, we are developing a cell-based high-throughput assay to determine ACE2-mediated SARS-CoV-2 entry. We have identified animal ACE2 gene orthologs that will likely facilitate entry of SARS-CoV-2 using computational structural prediction analyses. We have developed HEK293 cells that stably express ACE2 orthologs from 60 species to investigate ACE2-mediated entry using authentic SARS-CoV-2 isolates and we assess susceptibility by immunofluorescence and TCID50 assays.

Results:
We confirmed ACE2 expression using immunoblot analysis and cellular localization of ACE2 was confirmed using immunofluorescence microscopy. ACE2 orthologs from different animal species differentially facilitated the entry of SARS-CoV-2.

Conclusion:
This is the first step to systematically identify and robustly validate ACE2 orthologues from animal species that are likely to become infected with SARS-CoV-2

Self-declaration of research alignment with additional themes (optional):
COVID-19 Pandemic Research, Response, and/or Outreach

Basic Science 3

33. SPECIFIC RNA OLIGONUCLEOTIDES COUNTERACT RNA BINDING PROTEIN DYSFUNCTION IN A MULTIPLE SCLEROSIS (MS) OPTOGENETICS MODEL OF NEURODEGENERATION

Presenter:	**Joseph-Patrick Clarke**
College:	**College of Medicine**
Collaborators:	Hannah E. Salapa, College of Medicine
	Patricia A. Thibault, College of Medicine
	Aravindhan Ganesan
	Subha Kalyaanamoorthy
Supervisor:	Michael C. Levin

Background:
Canada has one of the highest rates of multiple sclerosis (MS) in the world, with Saskatchewan being the highest in Canada. Current evidence indicates that neurodegeneration is a prominent feature of the pathogenesis of MS and the primary cause of disability in MS patients. Yet, knowledge of the molecular mechanisms of neurodegeneration in MS, as well as treatment options that reverse neurodegeneration are lacking. Previous data from our lab indicates that dysfunction of the RNA binding protein (RBP) heterogeneous ribonucleoprotein A1 (A1) correlates with neurodegeneration in MS brain and animal models of MS. Additionally, we previously showed that A1 dysfunction can be controlled in an optogenetic cellular model of MS, allowing us to study the molecular dysregulation of A1 in the pathogenesis of neurodegeneration. Utilizing this model, this study examines the effect(s) of RNA oligonucleotide (RNAO) treatment to prevent and/or reverse A1 dysregulation that may attenuate neurodegeneration, inhibit disability, and improve long-term quality of life in persons living with MS.

Methods:
In silico molecular modeling of RNAO interaction(s) with A1 was performed using RNA modeling, RNA-protein docking and molecular dynamics simulations. In vitro thermal shift assays were performed using A1 protein to assess the effect(s) of RNAO treatment on A1 structure. In vitro, reversible, blue light stimulated, optogenetic A1 protein expression plasmids, containing wild-type A1, tagged with both the optogene Cryptochrome 2 and mCherry, were transfected into HEK293T cells and used to examine the effects RNAO treatment on protein dynamics and downstream cellular pathway functions in real-time. Using this in vitro optogenetic paradigm of A1 dysfunction, we measured how RNAO treatment affects A1 self-association clustering kinetics and characteristics, A1-dependent cell stress activation and protein translation attenuation, as well as stress granule formation and morphology – all markers of neurodegeneration.

Results:

In silico molecular modelling revealed that binding of RNAOs to A1 is stabilized by sequence- and structure-specific RNAO-A1 interactions. Additionally, thermal shift assays showed that binding of the specific RNAO to A1 alters its structure, as indicated by a significant change in the melting temperature of A1. Utilizing our in vitro optogenetic MS model, we also show that treatment with a RNAO designed to bind A1 with a high degree of specificity significantly decreased the kinetics of cytoplasmic A1 cluster formation and the number of cells with A1 clusters. Furthermore, we found that this specific RNAO treatment caused a significant increase in quantity and decrease in size of A1 clusters. Finally, we demonstrate that in contrast to controls, RNAO treatment attenuated stress granule formation, inhibited cell stress activation, and restored protein translation, all of which are affected by A1 dysfunction.

Conclusion:

Using in silico molecular modeling, in vitro thermal shift assays, and an established in vitro optogenetic approach, this study presents evidence that sequence and structural specific RNAO treatment attenuates MS-associated A1 dysfunction and can attenuate aberrantly affected downstream mechanisms of neurodegeneration (i.e., abnormal formation of stress granules, activation of cell stress and perturbation of protein translation). These results indicate a potential therapeutic avenue to develop A1-specific therapies that attenuate neurodegeneration, inhibit disability and improve the quality of life of persons living with MS.

34. EVALUATING THE NEURODEGENERATIVE MECHANISMS OF DYSFUNCTIONAL HETEROGENEOUS NUCLEAR RIBONUCLEOPROTEIN A1 IN MULTIPLE SCLEROSIS

Presenter:	**Miranda Messmer**
College:	**College of Medicine**
Collaborators:	Hannah E Salapa, Bogdan F Popescu, Michael C Levin
Supervisor:	Michael C Levin
Co-supervisor(s):	Bogdan F Popescu

Background:
Neurodegeneration, the death of neurons, is the cause of disability in people with multiple sclerosis (MS). MS pathology is prevalent in the brain, including in the gray matter, which contains neuron cell bodies and their processes. Pathologic features of the gray matter in progressive MS includes cortical demyelination (loss of the protecting coating around neuronal processes), and RNA binding protein dysfunction, including heterogeneous nuclear ribonucleoprotein A1 (A1). We hypothesize that A1 dysfunction contributes to neurodegeneration in MS.

Methods:
Post-mortem cortical tissue from people with progressive MS and healthy control were analyzed using immunohistochemistry to detect gray matter demyelination and pathologic A1 phenotypes in normal appearing gray matter (NAGM) and areas of cortical demyelination. MS cases were separated into two groups based on the frequency of neurons with A1 pathology compared to control (high and low A1 pathology). Neurodegeneration was compared between control and MS cases with high and low A1 pathology by staining for NeuN, a protein important for neuronal function, and quantifying the density of NeuN+ cells.

Results:
There was no significant difference in pathologic A1 phenotypes in cortical demyelination areas compared to NAGM regions. However, severity of A1 pathology positively correlated with cortical demyelination frequency ($p=0.003$) and the loss of NeuN expression ($p=0.02$). MS cases with high A1 pathology had significantly fewer NeuN+ cells in the NAGM compared to MS cases with low A1 pathology and controls.

Conclusion:
Neuronal A1 dysfunction in MS correlates with increased cortical demyelination frequency and loss of NeuN expression. A high degree of cortical demyelination is indicative of more severe disease progression. Similarly, loss of NeuN expression occurs in other neurodegenerative diseases with A1 dysfunction. Therefore, A1 dysfunction may underlie both neurodegeneration and disease progression in MS.

35. DID TRANS-REGULATORY CHANGES TO SOX9 AND RUNX2 DECREASE CARTILAGE GENE EXPRESSION DURING OSTEOBLAST EVOLUTION?

Presenter:	**Marziyeh Hassanzadeh**
College:	**College of Medicine**
Collaborators:	Patsy Gomez Picos, College of Medicine
	Katie Ovens, College of Arts and Science
	Ian McQuillan, College of Arts and Science
Supervisor:	Brian Eames

Background:
Changes in transcription factors (trans-regulatory elements) and/or their binding sites (cis-regulatory elements) can cause evolutionary changes in gene expression. In the vertebrate skeleton, limited data suggest that bone-forming osteoblasts of earlier-diverged vertebrates express more genes typical of cartilage-forming chondrocytes than osteoblasts of later-diverged vertebrates. We hypothesized that changes to the coding sequences of the critical skeletal cell transcription factors Sox9 and Runx2 caused osteoblasts to decrease chondrocyte gene expression during vertebrate evolution.

Methods:
To test our hypothesis, we first used laser-capture microdissection to compare in vivo transcriptomes of three main skeletal cell types in mouse, chick, and gar: 1) immature chondrocytes (IMM), which typically express Col2a1 and Acan; 2) mature chondrocytes (MAT), which typically express Col10a1 and Ihh; and 3) osteoblasts (OST). To evaluate whether changes were due to Sox9- and/or Runx2-related changes, we are transducing mouse pre-osteoblastic cells (MC3T3-E1.4) with recombinant FLAG-tagged Runx2 and Sox9 from four vertebrate clades: gar, frog, chick, and mouse. To figure out whether changes in Sox9 or Runx2 activity led to our observed evolutionary changes in OST gene expression and whether Sox9 or Runx2 from different species bind to the same genomic loci, we will perform RNA-seq and ChIP-seq analyses, respectively, on transduced cells.

Results:
In our in vivo study, pairwise differential gene expression analyses revealed that gar OST expressed significantly higher levels of some chondrogenic markers, including Col2a1, Acan, Sox6, and Col10a1, compared to mouse or chick OST. Gene co-expression network (GCN) analyses in gar showed increased positive correlations between genes in IMM and OST.

Conclusion:
Gene co-expression network (GCN) analyses suggest that IMM and OST are more similar in earlier diverged vertebrates compared to later diverged vertebrates. In addition to helping define changes to the gene regulatory network (GRN) underlying osteoblast evolution, this study will evaluate the relative contributions of trans-regulatory changes in cell type evolution.

36. DDX41 HELICASE IN P-BODIES FORMATION AND MYELOID MALIGNANCIES

Presenter:	**Lacey Winstone**
College:	**College of Medicine**
Collaborators:	Ananna Bhadra Arna, College of Medicine Ravi Shankar Singh, College of Medicine Shizhuo Yang, College of Medicine
Supervisor:	Yuliang Wu

Background:

Mutations in DDX41 cause myeloid malignancies, including myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML). MDS is characterized by unsuccessful hematopoiesis and peripheral blood cytopenia, and AML is characterized by the spread of immature myeloid cells. One-third of MDS will progress to AML, an aggressive blood cancer. DDX41 is a member of the DEAD-box helicase subfamily. It has been reported that DDX41 is involved in mRNA splicing, innate immunity, R-loop mediated genome maintenance, and ribosome biogenesis. The predominant mutation in DDX41 is R525H (67% in affected patients). However, the molecular pathogenesis of DDX41 mutations in MDS/AML are still unknown. Processing bodies (P-bodies) are cytoplasmic ribonucleoprotein granules comprised primarily of decapped mRNAs in complex with various proteins associated with translation repression and 5'-3'mRNA decay. P-bodies recruit mRNA decay-promoting components alongside translational factors and exist in normal conditions. Under stress conditions, P-bodies increase in size and frequency. Upon recovery, P-bodies start to disassemble. P-bodies follow a dynamic assembly and disassembly according to their local cellular condition. The dysregulation of P-bodies has been associated with various autoimmune diseases and cancers, including MDS/AML. Four proteins, EDC4 and DCP1A (two decapping complex components), LSM14A (mRNA decay factor), and 4E-T (translational repression factor), are considered vital for P-bodies dynamic continuation. Despite the increasing number of DDX41 clinical mutations, how DDX41 dysfunction results in myeloid malignancies such as MDS/AML is still unknown. Our preliminary results suggested that DDX41 is required for P-body formation; however, the molecular mechanism is not known.

Methods:

Cell culture: HeLa and HT1080 cells were grown in DMEM supplemented with 10% fetal bovine serum (Sigma-Aldrich) and penicillin/streptomycin (100 U/mL each, Sigma-Aldrich). CRISPR DDX41 knockout (KO) HeLa and HT1080 cell lines, and lentivirus over expression of DDX41-WT and DDX41-R525H genes in DDX41 KO background cell lines have been generated previously (Singh et al., Cell Rep 2022). Primary antibodies: The mouse monoclonal DDX41 (SC-166225) was from Santa Cruz. Rabbit polyclonal DDX41 (15076S), rabbit polyclonal EDC4 (2548S), and rabbit polyclonal 4E-T (2297S) were from NEB. Mouse polyclonal LSM14A (A118408) and monoclonal β-actin (A5441) were from Sigma. Mouse monoclonal G3BP1 (ab56574) was from Abcam. Immunofluorescence: Cells were washed twice with PBS and fixed with 100% methanol at −20°C for 30 min, then washed with PBS and blocked with blocking buffer (1% BSA in PBS) at room temperature for 1 h. The immunostaining was performed by first incubating cells with primary antibodies (1:1000) overnight at 4°C. After washing with PBS, the cells were incubated with secondary antibodies, Alexa Fluor 488 goat anti-rabbit IgG (1:1000, Invitrogen) and/or Alexa Fluor 594 goat anti-mouse IgG (1:1000, Invitrogen), for 1 h at room temperature. Cells were then washed with PBS and mounted with Prolong Diamond antifade reagent containing DAPI (Invitrogen) and cured at room temperature in the dark for 24 h. Immunofluorescence was performed on a Zeiss LSM 700 META inverted Axiovert 200 M laser scan microscope with a Plan-Apochromat 63×/1.4 oil DIC objective. Images were captured with a CCD camera and analyzed using LSM Browser software ZEN (Zeiss). For GFP-tagged proteins, once cells were fixed with

methanol and mounted with DAPI, they were observed directly under an LSM 700 microscope. Western blotting: The proteins were separated on 10% SDS-polyacrylamide gel using Tris-glycine buffer (with SDS). After electrophoresis, the proteins were transferred to PVDF membrane (Bio-Rad) at 150 V for 2 h at 4°C. The membrane was incubated with a blocking buffer (5% skim milk in PBS with 0.1% Tween 20) for 1 h at room temperature. The membrane was then incubated with respective primary antibodies (1:1000) in PBST with 1% skim milk at 4°C overnight. After washing with PBST five times (5 min each), the membrane was incubated with HRP conjugated secondary antibody (1:5000, Santa Cruz) in PBST with 1% skim milk for 1 h at room temperature, then washed five times with PBST (5 min each). The membrane was then treated with Clarity Western ECL Substrate (Bio-Rad) and visualized using a ChemiDoc MP Imaging System (Bio-Rad). Stress treatment: Sodium arsenite (Sigma-Aldrich) was dissolved in DMSO as a stock at a concentration of 100 mM. Cells were seeded in 6-well plates 24 h before treatment. Fresh DMEM media (2 mL/well) was used, and 10 uL sodium arsenite was added per well (final concentration at 0.5mM). Cells were collected at time points of 0, 15 min, 30 min, 1 h, 2 h, 4 h, and 8 h.

Results:

DDX41 is required for P-bodies but not stress granules formation. Using DDX41 KO cell lines that we have established and P-bodies marker EDC4 and stress granules marker G3BP1, we performed immunofluorescence and found that DDX41-depletion led to reduced P-body formation under stress (sodium arsenite), compared with WT cells. In contrast, stress granules remained same between DDX41 KO and WT cells. Our results suggest DDX41 is required for P-bodies formation but not stress granules. Increased P-bodies in mutant R525H-expressed cells. To understand the impact of DDX41 mutant R525H on P-bodies dynamics, we over expressed DDX41-R525H or DDX41-WT gene in DDX41 KO cells. Using EDC4 as a readout for P-bodies, we found that the mutant had increased P-bodies formation compared with WT under stress condition. DDX41 affects the expression pattern of EDC4 and 4E-T, but not LSM14A. To understand why DDX41 affects P-bodies formation, we used Western blotting to measure the expression of EDC4, 4E-T, and LSM14A proteins in WT and DDX41-KO cells. Overall, the expression of EDC4 and 4E-T was reduced in DDX41-KO cells compared to WT cells. In addition, both expressions peaked later than WT cells, namely 4 h in DDX41 KO and 2 h in WT cells. In contrast, the presence/absence of DDX41 did not influence LSM14A level. Therefore, DDX41 may regulate the expression of EDC4 and 4E-T. DDX41 does not affect the exogenous P-bodies proteins. Based on results above, we used 4E-T as another P-bodies marker, we preformed immunofluorescence and found that DDX41-KO cells have reduced 4E-T foci in all time points (15 mins-8 h). Furthermore, we transfected DDX41 KO and WT cells with GFP-DCP1A plasmid DNA, which is another P-bodies marker, and we found that there was no significant difference between KO and WT cells in terms of DCP1A foci formation, indicating DDX41 mainly affects endogenous genes' expression, but not exogenous genes.

Conclusion:

We have found that DDX41 is required for P-bodies formation but not stress granules, and DDX41 R525H mutation causes an increase in P-bodies formation. DDX41 influences the expression of P-bodies proteins EDC4 and 4E-T, not LSM14A. DDX41 affects endogenous P-bodies proteins than exogenous P-bodies proteins, potentially via mRNA splicing. Further experiments are undergoing to determine how DDX41 regulates mRNA splicing and what's the molecular defects in the patient mutant R525H. Taken together, our results enhance our understanding of MDS/AML pathogenesis and could lead to novel drug targets for the treatment of DDX41-mutated myeloid malignancies.

37. IMPACT OF THE ABUNDANCE OF THE LYME DISEASE BACTERIUM IN HOST TISSUES AND TRANSMISSION TO FEEDING TICKS

Presenter:	**Cody Koloski**
College:	**College of Veterinary Medicine**
Collaborators:	Georgia Hurry, Hesham Adam, Alexandra Foley-Eby
Supervisor:	Maarten Voordouw

Background:
In North America, Borrelia burgdorferi is transmitted to vertebrate reservoir hosts by the blacklegged tick (Ixodes scapularis). This tick-borne spirochete establishes a chronic infection in the host tissues. Experimental infection studies have shown that transmission of B. burgdorferi from infected hosts to feeding ticks (host-to-tick transmission; HTT) often decreases over the course of the infection, which suggests involvement of the acquired immune response. Mice with severe combined immunodeficiency (SCID) lack an acquired immune system and often have a higher pathogen abundance in their tissues compared to immunocompetent (IC) mice. Despite the amount of knowledge on Borrelia-host interactions, the effect of the acquired immune system on the lifetime host-to-tick transmission of the spirochete is not well characterized. In addition, there are very few studies that incorporate host sex into Borrelia-host interactions. The purpose of the study was to compare whether the difference in host tissue spirochete loads between SCID and IC mice influence HTT and the loads acquired by immature I. scapularis ticks and whether host sex is a contributing factor.

Methods:
Female and male, Immunocompetent/SCID mice (C57BL/6) were experimentally infected with B. burgdorferi via tick bite and were subsequently infested with I. scapularis larvae at weeks 4, 8, and 12 post-infection (PI) to measure host-to-tick transmission. Mice were euthanized and organs were dissected at week 16 PI. The infection status of the mouse organs and ticks were tested using qPCR.

Results:
The spirochete loads in the tissues of the SCID mice were 260x higher compared to WT mice. The infection prevalence of B. burgdorferi in immature I. scapularis ticks that fed on SCID mice was 100.0%, whereas it decreased in WT mice over time. The spirochete load in larvae that had fed on SCID mice was 24x higher compared to the larvae that fed on WT mice but declined to a 3-fold difference after the larvae had moulted into nymphs. We also found that male mice had higher tissue spirochete loads compared to female mice for both mouse genotypes.

Conclusion:
This research provides valuable insights into the intricate interplay between the acquired immune system and the abundance of spirochetes in host tissues, as well as the host-to-tick transmission of this tick-borne pathogen. Furthermore, it sheds light on the role of host sex in influencing Borrelia-host interactions.

38. EVALUATION OF LOCAL INNATE IMMUNE RESPONSES INDUCED BY NOVEL CYCLIC POLYPHOSPHAZENE, AND A KNOWN POLY[DI(SODIUMCARBOXYLATOETHYLPHENOXY)PHOSPHAZENE] ADJUVANT IN SWINE

Presenter: **Dylan Chand**
College: **School of Public Health**
Supervisor: George Mutwiri
Co-supervisor(s): Heather Wilson

Background:
Adjuvants induce local innate immune responses such as antigen uptake that influence the development of antigen-specific immune responses. Thus, selecting adjuvants to induce robust innate immune responses and understanding their mechanisms of action can improve vaccine efficacy and overall safety. The objective of this study was to evaluate the capacity of a novel adjuvant, cyclic polyphosphazene 75B (CPZ75B) to stimulate potent innate immune responses in swine and compare these results to a known adjuvant, poly[di(sodiumcarboxylatoethylphenoxy)phosphazene] (PCEP).

Methods:
Three groups of 4-week-old piglets (n=6/group) were immunized intradermally (ID). Each pig was injected four times on their limbs (for a total of 16 injections), with either 100 microliters of phosphate buffered saline (PBS), 100 micrograms of PCEP or 133 micrograms of CPZ75B at dissolved in 100 microliters of PBS. The day of euthanasia is considered day 0 and animals were immunized on day -7, day -4, day -2 and day -1 with one leg being immunized each day. Punch biopsies from each leg were collected for gene expression analysis (using reverse transcriptase quantitative polymerase chain reaction (RT-qPCR), immunohistochemistry (IHC), cytokine quantification (using Enzyme-linked immunosorbent assay (ELISA)), and kinome analysis.

Results:
Initial histological analysis has been mostly completed for the following IHC markers: CD107a (degranulating cells), CD3 (T lymphocytes) and antigen presenting cells via major histocompatibility complex class II (MHC II). The results indicate T cell recruitment was scant in response to CPZ75B, whereas there was modest recruitment observed in response to PCEP. MHC II positive cells were recruited at a modest number in response to CPZ75B, indicating that macrophage and dendritic cell recruitment may contribute to the local innate immune response. PCEP induced significant recruitment of MHC II positive cells relative to CPZ75B. We also observed recruitment of cells expressing CD107a in response to both adjuvants, which may contribute to local inflammation as a result of degranulation. Kinome analysis thus far has revealed differentially phosphorylated peptides at 96 hours and 24 hours post injection when comparing treatment groups to both each other and the control. The signaling pathways associated with these peptides are to be subjected to overrepresentation analysis (ORA) and validated using RT-qPCR by relative gene expression of targets in those implicated pathways. Cytokine expression will be quantified using a multiplex ELISA. Further analysis and data collection remains ongoing.

Conclusion:

CPZ75B appeared to induce unique immune responses relative to PCEP including distinct cellular recruitment patterns as determined by IHC and distinct kinase activity . This study aims to inform the mechanisms surrounding novel adjuvant cyclic polyphosphazene in stimulating innate immune effector mechanisms. From these conclusions we plan to establish a baseline to inform future studies about how CPZ75B contributes to the innate immune response and overall, how adjuvants work mechanistically.

39. MS-PATIENT DERIVED SOMATIC POINT MUTATIONS IN THE RNA BINDING PROTEIN HETEROGENEOUS NUCLEAR RIBONUCLEOPROTEIN A1 (A1) INDUCE NEURODEGENERATION.

Presenter: **Ansalna Ansari**
College: **College of Medicine**
Collaborators: Patricia Thibault, Hannah Salapa
Supervisor: Michael C. Levin

Background:
Multiple Sclerosis (MS) is an autoimmune disease of the central nervous system characterized by demyelination and neuronal and axonal degeneration, also known as neurodegeneration. MS disease progression and disability correlate with neurodegeneration; therefore, there is a need to understand the mechanisms underlying neurodegeneration in MS. We previously identified somatic point mutations in the RNA binding protein heterogeneous nuclear ribonucleoprotein A1 (A1) derived from MS patients, which may contribute to neurodegeneration. Therefore, this project will characterize the neurodegenerative consequences of two mutations in A1, including one within the nuclear localization sequence of A1 (A1P275S) and a second in the prion-like domain of A1 (A1 F263S) to test the hypothesis that neuronal A1 dysfunction drives neurodegeneration in MS.

Methods:
Mouse primary embryonic neurons were transduced with adeno-associated virus vectors, expressing wildtype A1 (A1WT) or mutant (A1P275S, A1F263S), the human synapsin 1 neuron promoter, and the cellular fluorescent tracker mCherry. A1 localization patterns, a phenotypic indicator of A1 dysfunction, were assessed via mCherry expression using ImageJ. Neurons were also examined for markers of neurodegeneration using immunocytochemistry, including changes in neuronal neurite length and complexity and FluoroJade-C, which stains degenerating neuronal cell bodies.

Results:
Examination of A1 localization across the groups revealed a 50% increase in nucleocytoplasmic mislocalization (**$p<0.01$) in neurons transduced with A1P275S, but not A1F263S, compared to A1WT. While A1F263S did not demonstrate significant mislocalization, both A1F263S and A1P275S induced neurodegeneration which was evident by significant reductions in total neuronal length by 25% (*$p<0.05$) and 41% (**$p<0.01$); neuronal complexity by 25% (*$p<0.05$) and 43%(*$p<0.05$), respectively, as compared to A1WT transduced neurons. Further, preliminary analysis showed both A1 mutants demonstrated a 2.5 times increase in FluoroJade-C cell body staining.

Conclusion:
Taken together, these results reveal that while MS-patient-derived mutations in A1 may manifest themselves through different phenotypes (i.e., mislocalization vs no mislocalization), both negatively impact neuronal health and lead to neurodegeneration. This study establishes a framework for understanding A1 dysfunction as a contributor to neurodegeneration in MS.

40. INVESTIGATING THE NUCLEAR PORE COMPLEX AND NUCLEOCYTOPLASMIC TRANSPORT IN MULTIPLE SCLEROSIS

Presenter:	**Todd Stang**
College:	**College of Medicine**
Collaborators:	Hannah Salapa, College of Medicine
	Joseph-Patrick Clarke, College of Medicine
Supervisor:	Michael Levin

Background:

Multiple Sclerosis (MS) is a debilitating central nervous system disease that disproportionately affects Canadian and Saskatchewan residents. While primarily known as a demyelinating disease, neurodegeneration, the loss of axons and neurons, causes progressive and permanent disability. Reducing and preventing neurodegeneration represents the next breakthrough in MS treatment, as currently, there are no treatments that impede disease progression and disability. Nuclear pore complexes, located within the nuclear membrane that surrounds the nucleus, allow for nucleocytoplasmic transport, which is vital for transporting cellular machinery between the nucleus and cytoplasm. In this study, we examined whether the nuclear pore complex and nucleocytoplasmic transport were disrupted in MS, which would prevent normal protein translation and function, leading to neuronal death.

Methods:

A cell line (differentiated Neuro2A cells) with knockdown of a critical protein called heterogeneous nuclear ribonucleoprotein A1 (hnRNP A1) was used as an in vitro MS model to determine if there was a connection between nuclear pore complex dysfunction and MS. Nucleocytoplasmic transport was also assessed using a fluorescent plasmid to establish a functional consequence of structural nuclear pore complex alterations. Post-mortem MS brain was immunostained to examine the nuclear pore complex to confirm disease relevance.

Results:

The nuclear pore complex structure was significantly altered after hnRNP A1 knockdown by staining multiple proteins of the nuclear pore complex (Lamin B $p=0.0195$, Nup98 $p=0.0045$, POM121 $p=0.0076$, RanGAP1 $p=0.0027$), confirming a connection between nuclear pore complex dysfunction and MS. Using a novel automated computer script and an ImageJ plug-in, three-dimensional images of the nucleus and nuclear pore complex following hnRNP A1 knockdown revealed quantitative morphological changes in the nuclear pore complex ($p=0.0069$). These results were similar to a standard qualitative method but reduced bias and provided high throughput analyses of quantitative changes in the nuclear membrane and nuclear pore complex. Further, nucleocytoplasmic transport was significantly altered in cells with hnRNP A1 knockdown by 30% ($p=0.0090$), illustrating functional consequences in addition to nuclear pore complex morphological changes. Finally, perturbations in the nuclear and nuclear pore complex structure were observed in neurons from post-mortem MS brains, similar to what was found in vitro.

Conclusion:

In an in vitro model of MS, neurons demonstrate alterations in the nuclear pore complex and nucleocytoplasmic transport, inhibiting homeostatic functions within the cell. Nuclear pore complex and nucleocytoplasmic alterations represent a novel neurodegenerative mechanism in MS, which may be targeted to reduce disability in persons living with MS.

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41. FEEDING RESPONSE IN MATED DROSOPHILA FEMALES IS REGULATED BY NUCB1'S EXPRESSION IN THE NERVOUS SYSTEM

Presenter:	**Narsimha Pujari**
College:	**Western College of Veterinary Medicine**
Collaborators:	Vanessa Browne, College of Arts and Science
	Adelaine K.W. Leung, Western College of Veterinary Medicine
Supervisor:	Adelaine K.W. Leung

Background:
In the common fruit fly, the sex peptide (SP) is an accessory protein transferred from the male to the female during copulation. Sex peptide binding to its receptor in the female initiates post-mating responses (PMR), which includes increased food intake to support the increased metabolic demand of reproduction. Recently, we discovered that the highly conserved nucleobindin-1 (dNUCB1) has anti-hunger properties, as observed in several mammalian systems. We hypothesize that dNUCB1 is involved in post-mating food intake increase in female flies.

Methods:
The effect of mating on dNUCB1 expression was tested using qPCR. Feeding in dNUCB1 knockdown flies was assessed with a dye-based assay. SP-null males were used for mating to test the effect of SP-transfer on dNUCB1 expression.

Results:
First, we confirmed that mating changes dNUCB1 mRNA level within the first 2 hours of mating and this is dependent on sex peptide transfer. Then, we evaluated the effect of pan-neuronal knockdown of dNUCB1 on food intake in virgin and mated females using a dye-based assay. We observed that this knockdown increased food intake only in mated females. dNUCB1-expressing neurons were identified in the hypocerebral ganglion of the nervous system.

Conclusion:
These results identify dNUCB1 as a key component of the post-mating feeding behaviour regulation. This regulation is shown to be mediated by the nervous system, likely via the neurons of the hypocerebral ganglion. These findings provide insights into the molecular and neural mechanisms of the complex behavioural and physiological induced by mating.

42. CAN METABOLOMICS BE USED FOR THE RAPID DIAGNOSIS OF POULTRY DISEASES?

Presenter: **Asha Ranaraja**
College: **Western College of Veterinary Medicine**
Supervisor: Susantha Gomis

Background:
In the Canadian broiler industry, outbreaks of infectious diseases cause significant economic losses as well as pose a threat to human food safety and quality. However, the lack of rapid diagnostic techniques to identify disease outbreaks within 1-2 days post-infection remains a major challenge. Moreover, the chicken industry lacks techniques to detect subclinical and simultaneous bacterial and viral infections. Metabolic biomarker-based diagnosis is a novel approach to detecting diseases by considering pathogen-induced early metabolic changes before clinical signs. In human medicine, metabolic biomarker-based studies are quite common, while in veterinary medicine, metabolic studies are still in their infancy.The objective of this study was to diagnose bacterial and viral infections in broiler chickens using metabolic biomarkers as a rapid diagnostic tool.

Methods:
Broiler chickens were experimentally infected with Escherichia coli or avian reovirus (ARV) using well-established animal models. And serum was taken 24 hours after infection and before clinical signs appeared. Untargeted metabolomics was performed on serum samples using liquid chromatography-mass spectrometry and spectral data was analyzed with Metaboanalyst 4.0 software.

Results:
The principal component analysis (PCA) revealed a clear separation of metabolites in birds infected with E. coli or ARV compared to non-infected birds. In comparison to non-infected chickens, broilers infected with E. coli exhibited 294 distinct metabolites in their serum. Similarly, ARV-infected birds exhibited 439 distinct metabolites in their serum compared to non-infected birds.

Conclusion:
These preliminary findings indicate the potential of serum metabolomics as an early disease diagnostic tool for broiler chickens with applications in preventing infectious disease outbreaks.

43. NOVEL DELIVERY OF PROBIOTICS IN CHICKEN EMBRYOS TO PROMOTE INTESTINAL IMMUNITY AGAINST INFECTIOUS DISEASES IN BROILER CHICKENS

Presenter: **Mihiprabha Rathnayake**
College: **Western College of Veterinary Medicine**
Supervisor: Susantha Gomis

Background:
Chicken Farmers of Canada (CFC) has withdrawn the use of category I and II antibiotics in the poultry industry and are phasing out the use of category III antibiotics to improve food safety and antimicrobial reduction. Thus, alternatives to antimicrobials and innovative technologies are needed to control bacterial diseases in the broiler industry. Enterococcus faecalis has been used as a probiotic in the broiler chicken industry to efficiently promote growth and feed conversion rate. This study aimed to study the possibility of delivering probiotics to chicken embryos to promote intestinal health against infectious diseases following hatch.

Methods:
An Enterococcus faecalis isolate was delivered to embryos using a novel technique. Bacterial broth maintained at 10oC was sprayed “three times” over eggshell for 30 seconds on days 12, 15, and 17. Two groups of eggs were sprayed “two times” on days 12 and 15 of incubation and on days 15 and 17 respectively. Two other groups were sprayed with bacterial broth “one time” on day 12 and day 17 respectively. Migration of E. faecalis through the eggshell into the intestine was measured by culturing the intestine of embryos at days 19 and 20 of incubation using selective media and identifying by MALDI-TOF mass spectrometry.

Results:
E. faecalis isolate was identified by culturing from 80% to 100% of embryos at day 19 and 20 respectively from the 3 times spray group. 100% of embryos of both “two time” sprayed groups were able to culture E. faecalis and non of the embryos in the “one-time” spray groups were able to culture E. faecalis. Analysis of the intestinal microbiome by metagenomics is underway to identify the entire microbial ecology of the intestine of chicken embryos. No mortality or effect on hatchability was noted with this spray application technique.

Conclusion:
This study demonstrated the possibility of delivering E. faecalis as a probiotic to embryos without interfering with embryo health. This is a non-invasive technique of delivering probiotics to chicken embryos and this technique is industry feasible. We hypothesize that delivery of probiotics during the incubation period of chicken embryos will prevent the colonization of pathogenic bacteria following hatch and will lead to reducing yolk sac infections in neonatal chickens.

44. OPPOSING ROLES OF BMPS DURING CHONDROCYTE MATURATION

Presenter: **Neethi Satish**
College: **College of Medicine**
Supervisor: Brian F. Eames

Background:
Osteoarthritis (OA) is a painful joint disease of degenerated articular cartilage, and studies of endochondral ossification hope to provide insights into OA progression. Cartilage is an excellent model to study OA as OA is characterized by ectopic cartilage maturation. Cartilage cells, or chondrocytes, are set in a proteoglycan-rich matrix, where they undergo a maturation process involving the expression of maturation markers, such as Ihh and Col10a1. Since OA involves ectopic cartilage maturation, molecular regulators of this process might serve as therapeutic targets for OA. Bone morphogenetic proteins (BMPs) are involved in many aspects of skeletal development, including induction of Ihh and Col10a1 during chondrocyte maturation. Recent work in our lab verified that inhibition of BMP signalling decreases chondrocyte maturation but exactly which BMP ligands are involved remains unclear. Bmp4 is elevated in OA patients, and Bmp7 has been used to treat OA patients to alter the cartilage phenotype implying Bmp4 and Bmp7 might exhibit opposing roles during chondrocyte maturation. We hypothesize that Bmp4 and Bmp7 play positive and negative roles, respectively, during chondrocyte maturation.

Methods:
To test the hypothesis in vitro, Bmp ligands are added to the chondrocyte cell line ATDC5, while to test in vivo, we created transgenic zebrafish that overexpress Bmp4 and Bmp7 specifically in cartilage. In both cases, we perform Alcian blue staining, col10 immunostaining, and alkaline phosphatase histochemical staining, which are indicative of chondrocyte differentiation, maturation, and matrix mineralization, respectively. We also performed quantitative real-time PCR to assay gene expression levels for Ihh, Col10a1, and Alpl.

Results:
Preliminary results from the histochemical and immunostaining assays demonstrate more staining for the Bmp4 and Bmp7 treated micromass cultures compared to the untreated control culture, which is indicative of both the proteins promoting chondrocyte maturation. From our gene expression assays on micromasses, in the presence of Bmp4, the chondrocyte maturation markers Ihh and Col10a1 might decrease, but the expression of Alpl might increase. In the presence of Bmp7, Col10a1, and Alpl appear similar to controls, but Ihh might decrease.

Conclusion:
Therefore, from the preliminary data generated, we can conclude that Bmp4 promotes chondrocyte maturation, which is consistent with the hypothesis, and Bmp7 also promotes chondrocyte maturation, which is not consistent with the hypothesis

45. THE YES ACTIVATED PROTEIN (YAP) INHIBITOR CELASTROL ANTAGONIZES TGFB-INDUCED GENE EXPRESSION IN HUMAN GINGIVAL FIBROBLASTS

Presenter: **Angha Naik**
College: **College of Dentistry**
Supervisor: Andrew Leask

Background:

Fibrosis, defined by the excessive accumulation of extracellular matrix (ECM) proteins, including type I collagen, results in a stiff scar tissue that impairs organ function and can cause death. The effector cells of fibrosis are myofibroblasts, an activated form of fibroblast. In gingiva (gums), fibrotic responses are predominantly hyperproliferative (hyperplasia), where transforming growth factor (TGF)-β is overexpressed, and via canonical signaling pathway acts on gingival fibroblasts to induce fibroproliferative genes such as cellular communications network factor 2 (CCN2). Targeting of TGFβ for therapeutic gain however is challenging due its pleotropic nature, and therefore identifying new and targetable alternatives is warranted. We have shown that mechanical tension as a result of stiff ECM increases TGFβ-driven expression of CCN2 in gingival fibroblasts. We further identified yes-activated protein (YAP1), a mechanosensitive transcription (co)factor that translocate to nucleus in response to mechanical tension in gingival fibroblasts. To test if YAP is the perpetuator of TGFβ-mediated fibroproliferative gene induction, I used celastrol, a recently identified YAP/TAZ (transcriptional co-activator PDZ-binding motif) inhibitor, whose anti-fibrotic potential is not yet elucidated -- projecting it as a potential treatment option for gingival hyperplasia.

Methods:

Human gingival fibroblasts (HGFs) in culture were serum starved overnight and pretreated with 500nM Celastrol or DMSO for 45 minutes followed by stimulation with or without TGFβ1 for 6 hours (RT-PCR) and 24 hours (Western blot). mRNA samples collected at 6 hours were also analysed using BulkRNAsequencing and functional cluster analysis. Celastrol's capacity to prevent the TGFβ induced proliferation of HGFs was investigated with BrdU cell proliferation assay. Celastrol's ability to block TGFβ-mediated YAP nuclear localization in HGFs was assessed using immunofluorescent staining of YAP protein.

Results:

In response to added TGFβ1, the gene and protein expression of fibrotic marker and mediator CCN2 (a known YAP1 target) was significantly downregulated in the presence of Celastrol. Similarly, of the 1364 genes induced by TGFβ1 (>2-fold), 657 genes showed a >50% decrease in the presence of Celastrol including genes involved in fibrotic responses such as type 4 collagen, interleukin 11, etc., Functional cluster analysis revealed ECM, Wnt/cytokine signaling, focal adhesion clusters induced by added TGFβ1, were downregulated in a Celastrol sensitive fashion. Also, basal level proliferation of HGFs was repressed in the presence of Celastrol. Additionally, Celastrol also successfully blocked TGFβ-mediated nuclear translocation of YAP as seen with immunofluorescence microscopy.

Conclusion:

These results are consistent with our hypothesis that targeting YAP/TAZ may be useful in blocking the profibrotic effects of TGFβ and that celastrol or other YAP inhibitors are eligible candidates as a treatment option for gingival hyperplasia.

46. NUCLEAR FACTOR ERYTHROID 2 RELATED FACTOR-1 (NRF1) ACT AS STRESS ADAPTIVE FACTOR TO REGULATE DOWNSTREAM TARGET GENES VIA ITS HETERODIMER PARTNERS

Presenter: **Alireza Ahadiabhari**
College: **College of Medicine**
Collaborators: Lei Li, College of Medicine
Supervisor: Scott Widenmaier

Background:
Nuclear Factor Erythroid-2 Related Factor 1 (NRF1) is an ER membrane-bound protein that belongs to the Cap'N'Collar family of basic-leucine zipper transcription factors and can, under stress conditions, undergo nuclear translocation to regulate gene transcription. We and others have shown NRF1 promotes hepatocyte homeostasis and resilience via transcriptional regulation of the proteasome, antioxidant defense, and other programs. It is known NRF1 must form a heterodimer with proteins such as small Mafs and bind to antioxidant response elements (AREs) in promoters to regulate transcription. However, the mechanism by which NRF1 is directed to AREs for proteasome, antioxidant defense or other gene targets and whether this is 'program' selective is unclear. We hypothesize distinct NRF1 heterodimers render selective gene program regulation.

Methods:
Establishment of knock-out cell lines for NRF1, MafK, MafG, and MafF using the CRISPR-Cas9 system Luciferase reporter assays Western blotting RNA isolation and quantitative real-time PCR Co-Immunoprecipitation

Results:
In this study, we identify the nutraceutical, Celastrol, as a stimulant for NRF1 activity in cultured Hep3B cells and proceed to investigate our hypothesis. Using CRISPR-based gene deletion, we identify Celastrol-induced proteosome-linked and antioxidant defense-linked genes dependent on NRF1. We show deficiency of MafK, MafG, or MafF impairs Celastrol-induced gene expression, with each deletion system having distinct and partial overlap with the effect of NRF1 deficiency. This finding supports a model whereby NRF1 heterodimerization with MafK regulates one gene sub-set, whereas heterodimerization with MafG or MafF regulates another.

Conclusion:
Thus, employing this experimental model system revealed a potentially key clue to stress defense gene programming by NRF1. Our goal is to continue gaining mechanistic insight and then relate this information to in vivo context in which NRF1 may have important translational impact, such as fatty liver disease and its progression to cirrhosis and hepatocellular carcinoma.

47. THE EFFECT OF THE HYPOXIA MIMETIC DIMETHYLOXYLGLYCINE (DMOG) ON GENE TRANSCRIPTION IN CULTURED HUMAN DERMAL FIBROBLASTS.

Presenter: **Asmaa Fadl**
College: **College of Dentistry**
Supervisor: Andrew Leask

Background:
Fibrosis is a pathological process characterized by the excessive deposition of extracellular matrix (ECM) components by connective tissue fibroblasts. Fibrotic diseases, which account for 45% of the health care costs and deaths in the developed world, are characterized by organ dysfunction, failure, and eventually, death. They include: idiopathic pulmonary fibrosis, liver cirrhosis, and scleroderma (systemic sclerosis, SSc). A priori, one would anticipate that SSc skin, as it is characterized by vascular damage, has a hypoxic microenvironment. Hypoxia stabilizes the transcription factor hypoxia-inducible factor (HIF)-1α, which, in the systems in which it has been studied, plays a critical role in cellular adaptation to hypoxia by activating genes that promote tissue homeostasis, cell proliferation, and ECM deposition. In normal conditions, HIF-1α activity is regulated by oxygen-dependent prolyl hydroxylation, which targets HIF-1α for degradation. These previous observations suggest that hypoxia, through HIF-1α, may contribute to the skin fibrosis observed in SSc. However, this hypothesis has yet to be tested and is the subject of my research project.

Methods:
Inhibition of prolyl hydroxylation by hypoxia mimetics, such as dimethyloxylglycine (DMOG), stabilizes HIF-1α and induces HIF-1α-dependent gene transcription. So, by studying the effect of DMOG on gene transcription in cultured human dermal fibroblasts (HDFs), I can investigate the contribution of hypoxia, via HIF-1α, to fibroblast-specific activation fibrogenesis. To test my hypothesis, an in vitro experiment was carried out using human dermal fibroblasts (HDFs) treated with or without DMOG (2 mM, up to 24h). RNA was extracted from the control and DMOG-treated cells and subjected to bulk RNA-sequencing and functional cluster analysis as an unbiased approach to identify differentially expressed genes that are induced in response to DMOG. Real-time quantitative polymerase chain reaction (RT-qPCR) was conducted to validate the RNA-sequencing results for specific genes. Western blot analyses were conducted to evaluate HIF-1α protein expression.

Results:
RNA-sequencing identified 759 genes (p-value < 0.05, fold change >1.3) that were induced in response to DMOG, including those involved in HIF-1α pathway, extracellular matrix, cell division, and cellular response to hypoxia. RT-PCR analysis confirmed the upregulation of several genes involved in the extracellular matrix, such as MMP2, FBLN7, SERPINE1, and P4HA1, in DMOG-treated cells compared to the control cells. In addition, western blot results verified that DMOG induced HIF-1α protein expression. These findings pave the way for further research into targeting HIF-1α as a therapeutic strategy for HIF-1α-mediated fibrosis, such as scleroderma. Since these results showed that the fibrosis-related genes that control extracellular matrix deposition and organization were induced under DMOG-induced hypoxia, inhibiting HIF-1α would be an attractive pharmaceutical target for treating fibrosis.

Conclusion:

DMOG treatment changes gene expression in HDFs by stabilizing HIF-1α. These findings provide insights into the molecular mechanisms underlying the cellular response to hypoxia as well as SSc fibrogenesis. Consequently, this research has the potential to lead to the identification of therapeutic targets and new treatments for fibrotic diseases, which will significantly improve the quality of life of those affected.

48. PRECULTURING PIG PERIPHERAL BLOOD MONONUCLEAR CELLS TO IMPROVE ANTIGEN-SPECIFIC INTERFERON GAMMA RESPONSES TO LAWSONIA INTRACELLULARIS PROTEINS

Presenter:	**Haoming Liu**
College:	**Western College of Veterinary Medicine**
Collaborators:	Donaldson Magloire, Western College of Veterinary Medicine
	Pooja Choudhary
	Siew Ng
Supervisor:	Heather Wilson

Background:

Lawsonia intracellularis (LI) is an obligate intracellular bacterium that causes gastrointestinal diseases in pigs. Although the diseases lead to large economic loss in pig industries and increased antibiotic use, the diseases can be prevented by LI vaccines. Commercially available LI vaccines are live-attenuated (Enterisol® Ileitis, Boehringer Ingelheim) or killed (Porcilis® Ileitis, Merck & Co., Inc), and both of these vaccines require cultivation of LI. LI is fastidious bacterium that can only be cultured in murine fibroblast-like McCoy cells (ATCC CRL 1696) in a tri-gas-controlled condition with 83.2% nitrogen, 8.8% carbon dioxide, and 8% oxygen at 37°C. These requirements limit the output of LI vaccines and increase the cost of vaccine production. A recombinant subunit vaccine, on the other hand, would enable large scale vaccine production as recombinant subunits can be produced using genetically modified Escherichia coli (E. coli) that is much easier to cultivate in various laboratory settings. To develop a subunit vaccine, we first identify immunogenic LI antigens that trigger strong cell-mediated immune (CMI) responses, which are mediated mainly by a proinflammatory cytokine called interferon gamma (IFNγ) and are efficient for killing of LI inside infected host cells. Several studies about LI infection in pigs showed strong CMI responses were only observed in pigs, which had recovered from the LI infection followed by high IFNγ level. We reason that by finding out which LI antigens trigger high IFNγ productions, we can identify LI antigen candidates in the entire LI proteome for constructing a recombinant subunit vaccine. In our preliminary studies, we used peripheral blood mononuclear cells (PBMCs) collected from LI-vaccinated pigs to screen the entire LI proteome in vitro for immunogenic LI antigen candidates. However, the PBMCs produced low IFNγ productions to all LI antigens, which impeded antigen identification. To overcome this limitation, we looked into literature and found that human peripheral blood monocytes can be precultured with human recombinant granulocyte-macrophage colony-stimulating factor (GM-CSF), IFNγ and lipopolysaccharide (LPS) to generate monocyte-derived inflammatory macrophages for strong CMI responses. Therefore, we modified the preculture method and hypothesized that preculturing the PBMCs with two porcine recombinant cytokines, rpGM-CSF and interleukin-2 (rpIL-2), and E. coli expressing LPS could amplify subsequent antigen-specific IFNγ responses to antigen restimulations in vitro.

Methods:

We obtained PBMCs from one pig vaccinated with killed LI vaccine. We precultured the PBMCs in three groups based on three media compositions: (1) NE group with no E.coli; (2) EC group containing E.coli; or (3) Flic-EC group containing E.coli that expressed a recombinant LI antigen called Flic (rFlic) and LPS used as an adjuvant to enhance the phagocytosis of rFlic. All three groups were cultured with rpGM-CSF and rpIL-2. After 7-day incubation, we isolated the precultured PBMCs from the old culture media containing exogenous IFNγ that would mess up with the downstream quantification of antigen-

specific IFNγ productions. We restimulated the precultured PBMCs with rFliC in fresh cytokine-and-E.coli-free media for 2 days. To verify whether the three preculture conditions could augment the antigen-specific IFNγ productions in shorter incubation period, we also restimulated the non-precultured autologous PBMCs with rFlic under the three preculture conditions for 2 days and then quantified the IFNγ secretions from both precultured and non-precultured PBMCs.

Results:

Preculturing the PBMCs in EC group ($P = 0.005$) and NE group ($P = 0.038$), significantly augmented rFliC-stimulated IFNγ productions above the background IFNγ level. Precultured PBMCs in EC group secreted greater amount of rFlic-stimulated IFNγ than that from the non-precultured PBMCs under the same preculture condition ($P = 0.009$). The results indicated that the rFlic-stimulated IFNγ productions increased to a significant level where we would be able to identify antigen candidates among thousands of antigens in the LI proteome.

Conclusion:

The results of our study suggest that preculturing PBMCs with rpGM-CSF, rpIL-2 and E.coli for 7 days prior to antigen restimulations lead to high antigen-specific IFNγ production. We anticipate that preculturing PBMCs with rpGM-CSF, rpIL-2 and E.coli will help us identify LI antigen candidates from the LI proteome for our subunit vaccine development.

Basic Science 5

49. INVESTIGATING THE IMPACTS OF PHENOLIC SYNERGY ON CELLULAR HEALTH AND LONGEVITY

Presenter: **Morgan Fleming**
College: **College of Agriculture and Bioresources**
Supervisor: Christopher Eskiw
Co-supervisor(s): Nicholas Low

Background:
Diet is the most influential environmental factor governing health and disease. Phenolics are a diverse group of compounds ubiquitously present in plant-based foods, as such, phenolics contribute to a healthy diet and the maintenance of cellular/organismal health. Haskap berries contain high concentrations of phenolics (blueberry phenolic concentrations range from 274.48 - 694.60 mg gallic acid equivalents (GAE)/100 g of fruit, where as haskaps range from 451.5 - 778.0 mg GAE/100 g of fruit) and therefore are of great interest in novel nutraceutical and nutrigenomic research. Modern research has demonstrated that phenolics have health promoting functions beyond antioxidant activity, impacting the levels and activity of proteins associated with health and longevity such as the deacetylase enzyme sirtuin 1. Intriguingly, select phenolic combinations have greater impacts on the levels and activities of these proteins, suggesting that phenolic compounds can interact synergistically to promote health. However, it is not well understood which structural features of phenolics contribute to these functions or to synergy. We hypothesized that select, structurally diverse haskap phenolics, and their combinations will differentially and synergistically impact readouts of cell health such as, population doubling time, oxidative potential, and sirtuin 1.

Methods:
To address this knowledge gap and test our hypothesis we have selected four phenolics, naturally present in haskap berry, based on their structural differences. The selected phenolics are: (1) caffeic acid (CA); (2) cyanidin (CY); (3) kaempferol-3-O-glucoside (K3G); and (4) gentisic acid (GA). These phenolics were tested individually and in equimolar combinations on non-diseased primary human dermal fibroblasts (2DD) to investigate their impacts on readouts of health and if synergy occurs. Antioxidant activity was investigated employing the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay and compared to intracellular antioxidant activity investigated with the MitoTracker Orange assay. Impacts on cell population doubling times, viability, and levels of sirtuin 1 were investigated employing cell counts, trypan blue dye, and western blotting, respectively.

Results:
With respect to the antioxidant activities of the selected phenolics, we demonstrated that they were structure-dependent. In addition, we found that antioxidant activities were different when biological and chemical assays were compared. Antioxidant synergy was observed in the combinations of CA/GA, CA/K3G, and CY/GA in the DPPH assay. All phenolic treatments maintained high levels of cell viability and the presence of CY and CA resulted in the greatest increase in cell population doubling

times of 16.0 and 8.9 hours, respectively. Furthermore, these four phenolics demonstrated structure-dependent impact(s) on the levels of sirtuin 1 in 2DD cells, and these results did not positively correlate with those of the population doubling time assay.

Conclusion:

These results support the hypothesis that phenolics can function to positively impact read outs of cell heath, which is dependent upon chemical structure. To the best of this authors knowledge this is the first scientific information available on the impact(s) of caffeic acid, cyanidin, kaempferol-3-O-glucoside, gentisic acid, and their combinations on cell (2DD) population doubling times, viability, and levels of sirtuin 1.

50. REGULATION OF NUCLEAR RIG-I PATHWAY BY DUSP 11 IN INFLUENZA A VIRUS INFECTION

Presenter: **Gaurav Malik**
College: **Western College of Veterinary Medicine**
Collaborators: Darryl Falzarano, Western College of Veterinary Medicine
Supervisor: Yan Zhou

Background:
Retinoic-acid inducible gene I (RIG-I) is a key pattern recognition receptor (PRR) that provides innate recognition of Influenza A virus (IAV) leading to the induction of type I interferons and pro-inflammatory cytokines. RIG-I was previously known to be cytoplasmic but our recent studies have established a nuclear resident fraction of RIG-I (nRIG-I) that senses IAV replication in the nucleus and induces an anti-viral response similar to cytoplasmic RIG-I. Since the discovery of nRIG-I no regulators for it have been reported to date. In this study we investigated how RNA triphosphatase dual-specificity phosphatase 11 (DUSP11) regulates nRIG-I mediated IFN induction in response to IAV infection. We show that during IAV infection DUSP11 is recruited to the nucleus via its interaction with IAV Nucleoprotein (NP) where it hydrolyses IAV RNA 5' triphosphate to 5' monophosphate, thereby preventing the activation of nRIG-I and hence, supporting IAV replication. Mutation on the key residue in DUSP11 abolished its interaction with NP resulted in its cytoplasmic retention and loss of action on viral RNA editing. These results provide a deeper understanding of how viruses exploit cellular factors to avoid innate recognition.

Methods:
We used luciferase reporter assay to observe the effect of DUSP11 on nRIG-I mediated IFN induction followed by RT-qpCR (to determine viral RNA levels) and viral plaque assays (to determine viral titer) to see how DUSP11 affects viral fitness. We also employed Bioluminescence resonance energy transfer (BRET) to see how DUSP11 impacts nRIG-I conformation change.

Results:
DUSP11 negatively affects nRIG-I-mediated IFN induction. For DUSP11 to edit viral RNA in the nucleus, it is recruited to the nucleus by viral nucleoprotein and is able to prevent nRIG-I conformation change to the active conformation and is therefore proviral in nature.

Conclusion:
DUSP11 is proviral in nature by editing viral RNA and making it incompetent to activate nRIG-I and therefore benefits viral replication.

51. PORCINE GAMMA-DELTA T CELLS: BIG NUMBERS, UNIQUE FEATURES AND MANY UNANSWERED QUESTIONS

Presenter: **Leonie Bettin**
College: **Western College of Veterinary Medicine**
Supervisor: Volker Gerdts

Background:
Gamma-delta (γδ) T cells represent a prominent lymphocyte subset in pigs. However, their function and age-dependent differences remain largely unknown. Toll-like receptors (TLR) are key receptors for recognizing pathogens, and previous experiments revealed a co-stimulatory effect of TLR7/8 ligand on γδ T cells. However, the signaling pathways behind this increased cytokine responsiveness remained unclear.

Methods:
After purifying γδ T cells from 7-week-old pigs and sows, they were cultured under four conditions, including TLR7/8 ligand and cytokines for two days. Cellular kinase activities were quantified with a peptide microarray representing 282 phosphorylation events with nine technical replicates of each peptide. Gamma-delta T cell responses to the inhibition of certain peptides were measured by flow cytometry.

Results:
In the older age group, the activation of STAT proteins mainly occurred under cytokine stimulation and was intensified by the addition of the TLR7/8 ligand. Additionally, differences in phosphorylation of IRAK1, MEK1 and IKK were observed. Responses were more heterogenous in the younger age group, and no clear patterns were observed. The inhibition of selected peptides revealed that IRAK1/4, JNK and p38 are involved in the effect of TLR7/8 ligand on adult γδ T cells. Young γδ T cells seem to use an additional or different signaling pathway with little to no involvement of IRAK1/4 and JNK.

Conclusion:
We were able to confirm that the TLR7/8 expression by γδ T cells is indeed functional and serves as a co-stimulatory signal, likely through the myD88 signaling pathway. Moreover, γδ T cells showed both conserved and age-specific responses. Based on this data, porcine γδ T cells could be involved in an anti-viral response by recognizing viral RNA through TLR7/8 and subsequently enhancing the adaptive immune response.

52. ESTERIFICATION LINKS CHOLESTEROL ACCUMULATION TO LIPID DROPLET CHOLESTEROL CRYSTAL FORMATION

Presenter: **Jordan Bairos**
College: **College of Medicine**
Supervisor: Scott B. Widenmaier

Background:
Excess cellular cholesterol is toxic and must be handled appropriately to prevent pathological consequences. Accumulation of unesterified or "free" cholesterol (FC) within the endoplasmic reticulum membrane triggers the conversion of FC into cholesterol esters (CE) by sterol O-acyltransferase 1 and 2 (SOAT1 and SOAT2, also known as ACAT1 and ACAT2). A severe consequence of non-resolving FC accumulation is the formation of cholesterol crystals (CC). The presence of CC in atherosclerotic lesions has been recognized to increase atherothrombosis risk. Likewise, recent studies in patients with non-alcoholic steatohepatitis (NASH) indicate that CC in hepatocyte lipid droplets distinguish NASH from benign steatosis. Additionally, studies using scanning electron microscopy provide evidence that CC can nucleate intracellularly on lipid droplet surfaces. Although CC are present in advanced stages of atherosclerosis and NASH, the mechanistic factors that contribute to cholesterol crystallization are not well understood. Given that SOAT1 catalyzes CE formation for lipid droplet storage, we sought to investigate the role of esterification in this process. We hypothesized that cholesterol esterification and subsequent lipid droplet localization is required for CC formation.

Methods:
CC were detected in live immortalized cell lines using polarized light microscopy. We first established an in vitro model of cholesterol crystallization in cultured human hepatocytes and mouse macrophages. Cholesterol crystallization was induced by loading hepatocytes with excess cholesterol and by loading macrophages with acetylated low-density lipoprotein (acLDL). Localization of CC was assessed using fluorescence microscopy to detect lipid droplets and lysosomes.

Results:
We find that CC form in a dose- and time-dependent manner following cholesterol or acLDL loading. Remarkably, inhibition of SOAT1 drastically reduced CC formation in both cell types which was confirmed in SOAT1-deficient hepatocytes. Fluorescence imaging reveal that these crystals form exclusively at hepatocyte lipid droplets and not in the lysosome.

Conclusion:
In summary, we identify SOAT1-mediated esterification as a critical step preceding CC formation at the lipid droplet surface. Our results provide needed mechanistic insight into cholesterol crystallization and highlight a potential strategy to prevent CC formation.

53. DEGRADATION OF HISTATIN 5 BY PORPHYROMONAS GINGIVALIS AS A NOVEL BIOMARKER OF PERIODONTAL DISEASE

Presenter:	**Andrea Escalante-Herrera**
College:	**College of Dentistry**
Collaborators:	Lina Marin, College of Dentistry Walter L Siqueira, College of Dentistry
Supervisor:	Walter L Siqueira
Co-supervisor(s):	Lina Marin

Background:

Alterations in the oral environment led to dysbiosis, disrupting the homeostasis between the host and oral microbiota. Under dysbiosis, specific microorganisms that are part of the oral microbiota proliferate and predominate in specific environments, causing the development of highly prevalent oral diseases such as periodontitis and dental caries. Porphyromonas gingivalis is considered the key etiological agent in the pathogenesis and progression of the inflammatory events that occur during periodontal disease development. Salivary proteins display a wide variety of functions, with histatins being one of the main groups of proteins in saliva. Histatins are histidine-rich proteins with antibacterial and anti-fungal activity. Histatins are salivary proteins that are degraded by oral proteolytic enzymes produced by host and microbial cells. The degradation rate and mode of histatin 5 differs between healthy individuals and with periodontitis, indicating that proteases produced by periodontopathogenic microorganisms may play a crucial role in this degradation process. This study aimed to determine the specificity of the proteolytic enzymes produced by Porphyromonas gingivalis to degrade histatin 5. Specifically, the degradation rate and mode of histatin 5 were assessed when incubated individually with P. gingivalis, and with other non-periodontopathogenic bacteria, such as Fusobacterium nucleatum, Streptococcus mutans and Aggregatibacter actinomycetemcomitans. Proteolytic enzymes responsible for its degradation were also identified.

Methods:

To evaluate the degradation rate and mode of histatin 5, 100 µL of each bacterial inoculum was individually mixed with synthetic histatin 5, to a final concentration of 0.5 µg/µL and incubated for 0, 2, and 6 h. The negative control was prepared by reconstituting synthetic histatin 5 in 100 µL of PBS, to a final concentration of 0.5 µg/µL. Each suspension having P. gingivalis and F. nucleatum was incubated in anaerobic conditions at 37 °C, while the bacterial suspensions having S. mutans and A. actinomycetemcomitans were incubated in a water bath at 37 °C. At each time point, aliquot samples were boiled for 5 min to stop any proteolytic activity and then processed for cationic polyacrylamide gel electrophoresis (PAGE). The percentage (%) of histatin 5 degradation was determined by comparing the resulting band densities with a reference control having 0.5 µg/µL of histatin 5 in PBS. The experiment was done in triplicate. The supernatant of the samples was taken and desalted before Liquid Chromatography–Electrospray Ionization–Tandem Mass Spectrometry (LC-ESI-MS/MS) analysis to identify the enzymes that are naturally produced by bacteria. The acquired MS/MS spectra were queried against custom databases containing the whole proteome reported for each specific microorganism (Swiss Prot and TrEMBL) using the SEQUEST algorithm in Proteome Discoverer 2.4 software, filtering by natural peptides.

Results:
The results showed that histatin 5 was only degraded by P. gingivalis at all times evaluated, this protein showed a high degradation rate being completely degraded at 6 h (0 h: 20 %, 2 h: 25 % and 6 h: 100 %). In addition, histatin 5 degradation fragments were evident at 2 and 6 h. In contrast, F. nucleatum, S. mutans, and A. actinomycetemcomitans did not degrade histatin throughout the experiment. The MS analysis was done just for the supernatant of P. gingivalis because it was the only microorganism that degraded histatin 5. The main enzymes found in the supernatant of the P. gingivalis were key enzymes such as Cysteine proteinase alpha-gingivain (Q51818) were found at 2 hours and control. Peptidase M24 family (B2RJ88), Trypsin like proteinase PrtT (B2RI85) and Coproporphyrinogen III oxidase (B2RH78) were found at 6 hours. All of those are related to the destruction of the tissues in the host.

Conclusion:
All together, these findings demonstrate that P. gingivalis plays a key role in the specific degradation of histatin 5. The enzymes released by P. gingivalis can degrade histatin 5 and produce unique fragments. The degradation of histatin 5 by P. gingivalis may represent a novel, innovative and promising biomarker of periodontal disease. (Grant #5273 – Saskatchewan Health Research Foundation).

54. MODULATION OF PORCINE EPIDEMIC DIARRHEA VIRUS (PEDV) RNA TRANSLATION BY THE NUCLEOCAPSID PROTEIN

Presenter: **Lin Hao**
College: **School of Public Health**
Supervisor: Qiang Liu

Background:

Porcine epidemic diarrhea virus (PEDV) Porcine epidemic diarrhea virus (PEDV) is a member of the Coronaviridae family and causes the porcine epidemic diarrhea (PED), which is characterized by vomiting, diarrhea, dehydration, and anorexia in pigs of all ages, especially severe diarrhea in piglets, with a mortality rate of up to 100% (Debouck & Pensaert, 1980; Sun et al., 2015). PEDV was first described in England and later reported in Europe (Debouck & Pensaert, 1980). In 2010, China was reported to outbreak a high pathogenic PEDV strains caused-disease, resulting in 100% mortality in piglets (Li et al., 2012). PEDV first entered the United States (US) in April 2013 and rapidly spread throughout the country (Stevenson et al., 2013). As the highly pathogenic strains of PEDV are constantly spreading around the world, it brings problems to pig industry and causes significant economic losses (Stevenson et al., 2013). PEDV is an enveloped, positive-sense, single-stranded RNA virus, with a genomic length of approximately 28 kb (S. Li et al., 2020). A 5'-terminal cap, a 3'-poly (A) tail and seven open reading frames (ORFs), including ORF1a, ORF1b, S, ORF3, E, M, and N genes, making up the viral genome (Figure 1) (Duarte et al., 1993; Renfeng, 2016; Song & Park, 2012). PEDV contains four structure proteins, spike protein (S), envelope glycoprotein (E), membrane glycoprotein (M) and nucleocapsid protein (N), which are encoded by four ORFs. In addition to the other three ORFs, the ORF3 encodes an accessary protein that plays a role for viral replication and virulence. ORF1a and ORF1b encode two replicase polyprotein precursors, pp1a and pp1b, generating 16 non-structural proteins (nsps) by viral proteases (S. Li et al., 2020). Among these, pp1a encoded by ORF1a produces nsp1-nsp11, whereas pp1ab encoded by ORF1b produces the remaining five non-structural proteins (nsp12-16) (Subissi et al., 2014). S protein is a receptor binding protein that consists of two subunits, S1 and S2, which are responsible for viral entry, virus-host interactions and immunogenicity (S. Li et al., 2020). These nsps and structural proteins play important roles in PEDV viral replication and transcription. PEDV N protein N protein is an abundant protein that binds to the viral RNA genome (Satarker & Nampoothiri, 2020). It contains two structural domains: N-terminal domain (NTD) and C-terminal domain (CTD), which are both involved in RNA binding and CTD has also been reported to play a role in dimerization (Fung & Liu, 2018). The N-arm and C-tail are two intrinsically disordered regions (IDRs) on both sides of the NTD and CTD (Figure 2). Furthermore, a central linker region (LKR) connects two structural domains. Previous research has shown that the LKR of N protein is also important in RNA binding (Fung & Liu, 2018). Simultaneously, the N-terminal domain and serine arginine-rich (SR) domain of some coronavirus N proteins are typically phosphorylated, which is related to regulating N protein functions and contributing to increase RNA binding and replication (Chang et al., 2016). N protein is a highly conserved protein with only a few point mutations in different strains. During the early stage of viral infection, the amount of N proteins expressed in infected cells (Satarker & Nampoothiri, 2020). Like other coronaviruses N protein, PEDV N protein serves multiple functions. For example, as a structural protein, it plays a role in the nucleocapsid formation with viral genomic RNA. Moreover, it regulates viral replication, transcription, and assembly. At the same time, N protein is also a phosphorylated protein that involved in many viral processes (Chang et al., 2016). PEDV N protein has also been found to enhance PEDV replication in

Vero E6 cells (Z. Li et al., 2020). Although it has been shown that N protein plays an important role in both virus RNA synthesis and host cell processes regulation, the effect of PEDV N protein on viral translation is not well understood. Furthermore, there has been no information about whether phosphorylation of PEDV N protein has any effect on translation. The PI3K/Akt signaling pathway As a regulator involved in a range of cellular responses, Phosphatidylinositol-3 kinase (PI3K) has been identified to promote phosphorylation of several protein kinases, including Akt. PI3K can be activated by a variety of growth factors and cytokines or virus (Sale & Sale, 2008). After activation, PI3K generates phosphatidylinositol-3,4, 5-triphosphate (PIP3), which binds to the PH domain of Akt on the plasma membrane and activates Akt through phosphorylation of Thr308 and Ser473 (Sale & Sale, 2008). The phosphatidylinositol-3-kinase (PI3K)/Akt signaling pathway is well known as regulating some cell progresses, like cell growth, proliferation, inflammation, and cell survival/apoptosis (Kong et al., 2016). Moreover, PEDV has been demonstrated to activates the PI3K/Akt pathway and inhibiting PI3K activation promoted PEDV replication in Vero cells (Kong et al., 2016). Akt is a serine/threonine–protein kinase that is known as protein kinase B (PKB) due to similarities with protein kinase A (PKA) and protein kinase C (PKC) (Sale & Sale, 2008). It exists as three isoforms: Akt1, Akt2 and Akt3. Akt is a 57-kDa soluble cytosolic protein, containing three conserved domains: N terminal pleckstrin homology (PH) domain, central catalytic kinase domain and C-terminal domain (Sale & Sale, 2008). The domain structure of the three Akt isoforms is similar. The most well-studied is Akt1, which is found throughout the body and involved in PI3K/Akt/mTOR signaling pathway (Kindrachuk et al., 2015). Akt2 has been discovered to play a role in glucose homeostasis regulation and is predominantly expressed in insulin-responsive tissues (Sale & Sale, 2008). Both Akt1 and Akt2 have been found to be activated by virus infections and play a role in regulating viral replication or translation (Shi et al., 2016). Akt3 is highly expressed in the brain, but little is known about its function or the relationship between Akt3 and virus infection (Sale & Sale, 2008). Whether Akt isoforms influence PEDV translation remains unclear.

Methods:

Objective 1: The role of N protein in PEDV viral translation. For this objective, we constructed a reporter for PEDV RNA translation. This reporter contained nanoluciferase gene flanked by the 5'UTR plus the first 54 nucleotides of the ORF1a sequence and 3'UTR sequences of PEDV, with a poly-A at the C-terminus (Figure 3a). PEDV N protein coding sequence with a Flag-tag was cloned into an expression vector. To determine the role of N protein in PEDV viral translation, HEK 293 cells were co-transfected with plasmids expressing PEDV N protein, EGFP, GST or empty vector and the translation reporter. Objective 2: Identification of which domain of N protein is responsible for viral translation modulation. PEDV N protein consists of N-arm, N terminal domain, Linker, C terminal domain and C-arm. To identify which domain of N protein impacts on PEDV translation, we generated six mutants encoding different domains of N protein with Flag-tag (Figure 4a). HEK 293 cells were co-transfected with plasmids expressing full-length PEDV N protein or mutants, and translation reporters. Objective 3: Determine the effects of PEDV 5'UTR and 3'UTR on translation modulation by the N protein. To test this, we generated translation reporters with 5'UTR or 3'UTR deletions (Figure 5a). Objective 4: The role of three Akt isoforms in translation regulation by PEDV N protein. Akt has three isoforms: Akt1, Akt2 and Akt3. To investigate the effects of these Akt isoforms on translation regulation by PEDV N protein, we generated plasmids expressing porcine Akt isoforms with Myc-tag.

Results:

Objective 1: The role of N protein in PEDV viral translation. Compared with EGFP, GST and empty vector, expression of PEDV N protein showed a significant increase in nano luciferase activity. These

results suggested that N protein increases PEDV RNA translation (Figure 3b). The expression of PEDV N protein, EGFP and GST were confirmed by Western blotting using an anti-Flag antibody. The levels of beta-actin were determined by an anti-beta-actin antibody (Figures 3c). Objective 2: Identification of which domain of N protein is responsible for viral translation modulation. The data from luciferase assay showed different domains of N protein downregulate PEDV RNA translation, expect for N terminal domain and Linker which is similar with full length N protein. These results demonstrated that N terminal domain and Linker plays a role in modulating PEDV RNA translation (Figure 4b). The expression of different domains of N protein, EGFP and GST were demonstrated by Western blotting using an anti-Flag antibody (Figure 4c). Objective 3: Determine the effects of PEDV 5'UTR and 3'UTR on translation modulation by the N protein. Upon co-transfecting PEDV N protein with different translation reporters in HEK293 cells, we found that N protein significantly increases RNA translation when only the 3'UTR was present. in contrast, N protein expression decreased translation when the 5'UTR was present in comparation with full-length reporter (Figure 5b). These results suggested that PEDV N protein increases RNA translation in the presence of 3'UTR. The expression of N protein, EGFP and vector were confirmed by Western blotting using an anti-Flag antibody (Figure 5c). Objective 4: The role of three Akt isoforms in translation regulation by PEDV N protein. PEDV N protein and three Akt isoforms were co-transfected in HEK293 cells, as shown in Figure 6a, compared with empty vector, Akt1 significantly increases RNA translation, Akt2 has no effect on the nano luciferase level, Akt3 downregulates RNA translation. These results suggested that Akt1 increases RNA translation enhancement by N protein. The expression of N protein and vector were confirmed by Western blotting using an anti-Flag antibody. The expression of three Akt isoforms were confirmed by Western blotting using an anti-Myc antibody (Figure 6b).

Conclusion:

N terminal domain and Linker plays a role of modulating PEDV RNA translation. PEDV N protein increases RNA translation in the presence of 3'UTR. Akt1 has been demonstrated to further increase PEDV translation by N protein.

55. INVESTIGATING ANTIVIRAL TYPE I INTERFERON RESPONSES IN BATS USING RECOMBINANT IFNB

Presenter: **Victoria Gonzalez**
College: **Western College of Veterinary Medicine**
Collaborators: Andrew Doxey, Linfa Wang
Supervisor: Arinjay Banerjee

Background:
Bats are reservoirs of emerging viruses that cause severe and often fatal disease in humans. These include Marburg filovirus, Nipah and Hendra paramyxoviruses, and coronaviruses that are closely related to SARS-CoV-2 and MERS-CoV. Remarkably, naturally or experimentally infected bats do not demonstrate clinical signs of disease. In mammals, interferons (IFNs) are the first line of defense against invading viruses. Type I IFNs, such as IFNβ, are induced upon viral infection to protect infected and neighboring cells through the induction of interferon stimulated genes (ISGs). Preliminary data in our laboratory indicate that bat cells mount a more robust type I IFN response to SARS-CoV-2, compared to human cells.

Methods:
To discover the full spectrum of type I IFN responses in bat cells, we synthesized recombinant IFNβ from Pteropus alecto, Eptesicus fucus and humans using Drosophila S2 cells. P. alecto (PaKiT03), E. fuscus (Efk3B), and human (Calu-3) cells were then treated with their species-matched recombinant IFNβ for various lengths to assess protection against vesicular stomatitis virus (VSV) and the IFNβ signaling pathway.

Results:
Treatment of bat and human cells with IFNβ for 6 hours led to complete protection against vesicular stomatitis virus (VSV). The induction of an antiviral state was attained by 2 hours in E. fuscus versus 4 hours in P. alecto and human cells. Upon evaluation of the IFNβ signaling pathway, phosphorylation and translocation of the STAT1 signaling molecule occurred similarly in P. alecto and human cells, while basal levels of phosphorylated STAT1 were evident in E. fuscus cells, supporting the difference in kinetics observed.

Conclusion:
Through the generation and treatment with species-matched recombinant IFNβ, we observed a potent response in bats, rapidly leading to protection against VSV infection. Future studies will assess the antiviral efficacy of human and bat IFNβ against Betacoronavirus infection and identify novelties in ISG expression. By understanding how bats control viral pathogenesis, we can gain important insights that will aid in the development of antiviral therapeutics.

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56. EMPLOYEE EXPERIENCES PROVIDING NUTRITIONAL CARE DURING THE COVID-19 PANDEMIC

Presenter:	**Heather Alford**
College:	**College of Pharmacy and Nutrition**
Collaborators:	Allison Cammer, College of Pharmacy and Nutrition Paulette Hunter
Supervisor:	Allison Cammer
Co-supervisor(s):	Paulette Hunter

Background:
Nutritional care is a critical yet often overlooked component of quality care in long-term care that is linked to culture, socialization, psychological and physiological well-being. Relationship-centred care during mealtimes improves nutritional outcomes and quality of life in long-term care. Given that several COVID-19 infection control protocols disrupted nutritional care practices, this study aimed to understand employees' experiences of these changes.

Methods:
Seven semi-structured interviews were conducted with Saskatchewan healthcare employees, each of whom had a role in supporting pandemic nutrition care in long-term care from continuing care aid, nursing, director, and CEO positions. Interviews were audio-recorded, de-identified and transcribed verbatim, and analyzed using reflexive thematic analysis.

Results:
Three main themes characterized interviewees' reflections: 1) Regression to an Institutional Mealtime Environment, 2) Unrealistic Expectations, and 3) Concern for Residents.

Conclusion:
The ethical implications of inadequate pandemic nutrition care highlight the need for improved resourcing for food service departments in long-term care. Given its centrality to quality of life, strategies tailored to support staff in providing relationship-centered nutrition care must be further articulated to maintain standards of care for long-term care residents in future outbreaks and epidemics.

Self-declaration of research alignment with additional themes (optional):
COVID-19 Pandemic Research, Response, and/or Outreach

57. IMPAIRED SLEEP, MULTIMORBIDITY, AND SELF-RATED HEALTH AMONG CANADIANS: FINDINGS FROM A NATION-WIDE SURVEY

Presenter:	**Shirmin Bintay Kader**
College:	**College of Medicine**
Collaborators:	Nahin Shakurun, College of Medicine Bonnie Janzen, College of Medicine Punam Pahwa, College of Medicine
Supervisor:	Punam Pahwa
Co-supervisor(s):	Bonnie Janzen

Background:

Self-rated health (SRH) is a validated measure of health status that is used worldwide and is strongly associated with many objective measures of well-being, including mortality. Both impaired sleep and the presence of multimorbidity are related to poorer SRH, but the precise nature of these associations remains unclear. Recent studies have suggested a possible mediation effect for multimorbidity in the relationship between impaired sleep and SRH, but further research is required. This study explored the association between impaired sleep (IS), multimorbidity, and SRH among Canadian adults.

Methods:

We used 2017-18 Canadian Community Health Survey (CCHS) data for this study which included Canadians 12 years of age and older. The dependent variable, SRH, required participants to rate their health on a 5-point Likert scale ranging from poor to excellent; SRH was dichotomized for the present study into good/very good/excellent and fair/poor. The primary predictor, IS, was derived from two variables (number of sleeping hours per night and trouble sleeping) and categorized into four groups: no sleep issues; fewer sleeping hours (<7 hours) only; trouble sleeping only; and fewer hours & trouble sleeping. Multimorbidity was present (yes/no) if a participant indicated being diagnosed with two or more chronic conditions. We have considered other socio-demographic variables in our full model. A weighted multivariable logistic regression with a robust variance estimation technique was conducted, and mediation analysis was performed using GSEM in STATA.

Results:

Just over one in ten Canadians reported fair/poor SRH; approximately one-quarter had multimorbidity and experienced impaired sleep (few sleep hours in combination with trouble sleeping). The results of the multivariable analysis indicated greater odds of fair/poor SRH associated with the 40-64years age group(AOR:1.87,95% CI: 1.33-2.63), male sex (AOR: 1.18, 95% CI: 1.04-1.33), lower SES (as indicated by education (AOR: 1.47, 95% CI: 1.22-1.77)), income (AOR: 1.35, 95% CI:1.18-1.55) and employment status (AOR:2.82, 95% CI:2.44-3.27)), the presence of multimorbidity (AOR: 4.63, 95% CI: 4.06-5.28), and a combination of fewer sleep hours and troubled sleep (AOR: 4.05, 95% CI: 2.86-5.74). Forty-four percent of the total effect of IS on SRH was mediated by multimorbidity.

Conclusion:

Impaired sleep was associated with increased odds of fair/poor SRH in this study, and the presence of multimorbidity may play a significant mediating role in this relationship. Additional research with enhanced design (ie., longitudinal) and measurement is required to confirm these findings.

Self-declaration of research alignment with additional themes (optional):

Interdisciplinary/Interprofessional Collaboration

58. UNCOVERING THE HIDDEN TOLL OF INADEQUATE RESOURCES ON INDIGENOUS MAYAN CHILDREN: THE DEVASTATING IMPACT ON BONE GROWTH AND HEALTH

Presenter: **Michele Monroy-Valle**
College: **School of Public Health**
Collaborators: Ginny Lane
Supervisor: Hassan Vatanparast

Background:
Poor hygiene and insufficient access to clean water can result in persistent diarrhea and several diseases, which results in malnutrition that can impair growth of young children. The development and maintenance of strong bones depends on nutrients like calcium and vitamin D, which are often malabsorbed during periods of diarrhea or disease. We aimed to explore the impact of overcrowding, poor growth (stunting), access to clean water, and sanitary services on Mayan Indigenous children's bone health.

Methods:
We conducted a cross-sectional study of 133 Indigenous Mayan children living in Chichicastenango, Guatemala. Participating children were between 2 to 5 years old. We measured bone quality (Speed of Sound m/s) using a Sunlight MiniOmni Ultrasound Bone Sonometer thrice at both the left distal radius and midshaft tibia. We collected information on household characteristics, including access to clean water and sanitary services, along with child health data. Stunted children were those whose height for age < –2 SD of the WHO Child Growth Standard median. Binomial logistic regression was used to ascertain the association of bone quality with the availability of clean water and sanitary services, poor growth (stunted), household overcrowding (three or more persons per room), living in a rural area, and diet quality.

Results:
In our study, 53.0% (n=70) of children were stunted; [49.2%(n=30) of girls and 56% (n=40) of boys]. More than half of the children (61.4%) lived in rural areas, and 67% had access to tap water and sanitary service. Those children living in a house without access to drinking water and sanitary services were 3.1 (95%CI 1.3, 7.0) times more likely to have low bone quality; while an overcrowded household posed a 2.3 (95%CI 1.1, 5.0) times higher risk; and stunted children had 2.8 (95%CI 1.3, 6) times higher odds of having low bone quality.

Conclusion:
Access to sanitary facilities and clean water are crucial for children's general health and development. Ensuring children have access to drinking water and sanitation prevents infections and diarrhea, as well as supports adequate growth and bone health. Living in an overcrowded household may be an overall indicator of poverty, which limits access to nutritionally dense foods to support healthy growth and development.

Self-declaration of research alignment with additional themes (optional):
Sustainable Progress

59. A COMPARATIVE STUDY OF THE EFFECTIVENESS OF A LOCALLY PRODUCED FORTIFIED FOOD AND PEANUT-BASED READY-TO-USE THERAPEUTIC FOOD (RUTF) FOR THE OUTPATIENT TREATMENT OF MATERNAL AND CHILD MALNUTRITION: A COMMUNITY TRIAL IN URBAN EL SALVADOR

Presenter: **Samy Abdullah**
College: **School of Public Health**
Collaborators: Ana Beatriz Sanchez, Michele Monroy Valle
Supervisor: Hassan Vatanparast
Co-supervisor(s): Michael Szafron

Background:
Acute malnutrition is caused by a deficiency in energy or nutrient intake that exacerbates immune and physical development. This rapid weight loss contributes to wasting, described as a Weight-for-Height Z-score (WHZ) <=-2 SDs of the WHO child growth standards. Acute malnutrition can vary by severity with those WHZ <=-2 and WHZ <=-3 classified with Moderate and Severe Acute Malnutrition (MAM & SAM) respectively. These burdens presently effect 45.4 million children under five worldwide, with a low prevalence in Central America (0.9%). However, El Salvador is currently suffering over double the burden at 2.1% contributing to UNICEF deploying the Ready-to-use Therapeutic Food (RUTF). The RUTF is a peanut-based nutrient dense paste that benefits from an extended shelf-life and preparation-free distributable packaging. However, it is limited by lack of regional taste adherence and production costs that limit its optimal utilization. Contrarily, Biofortik, a cereal blend made of biofortified maize, and sorghum replicates the Central American porridge drink, Atole. The domestic formulation of this treatment in El Salvador was studied to address malnutrition in children and pregnant women. Ultimately, the equivalence of these foods will demonstrate the potential for continued co-deployment of these treatments.

Methods:
To effectively compare these foods in an intervention science community setting, a quasi-experimental design from January-September 2021 was undertaken. In this period health clinics within the metropolitan region of El Salvador were randomly assigned to provide attending acutely malnourished children (WHZ<=-2) the RUTF or Biofortik. Children underwent an appetite test prior to receiving each food product and were required to visit the clinic for regular check-up sessions during the study period to monitor response treatment. Parents were provided instructions on how to prepare and serve each of the treatments to children. Once a child achieved a WHZ greater than -2 SD, they were discharged from the trial. The study was continued until their respective treatments finished or until achieving recovery. The objective was to determine differences in the proportions of recoveries in each treatment group and variations in weight gain rates (g/kg/day) and length of stay in the program.

Results:
Our findings demonstrated significant increases to WHZ scores in both the Biofortik and RUTF groups ($p<0.05$). At enrollment, children receiving Biofortik displayed a lower mean WHZ score of -2.86 when

compared to the RUTF average of -2.44 ($p<0.001$). However, at discharge there was no observed difference in WHZ scores, with a mean WHZ score of -1.87 and -1.65 for children who received the RUTF or Biofortik respectively ($p=0.75$). Additionally, both treatments contributed to similar observed for weight gain rates and length of stay in the program ($p>0.05$). When considering contextual group differences, those living in a rural setting were 4.8 times more likely to receive Biofortik compared to the RUTF ($p<0.05$). However, this was not shown to contribute to final recovery status among this sample ($p=0.33$). Among the covariates of recovery, low birth weight children, and those with SAM were less likely to escape acute malnutrition. Conversely, those who were breastfed for the first six-months after birth were shown to be nearly five-times more likely to recover than those who were not ($p=0.004$).

Conclusion:

Our findings support the hypothesis that Biofortik demonstrated nutritional equivalency to the existing RUTF to treat children with acute malnutrition. The present study was limited by a small samples size necessitating the implementation of methods to adjust for the low power. However, preliminary results indicate the potential for expanded malnutrition treatment within high-burden settings.

Self-declaration of research alignment with additional themes (optional):

Sustainable Progress

60. IS OBESITY ASSOCIATED WITH MIGRAINE IN CANADIANS? IDENTIFYING VULNERABLE SUBGROUPS

Presenter: **Ladan Kashaniamin**
College: **College of Medicine**
Supervisor: Punam Pahwa
Co-supervisor(s): Bonnie Janzen

Background:
Migraine is a widespread, recurring, and debilitating neurological disorder. Previous research suggests that obesity may be associated with an increased risk of migraine but it is unclear whether this association may vary for particular subgroups of the population. This study aimed to investigate the association between obesity and migraine, with consideration of sociodemographic factors and comorbidities as potential confounders and/or effect modifiers.

Methods:
Using data from the 2015-2016 Canadian Community Health Survey, the prevalence of migraine was determined for those 12 years of age and older, overall and stratified by the presence of obesity and other covariates. Multiple logistic regression was used to determine the association of migraine with obesity and other covariates; product terms were introduced into the model to investigate effect modification.

Results:
The prevalence of migraine was found to be higher among obese Canadians compared to non-obese. The odds of migraine were higher among participants aged 20 to 50 years old (compared to participants 50 years old and older), those with less than $20,000 income (compared to $80,000 or more), and those reporting mood and anxiety disorders. Significant interaction effects were observed between age and sex, and between age and BMI, with obesity more strongly associated with increased migraine among females and those younger than 20 years of age.

Conclusion:
The results of this study suggest that the association between migraine and obesity may vary by sociodemographic factors, highlighting the need for tailored prevention and management strategies with vulnerable subgroups of Canadians.

61. GENETIC DETERMINANTS OF OXYTETRACYCLINE RESISTANCE IN PAENIBACILLUS LARVAE FROM SASKATCHEWAN BEEKEEPING OPERATIONS

Presenter:	**Oleksii Obshta**
College:	**Western College of Veterinary Medicine**
Collaborators:	Michael Zabrodski, Western College of Veterinary Medicine
	Fahim Raza, Western College of Veterinary Medicine
	Tayab Soomro, Western College of Veterinary Medicine
Supervisor:	Elemir Simko
Co-supervisor(s):	Sarah Wood

Background:

Oxytetracycline (OTC) metaphylaxis is widely used against American Foulbrood (AFB) in North America, resulting in sustained selective pressure for resistance in the causative agent of AFB, Paenibacillus larvae. Previously, we identified 65 oxytetracycline-resistant P. larvae isolates from 8 commercial beekeeping operations in Saskatchewan (SK) which were located within the northeast and northwest regions of the province. Moreover, tet (L) and tet (K) genes have been previously shown to encode OTC-resistance in P. larvae in USA, Italy, Argentina. The objective of this research was to investigate the genetic determinants of OTC-resistance in P. larvae in Saskatchewan and investigate the phylogenetic relatedness of resistant isolates.

Methods:

Whole genome sequence (WGS) data was obtained for 17 P. larvae isolates representing a range of resistance phenotypes and geographic origins in SK. WGS analysis was performed for identification of antimicrobial resistance genes (ARGs), and determination of multilocus sequence type, and assessment of phylogenetic relatedness.

Results:

9 out of 11 OTC-resistant P. larvae isolates sequenced carried a tet (L) gene for OTC-resistance. However, known ARGs were not identified in 2/11 OTC-resistant P. larvae isolates. All 17 sequenced P. larvae isolates belonged to sequence type 15 and phylogenetic analysis demonstrated a high degree of similarity among P. larvae isolates, with clustering of isolates according to AMR phenotype and geographic origin.

Conclusion:

The genetic determinants of OTC-resistance in P. larvae in Saskatchewan include the tet (L) gene, as well as a possible novel ARG or non-genetic determinants of AMR in P. larvae isolates. The high degree of genetic relatedness among P. larvae in SK raises concern for potential spread of OTC-resistant isolates across the province.

Social & Population Health 2

62. EXPLORING SOCIAL ENGAGEMENT IN THE 'COGNITIVE KITCHEN': A VIRTUAL CULINARY NUTRITION INTERVENTION FOR DEMENTIA PREVENTION

Presenter: **Julie Beitel**
College: **College of Pharmacy and Nutrition**
Supervisor: Allison Cammer

Background:
Primary prevention efforts to reduce the risk of dementia have become a public health priority, given the significant financial and non-monetary costs for individuals and the health care system, and present lack of cure. Social activity and nutrition are among the modifiable lifestyle factors identified to play a role in preventing or delaying the onset of some forms of dementia. Supporting adoption of evidence-based dietary patterns, such as the Mediterranean diet and Mediterranean-DASH Intervention for Neurodegenerative Delay (MIND) diet remains a challenge. Participatory cooking programs encourage social contact while allowing for the natural integration of evidence-based nutrition information with practical recipe preparation. However, few recommendations exist for virtual delivery of cooking programs; virtual delivery may reduce previously reported barriers to participation related to travel, time, and inclement weather. This study aimed to examine facilitators and barriers to social engagement within the 'Cognitive Kitchen: Virtual Culinary Nutrition Intervention for Dementia Prevention' and understand the function of social interaction for older adult participants.

Methods:
Two Registered Dietitians led two weekday offerings of a six-week virtual nutrition education and applied cooking demonstration program focused on dementia risk reduction strategies. Adults 55 and over from 20 households participated on their preferred weekday. Most participants joined independently; a few signed on with their partners for some or all of the sessions. Data included facilitator session notes, weekly virtual participant journal entries, two virtual focus groups in the last week of the program, and post-program individual interviews with 15 participants. An interpretive description methodological approach was undertaken, and thematic analysis facilitated the identification of patterns in participants' experiences and facilitator observations.

Results:
Participants reflected on the unique opportunities associated with virtual delivery, including flexibility around their level of participation (e.g., they could cook along or prepare in advance), and ease of adjusting recipes to accommodate their tastes and dietary restrictions. Interruptions due to technical issues were minimal and the program had an overall attendance rate of 83.4%. Several participants felt increased accountability for meal preparation during the program, and many recorded notes on practical culinary and nutrition strategies shared by other group members, suggesting peer learning

was a valuable component. Though casual social contact that would normally occur in in-person programs could not be replicated in the virtual setting, discussion questions included to encourage social interaction were appreciated.

Conclusion:
Virtual delivery of a culinary nutrition intervention provided flexibility for participants and reduced several barriers to participation while maintaining important functions of in-person social groups such as accountability and peer learning. Additional effort by facilitators is required to encourage opportunities for social engagement in virtual cooking programs.

63. DETERMINANTS OF COVID-19 SEVERITY AMONG NORTHERN SASKATCHEWAN FIRST NATIONS

Presenter: **Igbaver Isaac Ieren**
College: **College of Medicine**
Collaborators: Nnamdi Ndubuka, College of Medicine
Supervisor: Nnamdi Ndubuka
Co-supervisor(s): Maureen Anderson

Background:
The Severe acute respiratory syndrome due to Coronavirus-2 (SARS-CoV-2), known as Coronavirus disease 2019 (COVID-19), remains a global public health threat, with cases still reported globally. Demographic and medical factors, such as vaccination status, have been reported to influence the disease burden. Reported inequities in social determinants of health further exacerbate the severity of COVID-19 in Indigenous populations. The impact of COVID-19 on Indigenous people in Canada remains understudied. This study aims to determine the association between demographic factors and vaccination with the severity of COVID-19 among Northern Saskatchewan First Nations.

Methods:
The study analyzed secondary quantitative data from 8478 laboratory-confirmed COVID-19 cases in Saskatchewan First Nations communities from March 2020 – December 2022. Binary logistic and negative binomial regressions were deployed to test the association between risk factors (Age, sex, housing, vaccination status), hospitalization, and length of hospital stay (LOHS), respectively, at $p<0.05$ and 95% confidence interval.

Results:
The risk of hospitalization among infected persons aged 30-59 years and those unvaccinated was higher than those 60 years and above (OR=0.37 95%CI 0.20-0.70) and those vaccinated (RR=0.59 95%CI 0.42-0.83) respectively. Individuals aged 30-59% were 24 times more likely to stay longer on admission than those 60 years and above (95%CI 15.2 – 38.6). Patients hospitalized in the Northcentral communities were 26 times more likely to be hospitalized for an extended period than those in the Northeast (95%CI 0.07 – 0.93). Furthermore, those who lived in crowded housing (RR=1.97, 95%CI 1.05-3.07) and those not vaccinated (RR=0.43 95%CI 0.23-0.80) were likelier to stay longer on hospital admission than those who lived in non-crowded housing and those vaccinated respectively.

Conclusion:
Being middle-aged (30-59 years old) and non-vaccinated were significant risk factors for severe COVID-19 infection that required hospitalization. The LOHS was longer in individuals aged 30-59, unvaccinated individuals, those living in crowded housing, and individuals in the Northcentral zone. These findings should provide information that will guide the review of the COVID-19 response in the face of pandemic uncertainty and for subsequent public health emergencies in the Northern Saskatchewan First Nations.

Self-declaration of research alignment with additional themes (optional):
Indigenous Health Research

64. FOOD SECURITY AND COVID-19: ASSESSING THE IMPLICATIONS FOR INDIGENOUS PEOPLES RESIDING IN URBAN AREAS OF SASKATCHEWAN, CANADA

Presenter: **Mojtaba Shafiee**
College: **College of Pharmacy and Nutrition**
Collaborators: Ginny Lane
Supervisor: Hassan Vatanparast

Background:
The COVID-19 pandemic and the measures taken to control it, such as lockdowns and business closures, have had a significant impact on various aspects of life, including food access and security. Certain populations, including Indigenous peoples living off-reserve, may be particularly vulnerable to these effects. In light of this, our objective is to examine the food security implications of the COVID-19 pandemic and associated lockdown measures among off-reserve Indigenous peoples in Canada.

Methods:
In partnership with Indigenous co-researchers, we created an online survey questionnaire consisting of four subscales: background information, Household Food Security Survey Module (HFSSM), food access, and traditional food consumption. The survey was administered through SurveyMonkey® to Indigenous adults residing in urban areas of Saskatchewan, Canada, from August 2021 to August 2022.

Results:
Out of 130 off-reserve Indigenous peoples who participated in our survey, 75.8% were females, 21.9% were males and 2.3% were other genders. The mean age of the participants was 36.2 ± 12.5 years. A significant portion of respondents were single (43.2%), had full-time employment (49.6%), held at least a bachelor's degree (34.1%), and lived in households with four or more members (43.2%). During the first four months of COVID-19 pandemic, 68.4% of off-reserve Indigenous people in Saskatchewan who participated in our survey experienced some degree of food insecurity (33.3% severe, 22.8% moderate, 12.3% marginal). Food price increases (47.1%) and limited food availability at markets (41.4%) were the main barriers reported in accessing food. Additionally, 41.8% of respondents reported facing challenges accessing traditional foods during the pandemic. Seeking assistance from food banks, welfare, and community agencies (40.7%) was the most commonly reported coping strategy for those experiencing food insecurity. Notably, 43.6% of respondents reported receiving no government financial support during the COVID-19 crisis.

Conclusion:
Our survey highlights that the short-term effects of COVID-19 pandemic have worsened existing disparities and significantly affected off-reserve Indigenous households already struggling to meet basic needs. Supply chain issues and food price increases are additional concerns. Immediate policy action is needed to address these challenges and support the food security of off-reserve Indigenous peoples in Canada.

Self-declaration of research alignment with additional themes (optional):
Indigenous Health Research

65. TITLE: RESPONSIVE METHODS OF PAIN ASSESSMENT IN A NORTHERN SASKATCHEWAN CREE COMMUNITY

Presenter:	**Tayah Zhang**
College:	**College of Medicine**
Collaborators:	Rachel Johnson, College of Medicine Elder Rose Dorian Sally Sewap
Supervisor:	Stacey Lovo

Background:
Pain assessment tools are used by health practitioners to quantify patients' pain. There are several forms of pain scales (numerical, facial expression-based and color-based), however, Indigenous Elder advisors in Pelican Narrows, a Cree community in Northern Saskatchewan, have indicated that Western pain scales may not be responsive tools for pain assessments within their community. This study looks to: (1) co-develop a Community Directed Pain Scale (CDPS) in collaboration with an Elder and Knowledge Keeper from Pelican Narrows, to allow clinicians and Indigenous patients to communicate in a more patient and community centered way about experiences with chronic musculoskeletal (MSK) pain; and (2) pilot the CDPS during virtual physiotherapy sessions for MSK pain in community and capture community members' voices regarding their experiences with pain communication and pain scale utilization.

Methods:
This study employed a mixed method research design that involved 2 phases. Phase 1 was the development of the pain scale with a Cree Elder. Qualitative data such as interviews, storytelling, and observations were obtained. Open discussion included the Elder, a nurse practitioner, student researcher, and the principal investigator (a physical therapist). A Knowledge Keeper provided Cree translation. Phase 2 was the piloting of the CDPS during virtual physiotherapy sessions for MSK pain. Participants completed pre-physiotherapy treatment questionnaires which included the utilization of the CDPS and Faces Pain Scale-Revised (FPS-R). Additionally, participants engaged in semi-structured interviews to express their perspectives regarding the pain scale. 27 participants completed the pre-physiotherapy treatment questionnaires and 10 participants were interviewed (9 community members; 1 healthcare provider). Quantitative data was analyzed using SPSS to assess for agreement and qualitative data was analyzed using thematic analysis with NVIVO software.

Results:
A weighted kappa analysis yielded k= 0.696 indicating a good agreement between the CDPS and FPS-R in terms of documenting participants' pain. Qualitative data from interviews with community members revealed three major themes: 1) Learnings Regarding Pain Scales, 2) Patient Centered Care; and 3) Strength-Based Solutions for Improving Pain Communication. Two themes were uncovered through conversations with the HCP: 1) Perspectives on CDPS and 2) Healthcare Provider Experiences Communicating about Pain. Each theme is composed of various subthemes that were gathered from the stories shared by the community members and the healthcare provider.

Conclusion:
Current pain assessment tools may not be culturally responsive for Pelican Narrows, a Cree community in northern Saskatchewan. Community engagement is the best approach in developing a pain assessment tool that best fits the needs of the members of this community. Moreover, a patient-centered approach is important to offer comprehensive pain assessments.

Self-declaration of research alignment with additional themes (optional):
Indigenous Health Research

66. CANADIAN YOUTH SMOKING BEHAVIOR PRE- AND POST-LEGALIZATION OF CANNABIS (2014-2015 & 2018-2019)

Presenter: **Tracey-Ann Stitchell**
College: **College of Medicine**
Supervisor: Nazeem Muhajarine

Background:
Following a sustained period of decreasing smoking prevalence by youth, in recent years, public health concern has grown regarding the effect of new product and legislation on the smoking behavior of youth. This study, therefore, sought to describe the national prevalence of cannabis, e-cigarette and tobacco cigarette use among Canadian youth before legalization of cannabis (in 2014-2015) and immediately after (in 2018-2019); and to identify factors associated with youth cannabis, e-cigarette and tobacco cigarette use before cannabis legalization (2014-2015) and compare it with factors associated with substance use immediately after legalization (2018-2019) among Canadian youth.

Methods:
Using two cycles of the cross-sectional survey (Canadian Student Tobacco, Alcohol Drug Survey) of grades 7-12 students, smoking preferences and correlates of other risky behavior were examined. Descriptive statistics and multinomial logistic regression were used to generate the weighted prevalence of various measures of product use.

Results:
The prevalence of using cannabis was unchanged, e-cigarette was increasing and tobacco cigarette was decreasing ($p=<0.0001$ for all three). The risk of cannabis use was higher in alcohol drinkers ($p<0.0001$), passengers of a driver under the influence of cannabis ($p=<0.0001$) and consumers of energy drinks ($p=0.024$) than non-users. Alcohol drinkers ($p=<0.0001$), passengers of a driver under the influence of cannabis ($p=<0.0001$) and consumers of energy drinks ($p=<0.0001$) versus non-users had higher risks of e-cigarette use. The risk of tobacco cigarette use was more likely in alcohol drinkers ($p=<0.0001$), passengers of a driver under the influence of cannabis ($p=<0.0001$), consumers of energy drinks ($p=<0.0001$) and drinkers of alcoholic beverages with energy drink names ($p=<0.0001$) than non-users. Multiple product use was popular and such students were more likely to drink alcohol, energy drinks, energy drinks with alcohol, alcoholic beverages with energy drink names and be passengers of a driver who was cannabis-impaired.

Conclusion:
There were multiple unhealthy risk behaviors associated with the smoking preferences and these patterns of behavior will be crucial in how health education, promotion and intervention strategies regarding smoking among youth are designed.

67. STREPTOCOCCUS ZOOEPIDEMICUS CAUSES SEPSIS IN PIGS WITHIN 2 HOURS OF INFECTION

Presenter:	**Barbara Cortez**
College:	**Western College of Veterinary Medicine**
Collaborators:	Arthur Nery, Western College of Veterinary Medicine
	Sulove Koirala, Western College of Veterinary Medicine
	Jessica Barbosa, Western College of Veterinary Medicine
Supervisor:	Matheus Costa
Co-supervisor(s):	Heather L. Wilson

Background:

Streptococcus equi subsp. zooepidemicus (S. zooepidemicus) is broadly recognized as a commensal bacterium of horses and occasionally dogs. Reports of this agent in occidental swine herds before late 2010s were rare, with only 6 isolated cases in the United States in 10 years. This scenario changed in 2019 when animal health professionals from North America reported cases of acute death of pigs where the causative agent was conclusively S. zooepidemicus. No commercial vaccine is available for this agent, leaving disease prevention based on biosecurity only. Prior to evaluating potential vaccine candidates, it is necessary to characterize the invasion mechanism employed by the pathogen and its effects on the host. The objective of this study was to evaluate whether the presence of S. zooepidemicus within the pigs organs changes as time passes.

Methods:

Twelve 8-weeks old pigs were inoculated intranasally with 2 mL of S. zooepidemicus ST-194 (109 CFU/mL). Pigs were euthanized and necropsied at 2, 4, 8 and 24 hours after inoculation (n = 3 pigs / time point) and submandibular lymph node (smLN), tonsil, lungs, liver, spleen, and mesenteric lymph node (mesLN) were collected and streaked in Columbia agar with 5% Sheep Blood for bacterial culture.

Results:

After 2 hours of inoculation, positive S. zooepidemicus cultures were obtained from liver (3/3 pigs) and spleen (2/3 pigs). After 4 hours of inoculation, at least one animal showed positive S. zooepidemicus culture in every organ. At 8 hours the mesLN, liver, and spleen were positive in all animals. At 24 hours the smLN was positive in all animals.

Conclusion:

S. zooepidemicus can cause sepsis in pigs within two hours of infection. Further studies characterizing bacterial migration within the host would be helpful for understanding the early pathogenesis of S. zooepidemicus in pigs.

Translational, Clinical, or Applied Science 1

68. GENDER-BASED SALIVARY PROTEIN SIGNATURES OF HEALTHY DOGS TO ASSESS INTER-INDIVIDUAL VARIABILITY USING MASS SPECTROMETRY

Presenter:	**Paras Ahmad**
College:	**College of Dentistry**
Collaborators:	Lina Maria Marin, College of Dentistry
	Candace Lowe, Western College of Veterinary Medicine
	Walter Luiz Siqueira, College of Dentistry
Supervisor:	Walter Luiz Siqueira

Background:

Dogs are relevant to biomedical research in connection both to veterinary medicine/dentistry for their role as pets and to basic investigations for their use as animal models in toxicology, pharmacology, and pathology studies. Dogs' saliva is a complex multifunctional biofluid, consisting of organic and inorganic components, that bathes the oral cavity to facilitate hard and soft tissue maintenance, buffering, lubrication, initiation of food digestion, and defense against pathogenic microbes. Saliva gained interest as a potential non-invasive source of biomarkers in humans and that interest initiates to be expanding also to other animal species including dogs. Hence, knowing the composition of dogs' saliva is extremely essential for identifying the presence of proteins that might be involved in physiological as well as pathological mechanisms of their oral cavity. Any investigation based on the proteomics analysis of saliva, be it for diagnostic purposes or understanding in-mouth mechanisms, requires considering variability in protein profiles within a group of participants. Hence, the present study aimed to investigate the inter-individual variability among healthy dogs via gender-based identification and characterization of salivary proteins using mass spectrometry (MS)-based proteomics analysis.

Methods:

Ten healthy dogs (5 males and 5 females), with similar ages (36 months old) and breeds (Beagle), were included in this study. A visual clinical inspection was performed by a trained veterinary dentist concerning the presence or absence of any oral disease. Using a saliva collection device (Micro•SAL™), ~1 mL of unstimulated saliva was collected from each dog. After processing and preparation of saliva samples, total protein quantification was performed by bicinchoninic acid assay with bovine serum albumin used as the standard, and the samples were stored at -80 °C until further analysis. Protein separation was performed using SDS-PAGE (10 µg protein from each saliva sample; 15% acrylamide gel), followed by staining of the gel with the Coomassie Brilliant Blue. In-solution trypsin digestion of 20 µg protein from each sample was performed. Finally, de-salting of each sample was conducted using C18 pipette tips for MS analysis. MS analyses were performed in triplicates for each sample with a nano-HPLC Proxeon which allows in-line liquid chromatography (LC) with the capillary column, 60 µm × 100 mm filled with C18 resin of 5 mm diameter and 200 pores sizes linked to the mass spectrometer using electrospray ionization in a survey scan in the range of m/z values 390–2000 tandem MS/MS. The acquired MS/MS spectra were compared to the Canis lupus familiaris protein

database (UniProt, using SEQUEST and Proteome Discoverer 1.3 software. To infer protein with high confidence, the SEQUEST filter criteria applied to MS/MS spectra were: 1.5; 2.5; 3.1; 3.1; 4.5 for the XCorr applied in addition to the Percolator filter. Search results were filtered at a false discovery rate of 1% using a reverse database search strategy. The functional enrichment analysis of the unique proteins was performed using the Reactome pathway enrichment analysis, while the protein-protein interaction (PPI) networks were analyzed using the STRING database.

Results:
Pooled saliva sample analysis found a total of 126 and 143 salivary proteins in male and female dogs, respectively, with a collective of 188 proteins. Among these, 45 proteins (24%) were exclusively present in male dogs, 62 proteins (33%) were found in female dogs only, and 81 (43%) proteins were common in both genders. Individual sample analysis revealed only 7 shared proteins among five male dogs (i.e., BPI fold containing family A member 2 [BPIFA2], BPIFB1 and BPIFB2, carbonic anhydrase 6 [CA6], lysozyme C, polymeric immunoglobulin receptor [PIGR], and prolactin-induced protein [PIP]) and 12 proteins among five female dogs (i.e., apolipoprotein A-I [APOA-I], BPIFA2, BPIFB1 and BPIFB2, CA6, chromosome 1 c6orf58 homolog, lysozyme C, phosphopyruvate hydratase 1, PIGR, PIP, and stratifin) (shown in the Venn diagram). Among males, lysozyme C was the salivary protein with the highest intensity, while BPIFA2 was present in the highest intensity among females (shown in the heatmap). The principal component analysis showed a high intra-gender as well as inter-gender salivary protein variability (based on the intensity). STRING database analysis of male and female salivary proteins revealed a total of 56 and 37 edges with PPI enrichment values of <1.0e-16 and 2.4e-06, respectively. In males, lysozyme C, associated with antibacterial activity and immune response, was the protein with the highest connectivity, whereas, APOA-I had the highest connectivity in females which is related to immune response.

Conclusion:
This study identified distinct differences in the salivary protein profiles of healthy male and female dogs. Gender-based variability should be accounted for when searching for salivary biomarkers or when investigating in-mouth biochemical mechanisms.

Self-declaration of research alignment with additional themes (optional):
Interdisciplinary/Interprofessional Collaboration

69. BONE DEFICITS PERTAIN TO THE TRABECULAR BONE ONLY IN CHILDREN WITH TYPE 1 DIABETES: SEX AND MATURITY MATCHED CASE-CONTROL COMPARISON

Presenter:	**Yuwen Zheng**
College:	**College of Kinesiology**
Collaborators:	Munier Nour, College of Medicine
	James (J.D.) Johnston, College of Engineering
	Saija Kontulainen, College of Kinesiology
Supervisor:	Saija Kontulainen

Background:
Children with type 1 diabetes (T1D) are experiencing a higher risk of fracture, which may be linked to impaired bone development. We aimed to assess differences in imaged bone and muscle characteristics, between children with T1D and typically developing children (TDC).

Methods:
Children with type 1 diabetes (T1D) are experiencing a higher risk of fracture, which may be linked to impaired bone development. We aimed to assess differences in imaged bone and muscle characteristics, between children with T1D and typically developing children (TDC).

Results:
At the distal radius, children with T1D had 5% lower trabecular number while cortical and tissue mineral density, cortical area, thickness and bone stiffness were 4-24% higher. At the distal tibia, children with T1D had 6% lower trabecular thickness while cortical density was 7% higher at the tibia shaft. Muscle density was 3% higher in children with T1D.

Conclusion:
Children with T1D had deficits in trabecular bone micro-architecture at the distal radius and tibia, while the distal radius cortex had higher area, density and thickness. Prospective, longitudinal data characterizing bone development in children with T1D vs. TDC, along with endocrine and lifestyle factors contributing to bone development, are warranted to clarify these seemingly contradictory cross-sectional observations.

Self-declaration of research alignment with additional themes (optional):
Interdisciplinary/Interprofessional Collaboration

70. INDUCTION OF IMMUNE RESPONSE AGAINST NECROTIC ENTERITIS BY A SINGLE INTRAPULMONARY LIVE CLOSTRIDIUM PERFRINGENS VACCINE AT HATCH TO PROTECT BROILER CHICKENS

Presenter: **Hemlata Gautam**
College: **Western College of Veterinary Medicine**
Supervisor: Susantha Gomis

Background:

Since past ten years, chicken production has increased dramatically to sustain the global food security. The chicken producers are making continuous efforts to enhance the food security and to supply safe and nutritious food to the people. However, the mandated limits on the use of antibiotics of human importance in Canadian broiler production led to a considerable increase in the infections of necrotic enteritis (NE). NE is one of the most important emerging diseases in the broiler chicken industry, caused by Clostridium perfringens (CP). NE associated economic losses estimated to be at $6 billion USD annually and no effective control available so far in the market. Moreover, CP residing in the gut of broiler chickens is capable of causing significant illnesses in human via contaminated poultry meat. According to Centers of Disease Control and Prevention, CP cause nearly 1 million people ill in the United States every year.

Methods:

Therefore, objective of this study was to develop a novel vaccination strategy against NE by modulation of the immune system of broiler chicken embryos with oligodeoxynucleotides containing CpG-motifs (CpG-ODNs) prior to delivery of a live CP vaccine by the intrapulmonary (IPL) route at hatch. Groups of broiler chickens (n=35) were vaccinated as: (1): no in ovo CpG-ODN, no CP vaccination; (2) CP challenge only; (3) in ovo CpG-ODN + live CP (IPL); (4) in ovo saline + live CP (IPL). Birds in groups 2-4 were challenged with CP at days 20 – 22 with a well-established animal model to study efficacy CP vaccine. Blood, intestinal mucosal scrapings, and sections of intestines were collected at day 23 of age for histopathology and to measure IgY and IgA against CP.

Results:

Birds vaccinated with CP antigens by the IPL route following in ovo delivery of CpG-ODN was protected against CP challenge at a significant level ($P<0.01$). Protection of birds against NE was correlated with IgY and IgA antibodies. It was demonstrated that broiler chickens were protected against NE with a single vaccination at hatch using this novel CP vaccine delivery by the IPL route. Moreover, this novel vaccination strategy using immunomodulation with CpG-ODN potentiate CP vaccine antigen presentation and immune responses against NE.

Conclusion:

This novel vaccination strategy will eliminate the current use of prophylactic antibiotics to control NE in the broiler chicken industry hence minimize antibiotic use and economic losses in the broiler chicken industry. In addition, reducing antibiotic use with biosecurity measures would help to strengthen the one health and bolster food security.

Self-declaration of research alignment with additional themes (optional):

Interdisciplinary/Interprofessional Collaboration

71. THE RELIABILITY OF DYNAMIC MULTIMODAL POSTURAL SWAY ASSESSMENTS IN UNIVERSITY AGED INDIVIDUALS

Presenter:	**Emmarie Racine Hallin**
College:	**College of Kinesiology**
Collaborators:	Lauren Lattimer, College of Kinesiology Isabella Crockower, College of Kinesiology
Supervisor:	Alison Oates
Co-supervisor(s):	Lauren Lattimer

Background:
Dynamic postural control requires an individual to accomplish movement tasks while maintaining an established base of support. In a dynamic environment, proper activation of muscles, a sense of location in space and the ability to predict and respond to our environment is imperative. Subjective symptoms of a concussion may resolve while deficiencies in postural control may exist and be undetected by clinicians. Postural control is often assessed with a static balance test where the stance configuration does not change. A static balance test, however, may not be sensitive enough to capture underlying impairments because of its lack of specificity to activity demands. Underlying deficits in postural control could put individuals at risk of injury and result in sub-optimal level of performance.

Methods:
Participants included healthy active university aged students (21.18±2.16 years). Repeated trials on force platforms were used to measure mean center of pressure sway velocity while participants performed four balance assessments. The four novel tests included a variety of upper extremity and lower extremity movement tasks combined with head and neck movements, stance positioning, and responses to auditory and visual cues using FitLight technology (STROOP, RECALL, YBAL1, YBAL2). Force plate data was measured in two identical sessions, separated by 2-5 days.

Results:
Reproducibility was calculated between both sessions using intra-class correlation coefficients (ICC 2,1). ICC estimates and their 95% confident intervals were calculated based on a mean-rating (k = 2), absolute-agreement, 2-way mixed-effects model. ICC analyses showed moderate reliability between the two data collection days for RECALL (ICC =0.753, p =0.019 95% CI =.111-.960). The remaining tests showed poor reliability STROOP (ICC =0.421, p =0.074 95% CI=-.166-.874), YBAL1(ICC =0.335, p =0.256, 95% CI=-.775-.878) and YBAL2 (ICC =0.380, p =0.212, 95% CI=-.566-.884.

Conclusion:
A multimodal postural control assessment that includes tandem stance, compliant surface and hand eye reaction task showed moderate reliability.

Self-declaration of research alignment with additional themes (optional):
Interdisciplinary/Interprofessional Collaboration

72. POLE WALKING INTERVENTION IN RETIREMENT CARE HOMES: A PILOT FEASIBILITY TRIAL

Presenter: **Mohsen Keramati**
College: **College of Kinesiology**
Collaborators: Mahdi Rostami Haji Abadi, College of Kinesiology
Supervisor: Saija Kontulainen
Co-supervisor(s):

Background:

Care home residents are at greater risk of falls and fractures than community-dwelling older adults. Exercise interventions are effective in reducing rates and risk factors of falls and fractures in community-dwelling older adults, yet their effectiveness in care home residents is unknown. This pilot study aimed to examine the feasibility of pole walking (i.e., Nordic walking with poles) and explore the related changes in fall risk factors in residents of retirement care homes.

Methods:

Four retirement care homes in Saskatoon, Canada participated in this patient-oriented single-arm pilot feasibility trial. We recruited 19 eligible participants (mean age 83.8 years; 84.2% female; 52.6% positive fall history) for the intervention. Intervention sessions (20-60 minutes) were offered 3 times/week over 12 weeks at each care home. Supervised group sessions included posture and balance warm-up, pole walking, strengthening exercises, and stretching tailored for each participant. Feasibility outcome measures were recruitment, retention, and attendance rates as well as reported adverse events and intervention acceptability, appropriateness, and feasibility. We assessed physical function by the timed “up & go” (TUG), 6-minute walk, 30-second chair stand (30CST), and handgrip strength tests. Participants filled out the following self-report scales: intervention acceptability (AIM), appropriateness (IAM), and feasibility measures (FIM), 36-item short-form health survey (SF-36), and falls efficacy scale. Pre-specified progression criteria to a randomized controlled trial (RCT) were as follows: recruitment rate ≥1 participant/site/month; retention rate ≥80.0%; mean attendance rate ≥70.0%; mean AIM, IAM, and FIM scores ≥4.0; and no intervention-related serious adverse event. We addressed the feasibility objectives using descriptive statistics and used paired-sample t-tests to examine the pre- to post-intervention changes in fall risk factors.

Results:

Recruitment, retention, and mean attendance rates were 2.7 participants/site/month, 84.2%, and 90.9%, respectively. Mean AIM, IAM, and FIM scores were 4.3, 4.0, and 4.1, respectively. Participants did not report any intervention-related serious adverse events. Compared with baseline, we observed significant improvements in basic functional mobility (mean change in TUG: -1.5 seconds; 95% CI: -2.6 to -0.4), lower limb muscle strength (mean change in 30-CST: 2.5 repetitions; 95% CI: 1.3 to 3.6), and SF-36 physical functioning score (mean change: 11.8; 95% CI: 2.9 to 20.7) after the 12-week intervention.

Conclusion:

Pole walking pilot in retirement care homes was feasible. Participants improved their physical function over the time the intervention was offered. Overall, these positive findings will guide the design of an RCT to test the efficacy and safety of tailored pole walking intervention in improving physical function and other risk factors of falls and fractures in retirement care home residents.

Self-declaration of research alignment with additional themes (optional):

Sustainable Progress

73. TARGETING REGULATORY T CELLS IN EXPERIMENTAL COLON CANCER WITH RADIOIMMUNOTHERAPY

Presenter: **Zhiwen Xiao**
College: **College of Pharmacy and Nutrition**
Supervisor: Ekaterina Dadachova

Background:
Colorectal cancer remains a formidable threat to the health of the People.Immunotherapy with immune checkpoint inhibitors became a game changer for cancer.The results in patients with colorectal cancer only a small number of patients experiencing long term progression-free survival. And Radioimmunotherapy (RIT) targets radiation at the molecular level utilizing radiolabeled monoclonal antibodies (mAbs) that bind to over expressed or uniquely cancer specific antigens located either on the cancer cell membrane or in the extracellular space of the tumor microenvironment. These targeted radiopharmaceuticals can precisely deliver highly cytotoxic "internal radiation" to localized or systemic cancer deposits, while reducing potential side effects.Several recent study have found that the transcriptome of ti-Tregs in different type of cancer include colon cancer showing the consistent up-regulation of the CCR8 gene.Which means that CCR8 can be a biomarker of ti-Tregs.

Methods:
1.Establishing Tumor Mouse Model and Micro SPECT/CT imaging Study (June-August 2022) We started this experiment by establishing CT26 (ATCC) colorectal tumors in balb/c mice and MC38 (ATCC) in C57Bl6 mice. Tumors were initiated by the subcutaneous injection of 0.5 x 106 CT26 or MC38 cells into the right flank. Tumor growthl was measured with electronic calipers every 2 days (volume = length × width2 /2). When tumors reached a volume of ~200 mm3 - the animals were injected IV with 200 uCi 111In-labeled anti-murine CCR8 mAb (Biolegend Clone-SA214G2) or control murine IgG (Biolegend) and imaged on microSPECT/CT at 24, 48 and 72 hours post antibody administration to ensure that there is a specific binding of anti-CCR8 mAb to ti-Tregs in the tumors. 2.Ac225 Labeled Anti-CCR8 Antibody Therapy Experiment and Mechanistic Study (Sept-Dec 2022) Once specific binding to CCR8 was confirmed, we performed simultaneous RIT and mechanistic study with 225Ac-labeled anti-CCR8 mAb to evaluate the effect of RIT on tumor progression and on the decrease of ti-Tregs cell population within the tumors. For this purpose, the tumors in mice were initiated as above and when they reach a volume of 100 mm3 - the tumor bearing mice were randomized into groups of 5 animals each and treated with: 1 - unlabeled anti-CCR8 mAb; 2 - 200 nCi 225Ac-anti-CCR8 mAb; 3 – 400nCi 225Ac-anti-CCR8 mAb; 4 - left untreatedAll the tumors were collected, fixed, and analyzed by immunohistochemistry for the presence of ti-Tregs, Briefly, the cells were fixed, then stored in 70% alcohol. IHC results were analyzed using Aperio Microscope and all the ti-Tregs were identified by staining with anti-Foxp3 antibody. CCR8+ ti-Tregs were identified by staining with anti-CCR8 antibody. Knowing at which time point post RIT the decrease in ti-Tregs numbers in the most pronounced help us to determine the optimal time of administering immunotherapy for combination treatment in next experiment. Certainly, I will collect all the tumor in the next experiment to confirm the decrease of ti-Tregs within the tumor by FACS. If IHC and FACS give us the same result, we can say with certainty that ti-Tregs are decrease.Anti-3.CCR8 RIT Combine with Anti-CTLA-4 Immunotherapy (March-May 2023) In this experiment we will investigate if the combination of RIT targeting CCR8 with anti-PD-1 antibody immunotherapy will produce an additive

or synergistic effect on the tumor progression. In these experiments, the mice with CT26 and MC38 tumors will be treated with a single dose of 200 nCi 225-Ac-anti-CCR8 mAb and will be followed by immunotherapy with anti-PD-1 mAb RMP1-14 which will start at time point determined in experiment 1. The experiment will be designed to include the following groups (5 animals per group): 1 - mice treated with RIT alone; 2- anti-PD-1 therapy alone (given three times with 3 days between the doses); 3 – combination of RIT and immunotherapy; 4 – left untreated.

Results:

First, we confirm that anti-CCR8 antibody does not bind to CT26 and MC38 cells by FACS and we established a reliable tumor growth curves by adjusting the number of the tumor cell injection. Then, we confirm that anti-CCR8 antibody can specific bind with ti-Tregs within the tumor by SPECT/CT imaging. Next, the animal experiment shows that our RIT works well in both two different mice model. Both two treatment group have significant different compare to the Cold Ab group and untreated group. Finally, we combined RIT with CTLA-4 therapy. We found that the combination group didn't have much more difference compare to the RIT alone group, because both two group the tumor are relatively small at the end point. But compare to the untreated group, more than 90 percent mice were surviving.

Conclusion:

As the results, we can say that Ac225 labeled CCR8 Ab can actually bind with the Ti-Treg, affected the tumor progression. And we didn't see any radio toxicity both in 400nCi and 200nCi Ac225 treatment groups. But during the combination study, Both two treatment groups' tumors are relatively small, so that we can't see any significant different between these two groups at the end point, however, we still can say that both the RIT and the combination therapy resist tumor progression compare to the untreated group. But if I want to confirm there is an additive or synergistic effect, maybe I need to low down the radioactivity of Ac225 to 100nCi or 200nCi at my next experiment.

Self-declaration of research alignment with additional themes (optional):

Sustainable Progress

74. VALIDATION OF MATURITY PREDICTION FOR CHILDREN AND ADOLESCENTS WITH TYPE 1 DIABETES

Presenter:	**Zahra Ghafouri**
College:	**College of Kinesiology**
Collaborators:	James (J.D.) Johnston, College of Engineering Munier Nour, College of Medicine Saija Kontulainen, College of Kinesiology
Supervisor:	Saija Kontulainen

Background:

At the same chronological age, the range in somatic maturity can be large, particularly around the adolescent growth spurt. Thus, validated estimates of somatic maturity are essential in clinical research when assessing skeletal growth and development. Predictive equations, based on predicting years from peak height velocity (i.e., maturity offset) have been validated for typically developing children and adolescents. The predictive accuracy of these equations has not been assessed in children and adolescents with type 1 diabetes (T1D). Validation is needed, as sex-specific impairments in growth velocity during the pubertal growth spurt have been reported in adolescents with T1D, which may affect predictive accuracy. The objective of this study is to assess agreement and differences between predicted and observed maturity in female and male children and adolescents with T1D.

Methods:

We obtained prospective measures of height from health records of participants (12 females and 10 males) in the Bone Strength Development Study in Children with Type 1 Diabetes (BSDS). We applied the Preece-Baines Model and defined age at peak height velocity (APHV) for 22 participants (12 females and 10 males). We calculated observed maturity offset by subtracting age at each measurement from APHV for male (N observations = 109, age 6-18 years) and female (N observations = 144, age 3-17 years) participants. We used Moore et al. maturity prediction equations to predict maturity offset at each measurement time. We used sex-specific linear regression models to report model-fit (R2) for the agreement and paired t-tests to report mean differences (Δ) with 95% confidence intervals, between observed and predicted maturity offsets. Significance was set to $p < 0.05$.

Results:

Predicted maturity offsets explained 86% and 87% of the variance in the observed maturity offsets in male and female children with T1D, respectively. Predicted and observed maturity offset means did not differ significantly in male (Δ 0.18, 95% CI -0.02 to 0.38) nor female (Δ 0.09, 95% CI -0.06 to 0.25) participants with T1D.

Conclusion:

The maturity prediction equation estimated maturity offsets within 2 months from the observed offset in both female and male children with T1D. These findings warrant cross-validation in a larger sample of children with T1D. Nevertheless, initial findings suggest that the commonly used Moore et al. equation may offer a reliable and practical solution to estimate biological maturity in studies assessing growth and development in children and adolescents with T1D.

Self-declaration of research alignment with additional themes (optional):

Sustainable Progress

Translational, Clinical, or Applied Science 2

75. USE OF A LIVE ATTENUATED VACCINE IN PIGS AGAINST DISEASE CAUSED BY STREPTOCOCCUS EQUI SUBSP. ZOOEPIDEMICUS

Presenter: **Sulove Koirala**
College: **Western College of Veterinary Medicine**
Collaborators: Arthur Nery da Silva, Western College of Veterinary Medicine
Jessica Barbosa
Lucie Amblard
Supervisor: Matheus de Oliveira Costa

Background:
Streptococcus equi subsp. zooepidemicus (S. zoo) ST-194 is associated with high mortality and morbidity in swine. It emerged as a novel disease in North America after the first outbreaks in farms in Canada and USA in 2019. Despite many efforts, no effective vaccine has been developed for this disease in swine. A clinical trial was conducted to evaluate the efficacy of vaccination with a live attenuated strain of S. zoo (H1) in preventing disease caused by S. zoo ST-194 in swine.

Methods:
Eleven pigs were randomly assigned to 2 groups: control (n = 5, vaccinated with placebo) and vaccinated (n = 6, vaccinated with H1). Each pig got 2mL bacteria orally and nasally for vaccination and challenge. Vaccinated pigs received 2 doses 23 days apart consisting of 10^7 CFU/mL. Both groups were challenged 14 days after the second dose with 106 CFU/mL of S. zoo ST-194. Pigs were euthanized seven days post-challenge or if welfare became a concern. Necropsy was performed, and samples plated on blood agar for microbiological analysis.

Results:
No mortalities were observed in the vaccinated group. Vaccinated group had a higher probability of survival (Log-Rank test, P = 0.03). All pigs in the vaccinated group recovered within 3 days after challenge, while the pigs in control group had pyrexia (> 39.7°C) until euthanized. Vaccinated group had less hemorrhagic submandibular LN (1/6), mesenteric LN (1/6) and no ascites (0/6) compared to control group, which had all (5/5) hemorrhagic LN and ascites (2/5). Control group had a higher proportion of culture-positive samples (Fisher's Exact test, P = 0.004).

Conclusion:
These results suggest that vaccination with H1 prevents clinical signs and death and may be a powerful tool in controlling the disease in North American herds.

76. ASSOCIATIONS BETWEEN REPRODUCTIVE HORMONE PRODUCTION AND MUSCULOSKELETAL HEALTH DURING THE MENOPAUSE TRANSITION

Presenter: **Manpreet Kaur**
College: **College of Medicine**
Supervisor: Angela Baerwald
Co-supervisor(s): Saija Kontulainen, Jaswant Singh

Background:
The relationships between musculoskeletal health and changes in reproductive hormone production during the menopause transition are poorly understood. The objective of this study was to test the hypotheses that LH and FSH would be negatively associated and Inhibin A, Inhibin B, Estradiol-17B, Progesterone and AMH would be positively associated with musculoskeletal health as women age.

Methods:
A prospective observational study was conducted in 26 ovulatory women of mid reproductive age (18-35 years, n=10) and Advanced Reproductive Age (45-55, n=16). Venipuncture was conducted every 1-3 days in each woman over one Inter-ovulatory Interval. A peripheral Quantitative Computed Tomography (pQCT) scan was conducted to quantify bone area, content and density within the radius, ulna, and tibia. A DEXA scan was conducted to quantify bone content, area, density at the tibia, lumbar spine and hip and total lean muscle mass. Associations between mean hormone concentrations over the IOI, DEXA and pQCT outcomes were analyzed using Pearson's rank correlation coefficient, to guide the development of univariate and multivariate regression analyses (SPSS V28.0.1.1).

Results:
Irrespective of age, estradiol positively correlated with trabecular density and Inhibin B negatively correlated with total area, trabecular area and content at the distal radius. LH positively correlated with trabecular density, while Inhibin B and AMH negatively correlated with total hip area. After adjusting for age, estradiol was positively associated with distal radius trabecular density, as well as lower limb muscle content and area. Estradiol was positively associated with lumbar spine bone mineral density in older women. LH was positively associated and Inhibin A was negatively associated with ulnar shaft total content in older women.

Conclusion:
Greater concentrations of serum estrogen were consistently associated with musculoskeletal outcomes in women of both reproductive and advanced reproductive age. Relationships between LH, Inhibin B, Inhibin A and AMH and musculoskeletal health require further investigation.

77. ADVANCEMENT IN UNDERSTANDING OVARIAN FOLLICULAR WAVE DYNAMICS: REPEATABILITY ACROSS MENSTRUAL CYCLES

Presenter: **Gabriella Antaya**
College: **College of Medicine**
Supervisor: Angela Baerwald

Background:
Ovarian follicles are fluid-filled sacs which contain oocytes or 'egg' cells. The number of ovarian follicles depletes with age and thus, is indicative of reproductive potential. Two or three waves of follicular development have been identified in the human menstrual cycle, consistent with domestic farm animals. In the bovine species, the number of follicular waves has been documented to be repeatable in 2/3 of estrous cycles. The objective of this study was to test the hypothesis that the numbers and patterns of follicle waves in women were repeatable over multiple menstrual cycles.

Methods:
22 healthy women of reproductive age underwent transvaginal ultrasonography every 1-3 days over two interovulatory intervals (IOIs). Women in the following categories were evaluated: reproductive age (RA, age 18-35, n=14), late reproductive age (LRA, age 36-44, n=4), and advanced reproductive age (ARA, age 45-55, n=4). The number of follicles on each day across the IOI were tabulated for the follicle diameter categories: 2-5mm, 2-10mm, ≥5mm, ≥4mm and 4-6mm. The size and location of all follicles ≥4mm were sketched each day during the IOI, and the growth profiles of individually identified follicles that attained a diameter of ≥6mm retrospectively determined and graphed. A wave was defined as an increase and subsequent decrease in the number of follicles in association with the growth of 1 or more ≥6mm follicles. Major waves were defined as those in which a dominant follicle was detected; minor waves were those in which a dominant follicle did not develop. The numbers and patterns of follicular waves across the IOI were compared between cycles and among age groups. An alpha level=0.10 was used for all statistical analyses (SPSS).

Results:
The mean number of waves developing per IOI was 3.3 + 0.14 (range=2-5 waves); neither cycle nor age group influenced the mean number of waves during the cycle (p=0.295 and 0.354, respectively). The proportion of women with 2-5 waves did not differ between cycles. However, women of RA had a greater proportion of 3 waves during the IOI compared to women of LRA and ARA (< 0.03). Similarly, RA women had a greater proportion of waves underlying the major ovulatory wave compared to LRA and ARA women. Irrespective of age, 13/22 (59%) of women exhibited repeatability in the number of waves across the IOI (2-5 waves). In contrast, only 6/22 (27%) women demonstrated repeatability in patterns of waves.

Conclusion:
Irrespective of age, 2-5 waves developed in women, with a repeatability in the number of waves across 2 cycles in most women. Younger women developed more waves within a given cycle compared to older women. Minor and major patterns of follicular waves were consistent in a minority of women. Knowledge about repeatability of ovarian follicular waves in humans is fundamental for optimizing ovarian stimulation protocols for couples undergoing fertility treatment.

78. INDUCTION OF TH2 CELL TOLERANCE AND CD4+CD40L+CD25+CD127- REGULATORY T CELLS BY HUMAN REGULATORY DENDRITIC CELLS FROM ALLERGIC DONORS

Presenter: **Sara Ness**
College: **College of Medicine**
Collaborators: Chris Rudulier, Melina Messing, Kelly McNagny
Supervisor: John Gordon
Co-supervisor(s): Don Cockcroft

Background:
In asthmatic individuals, otherwise innocuous stimuli (e.g., pollen) can induce inflammatory Th2 responses in place of the regulatory T cell (Treg) responses seen in healthy individuals. Several agents (e.g., IL-10) have been identified with the ability to skew differentiating dendritic cells (DC) towards regulatory phenotypes (DCreg), which then induce Treg responses among human Th2 cells and in murine models.

Methods:
Dendritic cells were differentiated from monocytes in the presence of cyclosporine (DC-CsA), vasoactive intestinal peptide+IL-10 (DC-VIP/10), or stimulatory agonists (DCstim) and loaded with specific allergen(s) before analysis of surface and secreted markers by means of FACS, ELISA and multiplex. Through collaborative efforts, we have thoroughly documented the physical characteristics of our induced DCreg/DCstim by means of CyToF and RNA sequencing. We also examined their abilities to suppress autologous Th2 responses of cells from mono- or dual allergen-sensitive allergic donors, driven by allergen-loaded DCstim (1.5x104 DCreg/1.5x104 DCstim/2x105 Th2 cells), their ability to convert these Th2 cells into Treg marker-positive cells. Generated Treg marker-positive cells were then examined for their suppressive activities on autologous Th2 cells (6x104 induced Treg/3x104 DCstim/2x105 Th2 cells).

Results:
Both DCreg expressed lower levels of HLA-DR, CD40 and CD86 than DCstim, but they also expressed DC-SIGN, ILT2, ILT3 and CCR4, as well as higher levels of IL-10 and TGFβ and higher IL-10:IL-12 expression ratios. CyToF and RNAseq analysis demonstrated distinct characteristics that were unique to each DC population. Both DCreg effectively suppressed allergen-specific Th2 proliferation and Th2-type cytokine expression (e.g., IL-5, IL-13; 84-99% reductions) by cells that were exposed to allergen-pulsed DCstim. They also induced CD40L+CD25+CD127- Treg, which in turn could effectively suppress activation of down-stream allergen-specific Th2 cell responses.

Conclusion:
We have shown that our DCreg are able to generate functionally suppressive CD40L+CD25+CD127- Treg. Our collaborative protocol involving CyToF and RNA sequencing techniques resulted in an in-depth phenotypic characterization of our novel (DC-CsA) and more established (DC-VIP/10) in-house DCreg, which sheds light on the possible mechanism(s) of action for these DCreg, and contributes to the goal of identifying and standardizing the ideal DCreg candidate for clinical use.

79. ANTIMICROBIAL RESISTANCE AND GENOMIC CHARACTERIZATION OF NORTH AMERICAN MELISSOCOCCUS PLUTONIUS ISOLATES

Presenter: **Fatima Masood**
College: **Western College of Veterinary Medicine**
Supervisor: Antonio Ruzzini
Co-supervisor(s): Sarah Wood

Background:
Melissococcus plutonius is a major bacterial pathogen of honey bee larvae, acting as the causative agent of European foulbrood (EFB) disease. In North America, antimicrobials are widely used to treat and control bacterial brood diseases, including EFB and American foulbrood. Thus, antimicrobial resistance (AMR) poses an imminent threat to North American apiaries, especially for cases of EFB disease since only a single antibiotic, oxytetracycline (OTC), is approved. Additionally, western Canada has seen an emergence of EFB disease despite OTC application. Little is known about the resistance profiles of M. plutonius and how these are related to the ability of drugs to clear infection in honey bee larvae.

Methods:
The objective of this study was to determine the AMR profiles of North American M. plutonius isolates through minimum inhibitory concentration (MIC) determination. Additionally, one sensitive and three resistant isolates were chosen to compare the utility of OTC, tylosin (TYL), and lincomycin (LMC) as alternative treatments for EFB using an in vitro larval infection model. Finally, pangenome analysis was performed for 71 M. plutonius isolates to evaluate AMR genes and assess relatedness.

Results:
We found that 67% of isolates were resistant to OTC (MIC ≥16 µg/mL) while 100% of isolates remained sensitive to TYL and LMC (MIC ≤4 µg/mL). We demonstrated that the efficacy of antimicrobial treatments in honey bee larvae were concordant with in vitro MIC measurements. Moreover, survival outcomes of larvae were not dependent on M. plutonius clearance, but on a strain-dependent reduction of their numbers within larvae. In the tested isolates, we did not find any genetic determinant of OTC-resistance, nor did phylogenetic analysis reveal patterns of genetic relatedness to explain resistance phenotypes.

Conclusion:
Overall, we demonstrated a high incidence of OTC-resistance in North American M. plutonius isolates, and that TYL and LMC may act as alternatives for EFB treatment in vitro.

80. SPARING EFFECTS OF SINGLE-LIMB LEG TRAINING DURING OPPOSITE LIMB IMMOBILIZATION

Presenter: **Aryan Kurniawan**
College: **College of Kinesiology**
Supervisor: Jonathon Farthing

Background:
Muscle strength and size losses occur immediately during periods of disuse due to a reduction in muscle protein synthesis. One novel approach to offset this decline is called “cross-education” training, where unilateral strength training can enhance the strength and muscle of the untrained, opposite limb. Recent studies show that cross-education can preserve strength and muscle size in an opposite immobilized upper limb, but the mechanism of these preservation effects is unclear. The proposed research will explore the mechanisms of these effects, for the first time using a lower-limb model. This allows the ability to acquire muscle biopsies to gain insight into changes in the muscle building processes. One hypothesis is that low level involuntary activation of the immobilized muscles during training of the opposite limb (called “mirror contractions”) influence the effects. Prior research shows that even low-level activation of muscle might influence muscle protein synthesis. The purpose of this work is to: 1) investigate the effects of single-limb, lower body strength training on preservation of the strength and muscle of the opposite immobilized limb; and 2) understand the contribution of mirror contractions to these effects.

Methods:
Twenty participants will have one leg immobilized for seven days, half of whom will train the opposite limb. Muscle protein synthesis will be estimated using a “heavy water” ingestion protocol that can be used to measure protein content via saliva, muscle, and blood samples. Imaging techniques will be used to measure muscle volume, along with measures of leg strength and muscle activation.

Results:
This study is a proposal and there are no results yet.

Conclusion:
By examining the role of cross-education in activating muscle protein synthesis, this research will offer a deeper understanding of mechanisms of disuse atrophy. This has societal implications for rehabilitation strategies after unilateral injury or impairment, allowing for a quicker and more complete recovery, reducing the costs and burden incurred by Canada’s health care system.

81. IS TIME SINCE PAIN ONSET RELATED TO SHOULDER KINEMATIC CHANGES IN PEOPLE WITH ROTATOR CUFF DISEASE: A PRELIMINARY ANALYSIS

Presenter: **Davidson Fadare**
College: **College of Medicine**
Collaborators: Lauryn Campbell, College of Medicine
Supervisor: Angelica Lang

Background:
Chronic shoulder pain is a leading musculoskeletal condition in Canada with the most common cause being injury or damage to the rotator cuff tendons. As rotator cuff disease progresses, pain in the shoulder becomes highly debilitative by impeding daily functioning and causing significant socioeconomic consequences. There are key biomechanical components to chronic shoulder disorders that may be related to the progression of this injury. However, research that aims to understand the relationship between shoulder biomechanics during functional tasks and time since pain onset for those with rotator cuff disease is minimal.

Methods:
A sample of 16 individuals between the ages 18 and 65 of any gender ranging from 1 - 8 years post injury were analyzed for this abstract. All participants tested positive on at least 3 of 4 impingement pain provocation tests and had pain for at least the last 3 months. Upper body motion (torso, humeri, and scapulae) was tracked using a 10-camera passive motion capture system. Participants performed a standardized protocol called the Work-Related Activities and Functional Tasks made up of seven tasks (comb hair, wash axilla, tie apron, overhead reach, forward transfer, floor lift, overhead lift). Marker trajectories were used to calculate thoracohumeral and scapular angles. Pearson correlation coefficients ($p < .05$) were used to quantify the relationship between each outcome (humeral elevation, humeral axial rotation, scapular protraction, scapular upward rotation, scapular tilt) and time since pain onset.

Results:
Preliminary data from 16 participants suggests that there is a linear relationship between time since pain onset and scapular and humeral kinematics for varying stages of rotator cuff disease. Time since pain onset was significantly associated with humeral elevation in the Overhead Reach. As time increased, humeral elevation decreased ($r = -.58$, $p = .01$). Time since pain onset was also significantly associated with humeral axial rotation in the Overhead Reach. As time increased, humeral internal rotation decreased ($r = -.51$, $p = .03$). Additionally, trends suggest scapular upward rotation in the Overhead Reach ($r = -.42$, $p = .08$) and scapular internal rotation in the Comb Hair ($r = .31$, $p = .21$) and Overhead Reach ($r = .35$, $p = .14$) may also change over time.

Conclusion:
These preliminary findings indicate a potential temporal component to scapular and humeral kinematics in different stages of rotator cuff disease. This information may aid in the identification of risk factors for rotator cuff disease to prevent the progression of chronic shoulder pain, but final recommendations will be made after completion of the full dataset.

Translational, Clinical, or Applied Science 3

82. COULD YAP INHIBITOR CELASTROL BE A NOVEL TREATMENT FOR SCLERODERMA FIBROSIS?

Presenter:	**Pratyusha Chitturi**
College:	**College of Dentistry**
Collaborators:	John Nguyen, Andrew Leask, Xu Shi-Wen, Bahja Ahmed Abdi, Richard J. Stratton, David E. Carter
Supervisor:	Andrew Leask

Background:

Fibrosis is the common end-stage pathway for many inflammatory conditions, including scleroderma. Scleroderma (systemic sclerosis, SSc) is an autoimmune inflammatory-fibrosis syndrome that damages the skin and internal organs. SSc is characterized by generalized activation of the innate and adaptive immune systems, in response to endothelial and epithelial injury, culminating in the recruitment and activation of fibroblasts into myofibroblasts, responsible for fibrosis. In SSc, persistently activated myofibroblasts are maintained by an excessive, autocrine mechanotranductive/pro-adhesive signaling loop. Drugs targeting this pathway are therefore of likely therapeutic benefit in SSc. The mechanosensitive transcriptional co-activator, yes activated protein-1 (YAP1), is activated in SSc fibroblasts. The terpenoid plant-derivative celastrol has recently been identified as a YAP inhibitor: however, if celastrol can alleviate SSc fibrosis, and its underlying mechanism of action, is yet unclear.

Methods:

We cultured human dermal fibroblasts sampled from healthy individuals and patients with diffuse cutaneous scleroderma (systemic sclerosis, dcSSc) and treated with or without transforming growth factor 1 (TGF1, 4ng/mL) in the presence or absence of celastrol (500nM). We also subjected C57BL6J mice to the inflammatory-driven bleomycin-induced model of skin SSc, in the presence or absence of celastrol. RNA expression was assessed using RNAseq, real-time polymerase chain reaction and spatial transcriptomic analysis. Protein expression was determined using Western blot and enzyme-linked immunosorbent assay. Skin fibrosis was monitored by hematoxylin/eosin and trichrome staining to assess skin thickness, collagen deposition and by indirect immunofluorescence analysis with anti-YAP and α-SMA antibodies to assess localization of YAP1 in myofibroblasts.

Results:

In dermal fibroblasts, celastrol impaired the ability of TGFβ1 to induce an SSc-like pattern of gene expression, including that of cellular communication network factor 2 (CCN2), collagen I and TGFβ1. Celastrol alleviated the persistent fibrotic phenotype of dermal fibroblasts cultured from lesions of SSc patients. In the bleomycin-induced model of skin SSc, activation of genes specific to epithelial and universal, papillary, and reticular fibroblast niches were notably activated. Celastrol inhibited fibrogenesis and blocked nuclear localization of YAP in myofibroblasts.

Conclusion:

Our data are consistent with the hypothesis that compounds, such as celastrol, that antagonize the YAP pathway warrant further consideration as potential treatments for SSc skin fibrosis.

83. ANTIMICROBIAL RESISTANCE SURVEILLANCE OF E.COLI CAUSING CANINE UTIS IN SASKATCHEWAN, BETWEEN 2018 - 2022

Presenter: **Yaasin Dulymamode**
College: **Western College of Veterinary Medicine**
Collaborators: Joseph E. Rubin, Western College of Veterinary Medicine
Supervisor: Joseph E. Rubin

Background:
E. coli is the most common cause of urinary infections in dogs. The emergence of antimicrobial resistance globally is resulting in limited therapeutic options available to veterinarians and physicians to treat their patients. The overall objective of this study was to analyze the antimicrobial susceptibility profiles and the epidemiology of resistance genes of E. coli isolates between Oct 2018-Oct 2022 (n = 601). Since 2013 we have been conducting a longitudinal passive surveillance project targeting E. coli causing UTIs in dogs; in the current study data from years 6 – 9 (Oct 2018- Oct 2022) will be described

Methods:
Laboratory records were reviewed, and one isolate per dog was included. Antimicrobial minimum inhibitory concentrations were then determined by broth microdilution and agar dilution. Based on the susceptibility profile, isolates were screened for broad-spectrum beta-lactamases (ESBL and AmpC enzymes) and plasmid mediated quinolone resistance determinants (PMQRs). Isolates possessing clinically relevant resistance genes were further characterized by MLST to identify resistant strains.

Results:
Over this period, 76.20% of the isolates were non-resistant while 4.33% were multidrug-resistant (resistant to 3 or more drug classes). Ampicillin resistance, identified in 13.97% of isolates, was the most common. Between 5-10% resistance was found in amoxicillin-clavulanate, cefazolin, chloramphenicol, nalidixic acid and tetracycline. Fewer than 5% of isolates were resistant to cefoxitin, ceftriaxone, cefepime, ciprofloxacin, gentamicin, amikacin, tobramycin, or trimethoprim-sulfamethoxazole.

Conclusion:
Resistance overall is fairly uncommon at this point. Our study supports the use of first-line therapies such as amoxicillin and trimethoprim-sulfamethoxazole. These data do not support the use of second-line drugs empirically in our region. Finally, continued surveillance is warranted to identify emerging resistance trends and guide future empiric therapy.

84. APPLICATION OF IN-FIELD MOTION CAPTURE PROTOCOL FOR EVALUATING UPPER BODY MOVEMENT; A VALIDATION STUDY USING WEARABLE IMUS SENSORS.

Presenter: **Opeyemi Akinluyi**
College: **College of Medicine**
Supervisor: Angelica Lang

Background:
Work-related musculoskeletal disorders (WMSD) are one of the leading causes of disabilities and injuries in Canada. WMSDs impose a significant economic burden on people, families, and social care systems. Better definition of the risk factors for WMSDs, such as postural exposures, could improve preventative measures for workers. However, before in-depth postural measurements can be conducted, novel measurement and assessment methods need to be validated. The main objective of this cross-sectional study is to validate the application of in-field motion capture (IMUs) for evaluating upper body movement in various settings.

Methods:
A convenience sample of 15 participants of any gender, between the ages of 18 and 65, and free from any upper limb impairments will be measured in three different in-field settings: home, work (on farms), and sport (training facility). Currently, three “home” setting participants have completed the study. Motion of the bilateral shoulders was tracked with inertial measurement units (IMUs) during the Work-Related Activities and Functional Tasks (WRAFT) protocol, consisting of seven tasks to replicate activities of daily living and work tasks (comb hair, wash axilla, tie apron, overhead reach, forward transfer, floor lift, overhead lift). Scapular and humeral were calculated and qualitatively compared to data from a previous in-lab study, consisting of 60 healthy individuals, also using the WRAFT protocol.

Results:
Preliminary data from three completed participants suggest that our novel in-field measurement methods are successful. It was observed that the scapular and humeral angles from in-lab kinematic data were similar to the in-field kinematic data for most angles. Humeral elevation and scapular upward rotation were the closest to in-lab values.

Conclusion:
The IMU system appears to be appropriate for capturing upper body motion during functional tasks. Based on the available data, the validity of the in-field measures were good compared to the conventional in-lab values. The results demonstrated that the tested IMU-based system had the required precision for postural assessments of upper body motion during work-related activities, but final recommendations for use will be made after the full dataset is completed.

85. THE EFFECT OF SELECTED BREAK ACTIVITIES ON REACTION TIME, BALANCE, AND PERCEIVED DISCOMFORT AFTER ONE HOUR OF SIMULATED OCCUPATIONAL WHOLE-BODY VIBRATION EXPOSURE IN HEALTHY ADULTS

Presenter:	**Dena Burnett**
College:	**School of Rehabilitation Science**
Collaborators:	Mike Tweten, School of Rehabilitation Science
	Udoka Okpalauwaekwe, College of Medicine
	Stephan Milosavljevic, School of Rehabilitation Science
Supervisor:	Stephan Milosavljevic

Background:
Negative health effects from occupational whole-body vibration (WBV) exposure during agricultural machinery operation include alterations in proprioception, vestibular function, reaction time, stress, sensory and motor response, and decrements in musculoskeletal health. In current practices, it is not possible to fully eliminate seated WBV exposure, but it may be possible to break up extended periods of such exposure with short breaks and activities. Ideally, short (<5 minutes) effective practical break activities would help protect against adverse effects of WBV exposure. The objective of this study is to determine if there are feasible and practical intervention activities that can minimize decrements in cognition, proprioception, and musculoskeletal discomfort related to seated WBV exposure during agricultural machine operation as evaluated in a controlled laboratory environment.

Methods:
Eleven healthy adults with a minimum of one year of operating agricultural or heavy machinery were recruited. Participants took part in four sessions of 1-hour of ecologically valid in-lab WBV exposure followed by one of four randomly selected 5-minute activities: sitting, walking, 2 minutes of gaze stabilization exercise (GSE) coupled with 3 minutes of trunk mobility exercise (GSE+MOBIL), or 2 minutes of GSE coupled with a 3-minute walk (GSE+WALK). Baseline and post-activity health effect outcomes (rating of perceived discomfort, balance and postural sway measurements, 5-minute psychomotor vigilance task test) were submitted to a paired t-test to determine the effect of intervention activities on physical, cognitive, and sensorimotor systems, and normalized post-intervention data were submitted to a repeated measures ANOVA to determine any differences across intervention activities.

Results:
Differences between baseline and post-activity reaction time (RT) outcomes were observed after sitting and walking activities, but not after GSE+MOBIL or GSE+WALK activities. After sitting, mean RT was 4% higher and the slowest 10% RTs were 8.6% slower. After walking, RT speed was 5.6% slower and the slowest 10% RTs were 7.3% slower. Across activities, there were no differences in normalized mean RT. Normalized fastest 10% RTs were slower after GSE+WALK than after all other activities and normalized slowest 10% reaction speeds were faster after GSE+MOBIL activity than all other activities. Rating of perceived discomfort (RPD) was higher after sitting and walking activities, but there were no differences between baseline and post-activity RPD after GSE+MOBIL or GSE+WALK activities. There were no notable differences across activities in balance or postural sway outcomes.

Conclusion:
After one hour of in-lab seated WBV exposure, an activity, such as walking, GSE, or trunk mobility exercises—either alone or in combination—as opposed to passively sitting for 5 minutes can provide some protection against decrements in reaction time, specifically related to slowest 10% RT outcomes. These selected break activities are easy to perform and do not require peripheral equipment, supporting potential implementation in field settings across various equipment types and tasks. A 5-minute break including an active intervention may be sufficient in protecting against negative health effects of seated WBV exposure. Further on-farm evaluation of typical operator behavior, involving both observational and qualitative studies, is necessary to further develop these break activities and implementation strategies to ensure feasible on-farm application.

Self-declaration of research alignment with additional themes (optional):
Interdisciplinary/Interprofessional Collaboration

86. “PLEASE DON’T CANCEL” PARTICIPANT REPORTED IMPACT OF THE VIRTUAL ‘NEUROSASK: ACTIVE AND CONNECTED’ PROGRAM FOR PEOPLE WITH NEUROLOGICAL CONDITIONS

Presenter:	**Stephen Patrick**
College:	**College of Medicine**
Supervisor:	Katherine B Knox
Co-supervisor(s):	Sarah Donkers

Background:

Neurological conditions account from more than half of Canadians requiring chronic care. Development of a self-management skillset is a critical component supporting those with chronic health conditions. The ‘NeuroSask: Active and Connected’ is a virtual chronic disease management program offering neuro-physiotherapist lead ‘active’ exercise twice a week, and knowledge exchange ‘connect’ sessions with guests. In response to restricted services and supports for people with neurological conditions, NeuroSask was launched May 2020. The program aims to provide seated physical activity, social interaction, and access to specialist expertise in neurological conditions and neurorehabilitation. The objective of this study was to evaluate the NeuroSask program from the participants’ perspectives.

Methods:

A qualitative approach was used to analyze open-ended responses to feedback surveys. All participants registered for the NeuroSask program were invited to complete optional surveys (Survey Monkey) circulated by email at 3 different time points post-program launch: 10 weeks, 1 year, and 2 years. Questions were co-designed by multi-stakeholder team members. Reflexive thematic analysis was completed by SP, KK and SD, with coding conducted in NVivo 12 Plus.

Results:

Survey response rates were as follows: 10-week survey 260/793, one year survey 326/1224, and 2-year survey 434/1989. The majority of participants reported being in the age categories of 40-59 years or above 60 years. 70% of participants reported a diagnosis of multiple sclerosis and 30% reported other neurological conditions. 75% of participants were female. All ten provinces were represented, with 45% of participants living outside of a large city. Three main themes, and eight corresponding subthemes were identified highlighting the perceived impact and key components of the NeuroSask program: Theme 1 “together in a positive and encouraging environment” (subthemes 1a: connection, 1b: empowerment); Theme 2 “access to enthusiastic qualified leaders from home” (subthemes 2a: leader characteristics, 2b: accessibility, 2c: program logistics); Theme 3 “being able to enjoy everyday life” (subthemes 3a: symptom benefits and beyond, 3b: carry-over, 3c: keep going, please don’t cancel).

Conclusion:

Participants reported that the combination of exercise followed by knowledge exchange between participants and experts helped to combat isolation and create a sense of empowerment. The NeuroSask virtual program was perceived as beneficial for fostering community and connection in an accessible, positive environment. The perceived benefits extended beyond symptom management to participant-reported improvements in function, daily life, and disease experience.

Self-declaration of research alignment with additional themes (optional):

Interdisciplinary/Interprofessional Collaboration

Special thanks

The organizing committee of the 30th annual Life and Health Sciences Research Expo would like to thank the following people for playing instrumental roles in the delivery and execution of this important University of Saskatchewan event.

Thanks to their commitment to supporting the next generation of health professionals, the expo has been able to continue its mission of bridging disciplinary gaps and enhancing discovery while championing efforts that can improve the health of people across Saskatchewan, Canada, and the world.

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